New Avenues in the Design of Sialyltransferase Inhibitors

Rémi Szabo
University of Wollongong

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New Avenues in the Design of Sialyltransferase Inhibitors

Rémi Szabo

Supervisors:
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UNIVERSITY OF WOLLONGONG
AUSTRALIA

This thesis is presented as part of the requirements for the conferral of the degree:

PhD in Medicinal Chemistry

School of Chemistry
University of Wollongong

2017
I, Rémi Szabo, declare that this thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, from the University of Wollongong, reports my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualification at any other academic institution.

March 2017,

Rémi Szabo.
Abstract

The presence of up to 30-40% more sialic acid (or hypersialylation) on the tumour cell surface compared to normal cells, along with a marked up-regulation of sialyltransferase (ST) activity is a well-established hallmark of cancer. Due to the critical role of these glycan-building enzymes in tumour growth and progression, ST inhibition has emerged as a potential new antimetastatic strategy for a range of cancers including pancreatic and ovarian cancer. Human STs are divided into four subfamilies or groups based on their linkages (ST3Gal, ST6Gal, ST6GalNAc and ST8Sia) with each subtype controlling the synthesis of specific sialylated structures each with a unique biological role. This has important implications for inhibitor development, as STs also play significant roles in immune responses, inflammation, viral infection and neurological disorders. Thus, to advance to the clinic it is essential to develop subtype selective ST inhibitors.

A wide range of ST inhibitors have been reported including inhibitors from design, from Nature and from high-throughput screening. In the absence of crystal structures of the human STs, the early inhibitors were designed and their activity optimised by using empirical observations for almost two decades. However, the recent publication of crystal structures of mammalian forms of the major enzyme subtypes ST3Gal, ST6Gal and ST8Sia, has furthered our understanding of the differences between the subfamilies and provided the opportunity to use computational tools for the design and optimisation of inhibitors.

All ST subtypes use CMP-Neu5Ac as the natural donor, and the most potent ST inhibitors to-date are all transition-state analogue inhibitors that essentially mimic the donor structure. As part of our study we published a major review focussing on cytidine-based inhibitors comprised of three key fragments: a nucleoside component and a sialic acid mimic linked by a phosphate group. Despite the nanomolar potency of the reported phosphate-linked inhibitors,
little is known about their selectivity towards the different ST subtypes or in vivo activity. Furthermore, their synthesis is often challenging and utilises expensive and low yielding methods. To facilitate an in-depth biological study of ST inhibitors, the design of novel compounds must be directed by the newly emerging structure activity relationships to create libraries of inhibitors via highly efficient and inexpensive synthetic routes.

Therefore, the current project was aimed at the convergent synthesis of triazole-linked inhibitors, which would allow the versatile ligation of nucleoside derivatives with a variety of functionalised sialic acid mimics. The choice of the building blocks for this strategy was guided by computational methods using the recently released ST crystal structures. A series of 19 α-hydroxyphosphonae esters were synthesised for the preparation of carbamate-based inhibitors and also examined for their own inherent anti-inflammatory activity. A series of 10 nucleoside derivatives and 15 alkynes fragments were also prepared, leading to 27 novel triazolonucleoside products. The preliminary testing of our library of triazole-based derivatives towards ST8Sia-II showed promising inhibition up to 98% when tested at a concentration of 100 μM. The incorporation of a fluorine atom on the nucleoside or the sialic acid mimic as well as heterocyclic structures provided the greatest inhibitory activity. Finally, the absence of toxicity of our candidates at concentrations of up to 300 μM allows for further cell-based evaluation of the effects of our compounds on tumour cell adhesion, migration and invasiveness.
Publications arising from this thesis (to date)

Journal articles


Conference abstracts


Szabo, R.; Ranson, M.; D. Skropeta, D., New Avenues in the Design of Sialyltransferase Inhibitors. RACI Organic Group One-Day Symposium, Canberra, Australia, 4 December 2014 (oral and poster presentation).

Szabo, R.; Ranson, M.; Skropeta, D., New Avenues in the Design of Sialyltransferase Inhibitors. RACI Organic Group One-Day Symposium, Brisbane, Australia, 1 December 2014 (abstract and poster presentation).

Szabo, R.; Zhang, J.; McCauley, J.; Ranson, M.; Skropeta, D., Novel α-hydroxyphosphonates as potential kinase inhibitors. RACI Biomolecular Division Conference, Leura, Australia, 14-17 July 2013 (abstract and poster presentation).

Szabo, R.; Zhang, J.; McCauley, J.; Ranson, M.; Skropeta, D., Novel α-hydroxyphosphonates as potential kinase inhibitors. RACI Organic Group One-Day Symposium, Sydney, Australia, 5 December 2012 (abstract and poster presentation).
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The content of this thesis is just the beginning of a greater story thanks to our collaborators contributing to the development of our ideas around the world. Many thanks to Prof. Rita Gerardy-Schahn and her group, in particular Jörg Ehrit at the University of Hannover, Prof. Susan Bellis and her team at the University of Alabama, Prof. Anne Harduin-Lepers at the University of Lille I and Mark Blaskovich, Namfon Pantarat and Johannes Zuegg at WADI. A lot of promising results are already on their way thanks to all of them.

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# Abbreviations

## General abbreviations

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<thead>
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<th>Full Form</th>
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<tr>
<td>δ</td>
<td>chemical shift</td>
</tr>
<tr>
<td>5-FU</td>
<td>5-fluorouracil</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>AcOH</td>
<td>acetic acid</td>
</tr>
<tr>
<td>ArC</td>
<td>aromatic carbon</td>
</tr>
<tr>
<td>ArH</td>
<td>aromatic hydrogen</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>Boc</td>
<td>t-butyloxycarbonyl</td>
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<tr>
<td>br s</td>
<td>broad signal</td>
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<tr>
<td>CDP</td>
<td>cytidine diphosphate</td>
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<tr>
<td>CHO</td>
<td>Chinese Hamster Ovary</td>
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<tr>
<td>CMP</td>
<td>cytidine monophosphate</td>
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<tr>
<td>CTP</td>
<td>cytidine triphosphate</td>
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<td>copper-catalysed azide-alkyne cycloaddition</td>
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<td>dd</td>
<td>doublet of doublets</td>
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<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco's modified eagle medium</td>
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<td>DMF</td>
<td>dimethylformamide</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>dt</td>
<td>doublet of triplets</td>
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<td>equiv.</td>
<td>equivalent(s)</td>
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<td>ESI</td>
<td>electrospray ionisation</td>
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<td>ethyl acetate</td>
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<td>ethanol</td>
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<td>fluorescein isothiocyanate</td>
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<td>h</td>
<td>hours</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
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<tr>
<td>HTS</td>
<td>high throughput screening</td>
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<tr>
<td>IC₅₀</td>
<td>half maximal inhibitory concentration</td>
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<tr>
<td>Kᵢ</td>
<td>inhibition constant</td>
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<td>lit.</td>
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<td>m</td>
<td>multiplet</td>
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<tr>
<td>M</td>
<td>molar</td>
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<td>[M⁺]</td>
<td>molecular ion</td>
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<tr>
<td>MDR</td>
<td>multi-drug resistance</td>
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<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption/ionisation</td>
</tr>
<tr>
<td>min</td>
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<tr>
<td>mmol</td>
<td>millimole</td>
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<td>m. p.</td>
<td>melting point</td>
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<td>MS</td>
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<td>MsCl</td>
<td>methanesulfonyl chloride</td>
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<td>mass to charge ratio</td>
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<td>NMR</td>
<td>nuclear magnetic resonance</td>
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**Amino acid abbreviations**

- Arg: arginine
- Asp: aspartic acid
- Cys: cysteine
- Gly: glycine
- His: histidine
- Lys: lysine
- Phe: phenylalanine
- Ser: serine
- Trp: tryptophan
- Tyr: tyrosine
- Val: valine

**Saccharide abbreviations**

- CMP-Neu5Ac: Cytidine-5′-monophospho-N-acetylneuraminic acid
- CMP-3F-Neu5Ac: Cytidine-5′-monophospho-N-acetyl-3-fluoroneuraminic acid
- Gal: galactose
- GalNAc: N-acetylgalactosamine
- GlcNAc: N-acetylglucosamine
- GM3: monosialodihexosylganglioside
- GT3: trisialylactosylceramide
- KDN: 2-keto-3-deoxy-D-glycero-D-galactonononic acid
- LacNAc: N-acetyllactosamine
- ManNAc: N-acetylmannosamide
- NANA: N-acetylneuraminic acid, sialic acid
- Neu5Ac: N-acetylneuraminic acid, sialic acid
- Sia: N-acetylneuraminic acid, sialic acid
- SLe$^a$: sialyl Lewis A
- SLe$^x$: sialyl Lewis X
### Enzyme abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>glycosylhydrolase</td>
</tr>
<tr>
<td>GT</td>
<td>glycosyltransferase</td>
</tr>
<tr>
<td>PKA</td>
<td>protein kinase A</td>
</tr>
<tr>
<td>ST</td>
<td>sialytransferase</td>
</tr>
<tr>
<td>ST3Gal</td>
<td>sialytransferase catalysing an α-2,3-linkage onto a galactose residue</td>
</tr>
<tr>
<td>ST6Gal</td>
<td>sialytransferase catalysing an α-2,6-linkage onto a galactose residue</td>
</tr>
<tr>
<td>ST6GalNAc</td>
<td>sialytransferase catalysing an α-2,6-linkage onto a GalNAc residue</td>
</tr>
<tr>
<td>ST8Sia</td>
<td>sialytransferase catalysing an α-2,8-linkage onto a sialic acid residue</td>
</tr>
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CHAPTER 1

Sialyltransferases
Chapter 1: Sialyltransferases

1.1. Sialic acids

1.1.1. Background

Sialic acid is considered to be the second most important sugar in the human body, next to glucose.\(^1\)\(^{-3}\) It belongs to a family of sugars containing more than 50 derivatives,\(^4\)\(^{-5}\) that are found in all vertebrates and are also present in bacteria, plants and animals. Sialic acids all share a nine carbon backbone bearing a carboxy group at the 1-position and a hydroxy group at the 2-position (fig. 1.1).\(^6\)\(^{-7}\) One subfamily of sialic acids is N-acetylated to form N-acetylneuraminic acid 1 (Neu5Ac, NANA, Sia), which is the most ubiquitous member. Sialic acids are typically found on the extremity of non-reducing glycan chains on the surface of cells, where they are linked to the 3- or 6-hydroxyl groups of galactose (Gal) moieties, or to the 6-hydroxyl group of N-acetylglucosamine (GlcNAc) or N-acetylgalactosamine (GalNAc, fig. 1.1). They can also form polysialic chains by linking at the 8-hydroxyl group.\(^8\)

Figure 1.1. Structure of sialic acid derivatives and O-sialosides on the cell surface.\(^9\)
1.1.2. Cell surface glycans

Sialylated oligosaccharides have long been predicted to be information–containing molecules and critical determinants in cell-cell recognition processes, cell-matrix interactions and in the maintenance of serum glycoproteins in the circulation as a result of their terminal position on oligosaccharide chains.\textsuperscript{10-12} The cell surface sialic acid residues, which are negatively charged at physiological pH, have been known to act as receptors for the influenza virus.\textsuperscript{13} They often function in the “antirecognition” or masking of the carbohydrate groups they terminate.\textsuperscript{11} The highest concentration of sialic acid (as N-acetylneuraminic acid) in the human body occurs in the brain where it is an integral part of ganglioside structure.\textsuperscript{14} Consistent with this finding, human milk was also found to contain a high concentration of sialic acid, associated with an increase of gangliosides in the brain and improved learning ability.\textsuperscript{15} Conversely, sialoside reduction is correlated to erythrocyte destruction.\textsuperscript{16}

Glycan structures of cell surface glycoconjugates have pivotal functions in various cellular recognition systems involving cell differentiation, development,\textsuperscript{17,18} inflammation,\textsuperscript{19,20} immune response,\textsuperscript{21} tumour metastasis, bacterial and viral infections, and many other intercellular communication and signal processes.\textsuperscript{22-24} However, glycan expression and its microheterogeneity in common glycoproteins cannot be predicted because protein glycosylation is not template-driven and is subject to multiple sequential and competitive enzymatic pathways.\textsuperscript{25-27} The biosynthesis of complex glycans is carried out by a variety of glycosyltransferases (GTs) sharing several sugar nucleotides as substrates in collaboration with many glycoside hydrolases (GHs) to define further synthetic pathways.\textsuperscript{28,29} In other words, it is extremely difficult to identify every enzyme responsible for synthesizing biologically relevant glycoforms. In the future, large-scale glycomics may help to reveal
whole human glycoforms and profile their structural alterations during cellular differentiation, proliferation, carcinogenesis, malignant alteration, and metastasis.\textsuperscript{30-33}

Specific and potent inhibitors of GTs would be useful tools for the investigation of structure-function relationships of the human glycome and the regulation mechanism of glycan biosynthesis as well as leading to the discovery of new classes of therapeutic agents. Extensive efforts have been invested to develop such inhibitors for biologically important GTs.\textsuperscript{34-36}

1.2. Sialyltransferases

1.2.1. Classification and occurrence

Sialyltransferases (STs) are a subset of GTs involved in the biosynthesis of sialylated glycolipids and/or glycoproteins. Depending on the glycosidic linkage formed and their monosaccharide acceptor, vertebrate STs are arranged in four families of proteins (ST3Gal, ST6Gal, ST6GalNAc and ST8Sia, table 1.1).\textsuperscript{37} ST families are further subdivided into 20 sub-families in mammals and more than 25 sub-families in lower vertebrates, each of them being characterised by conserved amino acid residues. All of them use CMP-Neu5Ac 2 as the activated sugar donor to catalyse the transfer of sialic acid residues to terminal non-reducing positions of oligosaccharide chains of glycoproteins and glycolipids (fig. 1.2).\textsuperscript{8,38} However, these enzymes differ in their substrate specificity, tissue distribution and various biochemical parameters. Enzymatic analysis conducted \textit{in vitro} with recombinant enzymes have revealed that one linkage can be synthesised by multiple enzymes.\textsuperscript{39} Moreover, even though eukaryotic STs share the same sugar donors, \textit{i.e.} CMP-Neu5Ac 2, and recognise identical acceptor substrates, they do not exhibit similar protein structure except for the three short and conserved consensus sequences present in the catalytic domain.\textsuperscript{40-41}
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Substrates</th>
<th>EC number</th>
<th>HGNC</th>
<th>Older names</th>
<th>Gene ID</th>
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<td>Gal-β-1,3-GalNAc-(protein)</td>
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<td>ST3O 8q24.2 (6482)</td>
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<td>CGS23, FLJ11867, NANTA3, STZ 11q23–q24 (6484)</td>
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<td>Gal-β-1,4-Glc-Ceramide</td>
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<td>SIAT9</td>
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<td>SIAT7H</td>
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<td>SIAT8C</td>
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<td>SIAT8E</td>
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<td>2.4.99.8</td>
<td>23317</td>
<td>SIAT8F</td>
<td>10p13 (338596)</td>
</tr>
</tbody>
</table>

Table 1.1. Nomenclature of the 20 human sialyltransferases (STs) described to-date.
These enzymes have been shown to be topologically restricted to the cisternae and the Golgi network of the Golgi apparatus, although catalytically active soluble forms can be generated \textit{in vivo} by proteolytic cleavage at the stem region, \textit{e.g.} serum soluble ST6Gal-I produced by proteolytic cleavage at the stem domain.\textsuperscript{43} ST3Gal-III has also been identified in post-Golgi locations in rat kidney tissue.\textsuperscript{44}

1.2.2. Sialyltransferase mechanism of action

The mechanism for the sialylation reaction was first proposed by Horenstein (fig. 1.2).\textsuperscript{45-49} The reaction undergoes mechanistic pathways similar to other GTs\textsuperscript{50} and uses the sugar-nucleoside conjugate CMP-Neu5Ac \textsuperscript{2}, with the CMP fragment of the donor behaving as a leaving group (fig. 1.2).\textsuperscript{51-55} The catalytic residues are usually histidine or tyrosine, however arginine residues have also been observed in some cases. These residues play a key role in the deprotonation of the hydroxyl group of the acceptor sugar and in activating the phosphate group. Most of the other residues in the active site help in positioning both the donor and the acceptor in their respective binding pockets.\textsuperscript{56} The transition-state proposed seems to be applicable for all ST subtypes. During the sialyl transfer, the acceptor approaches the anomeric centre of the sialic acid fragment by the side opposite to the CMP residue. At this stage, a planar carboxenium intermediate is formed in a S\textsubscript{N}2 fashion. The final products are CMP \textsuperscript{3} and the sialylated sugar (fig. 1.2). This proposed mechanism was recently revisited and assessed by Schmölzer \textit{et al.}\textsuperscript{57}
More than 90% of cancer deaths are caused by cancer metastasis, despite recent advances in cancer chemotherapy and the advent of tumour-targeted agents. Currently, treatment and chemotherapy are focusing on primary tumours rather than metastatic processes and antimetastatic drugs are often used in combination therapy. The metastatic process itself is highly complex, depending both on the tumour and its microenvironment. Therapeutic benefits in late-stage or elderly cancer patients are especially poor. The development of antimetastatic drugs emerging from current drug-screening pathways is thus urgently needed. In order to improve this approach, efforts on understanding and reversing the metastatic processes might have a great outcome on the effectiveness of chemotherapy. Human cancer metastasis is a long-evolving, multi-step process that can only be treated or controlled by drugs or immunomodulators to date. Research in the field has suggested that future antimetastatic therapies could
be strategically optimised according to the characteristics of metastatic processes in order to address these unmet therapeutic benefits.\textsuperscript{61-63}

Elevated plasma levels of STs have been observed in cancer patients since the 70's, and hypersialylation of cell surface proteins is now a well-established hallmark of cancer that forms the basis of several diagnostic cancer markers.\textsuperscript{64-68} One such example is the carbohydrate antigen CA19-9 for pancreatic cancer, which results from overexpression of ST3Gal-III, IV and VI leading to the production of large amounts of sialyl Lewis\textsuperscript{a}. This is typical of the involvement of the ‘incomplete synthesis’ mechanism in the enhanced expression of sialyl Lewis X (SLe\textsuperscript{X}) and sialyl Lewis A (SLe\textsuperscript{a}) in cancers.\textsuperscript{67,56} Sialylated glycans have long been predicted as cell-type specific markers and critical determinants in various cellular recognition processes (cell–cell recognition, adhesion, protein targeting, blood coagulation, fertilization, and other biological events).\textsuperscript{69} They also play significant roles in immune responses,\textsuperscript{70} inflammatory diseases, viral infection\textsuperscript{71,72} and tumour proliferation,\textsuperscript{73} migration, and metastasis.\textsuperscript{74-76}

STs have also been described as “engines of self-fuelling loops in cancer progression”.\textsuperscript{77-79} Hypersialylated integrins are believed to facilitate cell migration in many different cancer types,\textsuperscript{80} such as colon,\textsuperscript{81-82} lung \textsuperscript{83-84} and ovarian cancer.\textsuperscript{85} Aberrant sialylation at the non-reducing end of glycoproteins or glycolipids has been shown to promote events involving abnormal stimuli in the interaction of neoplastic cells with extracellular matrices.\textsuperscript{33} The biosynthesis of sialylated glycoprotein, mediated by ST6Gal-IV, was also reported on various kidney cancer cell lines.\textsuperscript{86} As well as considering membrane sialylated glycoproteins as a target for treating cancer, these molecular patterns are also useful in the prognosis of cancer\textsuperscript{87-88} and other diseases.\textsuperscript{89} Tumour cell migration and invasion are regulated by sialylated glycoforms of integrins. Specifically, β1 in colon adrenocarcinomas\textsuperscript{81-82} and lung,\textsuperscript{83-84} where ST expression, in particular ST6Gal-I,\textsuperscript{90} ST3Gal-I,\textsuperscript{91-92} ST3Gal-III,\textsuperscript{74-90} ST3Gal-IV,\textsuperscript{74-93}
ST6GalNAC-I\textsuperscript{94} and ST6GalNAC-V\textsuperscript{95} are strongly correlated with tumour invasiveness. This is also the case in numerous other cancers including, bladder,\textsuperscript{92} breast,\textsuperscript{96} pancreatic,\textsuperscript{97} ovarian,\textsuperscript{85} gastro-intestinal\textsuperscript{98} cancers and leukemia.\textsuperscript{99} The level of gene alteration observed for the 20 ST subtypes is summarised in fig. 1.3 for melanoma, lung, ovarian, prostatic, breast and pancreatic cancers.

**Figure 1.3.** Gene alteration frequency of sialyltransferase subtypes in various cancers.\textsuperscript{100}

**Lung Cancer:** Sialyl Lewis x (SLe\textsuperscript{x}) is a well-known tumour-associated antigen that is found in elevated levels in lung cancer. It facilitates cell adhesion to the endothelium, initiating the process of extravasation and metastasis. ST3Gal-III, IV and VI enzymes play a key role in the synthesis of SLe\textsuperscript{x}. In lung cancer, expression levels of both SLe\textsuperscript{x} and ST3Gal-III are inversely correlated with patient survival.\textsuperscript{101} Hypersialylation and increased expression of SLe\textsuperscript{x} is also observed in the mucins of cystic fibrosis patients,\textsuperscript{102} while over-sialylation of several other proteins including integrins has also been shown to enhance cell invasion in ovarian, colon and lung cancer. ST3Gal-I is not detected in normal human bronchial epithelial cells, but is over-expressed in highly metastatic lung cancer cells such as A549 and CL1-5.\textsuperscript{84} Thus, inhibition of
STs, in particular the ST3Gal subfamily, has emerged as a new target for the treatment of early lung cancer, and to aid in understanding the progression to more aggressive phenotypes.

**Ovarian Cancer:** Increased serum glycoprotein sialylation in a murine ovarian carcinoma model has been found to correlate with inflammation and tumour progression, with increased expression of ST3Gal-I and ST6Gal-I. The latter also regulates ovarian tumour cell survival mechanisms and confers cisplatin resistance. CA125, the clinically used ovarian cancer marker is a heavily glycosylated mucin (MUC16). Levels of sialyl-Tn antigen, expressed on MUC16 in patients, is found to differ between endometriosis and ovarian cancer raising the potential for its use in diagnosis, and evaluating the clinical stage, cytological grade, and histological type of ovarian cancer.

**Breast Cancer:** STs have been found to play a key role in breast cancer progression, aggressiveness, metastasis and drug resistance. Recent studies in triple-negative breast cancer patient-derived xenograft models treated with standard chemotherapy identified high levels of the sialyl-glycolipid stage-specific embryonic antigen 4 (SSEA4). This treatment-resistant breast cancer subpopulation also exhibited high expression of ST3Gal-II, which is required for the biosynthesis of SSEA4. This could be used as a predictive biomarker of poor clinical outcomes in both breast and ovarian cancer patients treated with standard-care genotoxic chemotherapy. Gene expression analysis coupled with functional analysis of clinical samples has also identified ST6GalNAc-V as a key mediator of breast cancer metastasis to the brain. Atypical expression of this brain ST in breast cancer cells is thought to facilitate their passage through the blood-brain barrier. A strong correlation between the expression of the transmembrane tumour-associated glycoprotein Mucin 1 MUC1 and T antigen has been found in breast cancer tumours and breast cancer cell lines, along with the down-regulation of expression of ST3Gal-I. Expression analysis of 74 primary breast cancers showed a
significant correlation between ST3Gal-I and COX-2, indicating that malignant characteristics of the cyclooxygenase-2 expression may be related to tumour cell surface sialylation.\textsuperscript{112}

**Multiple Myeloma:** High expression of ST3Gal-VI has been identified in patients with multiple myeloma and correlated to poor patient prognosis. Knockdown of ST3Gal-VI in myeloma cell lines MM1S and RPMI-8226 significantly reduced α-2,3-linked sialic acids on the cell surface and reduced adhesion to bone marrow stromal cells and fibronectin. It further inhibited transendothelial migration \textit{in vitro} and reduced homing and engraftment, decreased tumour burden and increased survival \textit{in vivo} in a xenograft (SCID-Bg) mouse model.\textsuperscript{113}

**Leukemia and Glioma:** Elevated mRNA levels of ST expression have been found to positively correlate with a high risk of numerous cancers including paediatric leukemia, where it has been suggested as a potential biomarker.\textsuperscript{114} In particular, cell surface sialylation is emerging as an important feature of cancer cell multidrug resistance (MDR). Recently, the α-2,8-sialyltransferases ST8Sia-IV and ST8Sia-VI were found to positively correlate with the multidrug resistant phenotype in chronic myeloid leukemia cells, with ST8Sia-IV appearing to regulate the activity of PI3K/Akt signalling and P-gp expression.\textsuperscript{115-116} Overexpression of ST6GalNAc-V, a ganglioside-specific α2,6-sialyltransferase, inhibits glioma growth \textit{in vivo}.\textsuperscript{117}

**Metastasis and MDR:** Also of important consideration is metastasis, a major challenge in the treatment of cancer and is largely responsible for the high rate of cancer mortality. It is emerging that molecules conferring drug resistance may also promote metastatic invasion. This gives rise to the situation where metastatic disease is often more resistant to treatment.\textsuperscript{118}
The biological functions of sialic acids are well known and the literature provides a strong body of evidence for the role of sialylation in various medical conditions and in particular cancer. This growing knowledge on STs is illustrated in comprehensive reviews published by many experts in the field, including Schauer, Varki, Crocker, and Boons et al. Over the last decade, the therapeutic potential of targeting STs has also appeared in reviews focusing on GTs and altered glycosylation in cancer from Brooks et al., Brown et al., Compain & Partin, Gloster & Vocadlo, Hinou & Nishimura, Izumi et al., Kajimoto & Node, Roychoudhury & Pohl, Schnaar et al. and Zhou et al.

In summary, sialylated cell-surface carbohydrates are crucial in the course of cell development and differentiation. Anomalies in their expression is indisputably associated with cancer progression and metastasis as well as directing carbohydrate-mediated adhesion processes in angiogenesis and drug resistance. Therefore the development of antimetastatic drugs targeting aberrant sialylation has recently emerged as a new therapeutic avenue in cancer treatment.

1.2.4. Biological assays

A key challenge in the ST inhibitors field is the choice of biological assay employed to evaluate the effectiveness of newly developed inhibitors. There are no commercially available ST inhibitor screening assays at the time of writing and very few commercial sources of mammalian ST enzymes, those being rST3Gal-I (EC 2.4.99.4) from Merck (#566227) and hST6Gal-I (EC 2.4.99.1) from Merck (#566223) and Roche (#07-012-250-103). For this reason, most research groups clone, express and/or isolate their own ST enzymes, which are used in either crude, partially purified or purified form. As with all enzyme work, there are a wide range of enzymes used (e.g. predominantly human, rat, and bacterial STs).
and a variety of assays employed (colorimetric, fluorescent, radioactive, mass spectrometry and HPLC-based) to evaluate inhibitory activity, and this should be considered when comparing biological results.

**HPLC-based Assays:** Earlier studies into STs used asialofetuin or lacto-\(N\)-tetraose as the acceptor moiety and \(^{14}\)C or \(^{3}\)H-labelled CMP-Neu5Ac\(^{150}\) as the donor and the radioactive products were isolated by chromatography or precipitation.\(^{150-152}\) In 1998, R. R. Schmidt and co-workers introduced a HPLC-based assay using the \(p\)-nitrophenyl glycoside of \(N\)-acetyllactosamine as a UV labelled acceptor, and this continues to be one of the most widely used ST inhibition assays today.\(^{153}\) Recent extensions of this method, include a continuous all-in-one HPLC-based assay that enables the quantification of productive turnover, error hydrolysis and site selectivity of sialyltransfer and donor substrate hydrolysis, with the potential for high-throughput screening.\(^{154}\) Further HPLC-based assays include those reported by Kijahara et al. and involve a combination of HPLC analysis with a fluorescent analytical technique based on reacting sialic acid with 1,2-diamino-4,5-methylenedioxybenzene,\(^{155}\) or using 2-\(\{(2\)-pyridyl)aminoethyl\}-\(\beta\)-D-lactosamidine as a ST acceptor for potential use in fluorescent assays.\(^{156}\)

**Fluorescence-based Assays:** Rillahan et al. designed a fluorescence polarisation assay enabling high-throughput screening of sialyl- and fucosyltransferases.\(^{157}\) The method employs a CMP-Neu5Ac derivative bearing a fluorescein isothiocyanate (FITC) tag at the 9-position\(^{158}\) and asialofetuin as the acceptor. This technique has the advantage of being suitable for various enzyme subtypes by using a versatile acceptor, however, it does require the non-trivial preparation of the FITC-labelled CMP-Neu5Ac donor 4 (fig. 1.4). Kumagai et al. have reported a multi-enzyme system allowing the screening of potential inhibitors against a wide variety of GTs including glucosyl-, galactosyl-, glucuronyl-, xylosyl-, fucosyl- and sialyltransferases.\(^{159}\) Three kinase enzymes are used in order to provide glucose-6-phosphate...
(G6P) starting from the nucleoside monophosphate resulting from the glycosylation reaction. G6P dehydrogenase converts then resazurin, in a dose-dependant manner, to resorufin and the fluorescence of the solution is measured. This method has the advantage of allowing HTS of compound libraries. More recently, Preidl et al. designed a binding affinity assay in which the dissociation constant was determined by comparing the anisotropy changes in fluorescence polarisation.\textsuperscript{160}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure1.4}
\caption{Reagent 9''-FITC-CMP-Neu5Ac 4 used in sialyltransferase inhibition assays.}
\end{figure}

**MS-based Assays:** Hosoguchi et al. designed a MS-based protocol suitable for HTS of potential GT inhibitors. The authors monitored the inhibition rate by determining the amount of glycosylated acceptor using MALDI-TOFMS.\textsuperscript{161} An alternative technique consists of immobilising the acceptor sugar on a plate or a cellulose membrane. This method was described using \textit{N}-acetyllactosamine\textsuperscript{162} or trisialyl lactoside (GT3) by click-chemistry.\textsuperscript{163} Other assays using multi-enzyme systems combined with spectrophotometry\textsuperscript{81,164} or colorimetry\textsuperscript{165} have also been reported.

### 1.2.5. Structure of sialyltransferases

On the basis of sequence similarities, STs are grouped into five different GT families within the carbohydrate-active enzymes (CAZy) database.\textsuperscript{166} All eukaryotic STs, along with the viral enzymes, are found in a single CAZy family, GT29. Mammalian STs share a predicted \textit{N}-terminal membrane-anchoring region and four conserved sequence motifs denoted as
L (large), S (small), VS (very small) and motif 3.\textsuperscript{167-171} Although these ‘sialyl motifs’ have been instrumental in the identification and cloning of new STs over the last three decades, their structural context has remained unknown, with the only insights being those obtained by kinetic analysis of mutants.\textsuperscript{167,169}

Due to the tremendous therapeutic potential associated with STs, extensive efforts have been made to identity and understand the structural differences between the different ST subtypes. The ST crystal structures so far reported were usually obtained with an additive co-crystallised with the enzyme, such as CMP 3, CDP 5, CTP 6, the inhibitors CMP-3F-Neu5Ac\textsuperscript{172} 7 and simplified aromatic inhibitor 8, later described in section 1.3.4, selenomethionine and several sugars, including oligosaccharides as well as the modified acceptors such as 2-nitrophenylLacNAc 9 and the sialylated keratin sulfate 10 (α-Neu5Ac-2,3-β-Gal-1,4-(6S)-β-GlcNAc, fig. 1.5). The presence of these compounds provides some information about the location of the active site.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{images}
\caption{Additives found co-crystalised with sialyltransferases.}
\end{figure}
1.2.5.1. Bacterial Sialyltransferases

Bacteria have been found to be a good source for STs. For example, from *Campylobacter jejuni*, Cst-I\textsuperscript{173} and Cst-II\textsuperscript{174} were isolated and promote specific or multifunctional sialyl patterns. PM0188, an \(\alpha\)-2,6-ST from *Pasteurella multosida*, has been reported\textsuperscript{175-176} along with \(\Delta\)24PmST1 was also examined and shows \(\alpha\)-2,3-ST, \(\alpha\)-2,6-ST, sialidase and trans-sialidase acitivity.\textsuperscript{177-178} Some mutant versions of PmST1 were described,\textsuperscript{179} including D141N and M144D,\textsuperscript{180} which showed significantly decreased sialidase activity. In a similar approach, the crystal structure of the mutant bifunctional \(\alpha\)-2,3-ST and \(\alpha\)-2,6-ST \(\Delta\)29NST was obtained from *Neisseria meningitides*. \(\Delta\)16psp26ST is a truncated form of JT-ISH-224, mono-functional \(\beta\)-galactoside \(\alpha\)-2,6-ST, cloned from *Photobacterium phosphoreum*.\textsuperscript{181} \(\Delta\)Npp23ST, also a truncated form of JT-ISH-224, was later crystalised.\textsuperscript{182} Finally, the structures of \(\Delta\)15Pd2,6ST(N) and \(\Delta\)112Pd2,6ST(N) where reported, also \(\alpha\)-2,6-ST from *Photobacterium damselae*.\textsuperscript{52}

1.2.5.2. Mammalian Sialyltransferases

The first mammalian ST crystal structure was reported by Rao et al. in 2009\textsuperscript{183} The crystallised enzyme is a porcine ST3Gal-I variant, which shows 85% identity with human ST3Gal-I. In addition, kinetic studies of pST3Gal-I samples revealed similar profiles, *i.e.* \(K_M\) values on the micromolar scale, in comparison with STs obtained via eukaryotic expression systems and different animal species.\textsuperscript{171,184-186} From these data, the authors later proposed a mode of binding for the donor CMP-Neu5Ac 2 and the acceptor 2-nitrophenyl-Gal-\(\beta\)-1,3-GalNAc 9.\textsuperscript{187} More recently, both rat\textsuperscript{188} and human\textsuperscript{189} ST6Gal-I crystal structures were also reported, along with human crystal structure for ST8Sia-III.\textsuperscript{190} The superimposed ST crystal structures are represented in fig. 1.6, aligned to the nucleoside fragment (CMP 3) that was co-crystalised with them.
Figure 1.6. Superimposed ST crystal structures aligned to CMP 3 (in white sticks). The porcine ST3Gal-I (PDB: 2WNB, in green), the human ST6Gal-I (PDB: 4SJ2, in grey) and the human ST8Sia-III (PDB: 5BO6, in orange and PDB: 5CXY in dark orange).

The crystallographic data provided insight on the mode of action of STs and allowed the use of computational tools to assist structure-based design of inhibitors. The reported mammalian ST crystal structures are summarised in table 1.2. All reported PDB files are available on the RCSB Protein Data Bank website (www.rcsb.org).

Table 1.2. Reported crystallographic data for mammalian sialyltransferase subtypes and their corresponding PDB file entries.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Organism</th>
<th>PDB file</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST3Gal I</td>
<td>Sus scrofa (pig)</td>
<td>2WML, 2WNB, 2WNF</td>
<td>Rao, 2009183</td>
</tr>
<tr>
<td>ST6Gal I</td>
<td>Rattus norvegicus (rat)</td>
<td>4MPS</td>
<td>Meng, 2013188</td>
</tr>
<tr>
<td>ST6Gal I</td>
<td>Homo sapiens (human)</td>
<td>4JS1, 4JS2</td>
<td>Kuhn, 2013189</td>
</tr>
<tr>
<td>ST8Sia III</td>
<td>Homo sapiens (human)</td>
<td>5BO6, 5BO7, 5BO8, 5BO9</td>
<td>Volkers, 2015190</td>
</tr>
<tr>
<td>ST8Sia III</td>
<td>Homo sapiens (human)</td>
<td>5CXY</td>
<td>Volkers, 2016</td>
</tr>
</tbody>
</table>
Collectively this data confirms the mechanism for the reaction proposed by Horenstein (fig. 1.4).\textsuperscript{45-49} The conserved sialyl motifs frame the catalytic site, and a disulfide bond stabilises the two sialyl motifs (large, L, and small, S), a feature conserved throughout the ST family.\textsuperscript{191} A flexible loop missing from the electron density is proposed to form a ‘lid domain’ that participates in the binding of the donor substrate. The acceptor binding sites of different mammalian STs must vary in structure in order for them to accommodate different carbohydrate moieties such as galactose in ST3Gal and ST6Gal, N-acetylgalactosamine in ST6GalNAc or sialic acid in ST8Sia. Likewise different regiochemical outcomes must be encoded; thus, ST3Gal and ST6Gal need to bind the galactosyl moiety in different orientations relative to CMP-Neu5Ac \textsuperscript{2} in order to make $\alpha$-2,3 and $\alpha$-2,6 linkages, respectively. Previous mutagenesis studies revealed several conserved peptide sequences within each subfamily group, located between the L and S sialylmotifs, that were postulated to contribute to the different linkages and acceptor specificity.\textsuperscript{192} Two such submotifs are observed in the case of pST3Gal-I.\textsuperscript{187}

Several structural features of the mammalian ST family have previously been deduced from their primary sequences.\textsuperscript{191} All were known to have $N$-terminal signal anchor sequences that tether them to the Golgi membrane, and a flexible ‘stem’ region that is not required for catalytic activity. Various homologous regions have been identified in the catalytic domain, also called sialyl motifs, and were postulated to be involved in binding substrates and catalyzing the transfer of the sialic acid to the acceptor glycan.\textsuperscript{171,193-196}

1.2.5.3. Computational studies

The recent characterisation of mammalian ST crystal structures have further enabled visualisation of the substrate-enzyme interactions. As predicted, the donor binding site which is highly conserved between the different subtypes, remains consistent while the major
Chapter 1

variations occur in the acceptor site (fig. 1.7-A and B). Insight from crystal structures, such as rat and human ST6Gal-I show many similarities and inhibition of the rat-derived enzymes is thus likely to be translatable to the human form of the enzyme.

Figure 1.7. The sialyl donor CMP-Neu5Ac 2 (green sticks) docked into the human ST6Gal-I (A, PDB: 4JS2) and the porcine ST3Gal-I (B, PDB: 2WNB) with their respective acceptors LacNAc 11 and 2-nitrophenyl-Gal-β-1,3-GalNAc 9 (orange sticks).

Using recent crystallographic data, research groups have started developing new inhibitors by structure-based design. Kumar et al. designed non-charged inhibitors and docked them into the bacterial Cst-I enzyme (PDB: 2P2V). Chen et al. evaluated the binding site of potent fluorescent probes by docking into the bacterial PmST1 (PDB: 2IHZ) and pST3Gal-I (2WNB). Sujatha et al. established an homology model for the six human ST3Gal I-VI by aligning their sequence with the bacterial enzyme Cst-II. Grewal et al. later created homology models for the human ST6GalNAc-I, ST3Gal-III and ST8Sia-I using the crystal structure of the porcine ST3Gal-I as a template and evaluated potential binding sites for the natural products curcumin and resveratrol.
1.3. Sialyltransferase inhibitors

1.3.1. Reviews

The design and development of potent small molecules inhibiting ST enzymes was pioneered by Schmidt and co-workers, almost two decades ago. The synthesis and biological evaluation of ST inhibitors described at that time have been reviewed previously and focussed on α-2,3-ST and α-2,6-ST subtypes. In the intervening years since the first development of ST inhibitors, a rising number of biological and medical-related articles have appeared. This has expanded our understanding of the role of glycosylation in various disorders, as well as the role of altered sialylation in diseases, in particular metastatic cancers. In recent years, along with the mounting evidence of the critical role of sialylation in cancer and growing structural information on STs, several new potent ST inhibitors have been described. These have been derived from various scaffolds and thus increasing the molecular diversity of ST inhibitors in the literature. The biological data provided for the known inhibitors relies on a large array of assays (Cf. section 1.2.4), which provides intuitive trends for the compounds’ potency rather than enabling consistent comparison. A comprehensive review aiming at bridging the gaps between the chemical and biological aspects of ST inhibition was published by our group in 2016. The article also presents emerging techniques in the STs field such as computational modelling, recently made possible by the publication of the first crystal structures of mammalian STs (Cf section 1.2.5), and towards diagnostic tools using imaging and glycoengineering. Herein a brief history of the published review is presented.

1.3.2. Inhibitors from Nature

Screening natural products for their activity towards STs has provided a few new potent inhibitors. Three spirocyclic drimanes isolated from the fungus *Stachybotrys cylindrospora*
have been reported as ST inhibitors. The activity of these terpenoids was evaluated towards ST3Gal-I, ST3Gal-III, ST6Gal-I as well as some fucosyltransferases and galactosyltransferases.\textsuperscript{210} Mer-NF5003F or stachybotrydial 12, inhibited these enzymes in the low micromolar range (fig. 1.8). The Taiwanese cyanobacteria *Lyngbya Majuscula* was found to produce fatty amides, including isomalyngamide A-1 13 (fig. 1.8), which inhibits ST3Gal-I with an IC\textsubscript{50} value of 65.7 µM.\textsuperscript{211} The natural product 13 also significantly decreased the proliferation and migration of the metastatic human breast cancer cells MCF-7 and MDA-MB-231. Several flavonoids have been recently reported by Hidari *et al.*\textsuperscript{212} and moderately inhibited rat ST3Gal-I and both rat and human ST6Gal-I. Epigallocatechin gallate 14, a flavonoid present in green tea, was previously shown to inhibit rat brain ST3Gal-I with an IC\textsubscript{50} value of 2.7 µM.\textsuperscript{213} The compound 14 also inhibits fucosyltransferases in a similar order of potency.

The steroidal derivative soyasaponin I 15, extracted from soy beans, inhibits ST3Gal-I with a $K_i$ value of 2.3 µM\textsuperscript{214} and reduced the migration of breast cancer cells MCF-7\textsuperscript{215} and melanoma cells B16F10.\textsuperscript{216} The bile acid lithocholic acid 16 exhibits ST3Gal-I inhibition in the low micromolar range (IC\textsubscript{50} = 21 µM).\textsuperscript{217} The synthetic derivatives Lith-O-Asp 17\textsuperscript{83} and AL-10 18\textsuperscript{84} show improved activity (IC\textsubscript{50} = 21 µM and 0.88 µM respectively, fig. 1.8) and further biological evaluation revealed that their antimetastatic effects are mediated by inhibiting the sialylation of integrin-β1 in several cancer cell lines.

Most of these compounds are built on classical naturally occurring scaffolds and were found to exhibit activity towards various biological targets which could also be responsible for unwanted off target effects.
1.3.3. Inhibitors from high-throughput screening

High-throughput screening of synthetic combinatorial libraries is widely used for identifying hits in medicinal chemistry. In the context of ST inhibition, this method was used to screen libraries of synthetic peptides showing moderate activity for the hexapeptide GNWWWW 19 towards ST3Gal-I, with a $K_i$ value of 8.8 µM (fig. 1.9). The Paulson’s group evaluated a library of molecules against porcine ST3Gal-I, rat ST3Gal-III and human ST6Gal-I as well as the human fucosyltransferases FUT-6 and FUT-7. The compound JFD00458 20 was identified as a broad-
spectrum inhibitor and the compound 21 showed specificity for ST3Gal-III (IC$_{50}$ = 1.7 µM), while being competitive with the natural donor substrate site (fig. 1.9). The authors also stated that despite the possible existing similarities at the active site of the various enzyme subtypes, significant variations may allow differentiation for the design of selective inhibitors.

![Figure 1.9](image)

**Figure 1.9.** Examples of sialyltransferase inhibitors from high-throughput screening

### 1.3.4. Inhibitors by Design

Several approaches have been explored for designing ST inhibitors, based on the enzyme substrate structures. In the early days, the design was driven by empirical observation given the lack of crystal structures for the mammalian enzymes.

**Acceptor-analogue:** Since the early modified disaccharides reported by Hashimoto’s group$^{219-220}$ a few different types of acceptor-analogue have been designed.$^{204}$ As the enzyme subtypes are classified by their respective acceptor, using this as a template for designing ST inhibitors presents the advantage of targeting the different subtypes selectively. However, to date this has only given inhibitors of weak to moderate potency. Of these, inhibitors created from the structure of lactose, modified on the 6-position of the galactose unit, the 2-position of the glucose fragment or in the form of dimers, gave compound 22 as the best example, with some significant selectivity towards ST3Gal-I, with a $K_i$ value of 185 µM compared to 533 µM against ST6Gal-I (fig. 1.10).
**Bisubstrate inhibitors:** The bisubstrate analogue 23 prepared by Izumi’s group for a SAR study only showed weak inhibitory activity against recombinant rat ST3Gal-I (IC$_{50}$ = 1.3 mM) and ST6Gal-I (IC$_{50}$ = 2.4 mM). The compound structure consists of a CMP-Neu5Ac analogue in which the carboxylic acid group was reduced to an alcohol and linked to a glucose molecule via a phosphodiester moiety as shown in fig. 1.10.

![Figure 1.10. Examples of acceptor-analogues and bisubstrate inhibitors.](image)

In addition, their preparation involves challenging carbohydrate synthetic methods, resulting in long multistep sequences and leading to a low overall yield.

**Cytidine-based inhibitors:** Over the last decade, several research groups focused on the design of donor analogue inhibitors. Prevention of the glycosyl transfer is mediated by binding in the active site of the enzyme in competition with the natural glycosyl donor CMP-Neu5Ac 2. Most of the active compounds resulting for this approach are CMP-Neu5Ac derivatives which have been modified on the sugar fragment. Various scaffolds showed promising activity aligning with the expertise of the research groups where these inhibitors were synthesised. For example, the Horenstein’s group investigated some bicyclo[3.1.0]hexane derivatives to mimic sialic acid. The structure was obtained using the Meinwald rearrangement of norbornadiene promoted by a peracid. This scaffold offers an elegant way to imitate the elongated distance between the anomeric position of the sugar and the phosphate linker upon leaving. The prepared compound 24 displayed some inhibition in the low micromolar range towards ST3Gal and
ST6Gal (fig. 1.11). This work was continued by Niwayama et al. using a different synthetic pathway in milder conditions but the biological activity was not communicated.\cite{223}

![Transition-state analogues](image)

**Figure 1.11.** Bicyclic sialic acid mimic 24 as sialyltransferase inhibitors.

**Transition-state analogues:** Designing an inhibitor by taking inspiration from the transition-state of the enzyme often provides potent compounds.\cite{224-225} This concept was implemented in the Schmidt’s group, based on the S$_{N}$2-like mechanism,\cite{45-49} whereupon many transition-state analogues were designed and evaluated. This transition-state is believed to occur through an oxocarbenium ion-like structure on a distorted half-chair conformation ring. Using this strategy, a double bond was introduced at the anomic position of the sialic acid fragment in order to replicate the planar geometry of the oxocarbenium intermediate. An extra carbon atom was added to the structure to mimic the elongated distance between the reactive center and the phosphate group while the CMP moiety started to leave. As a result, molecules derived from unsaturated sialic acid analogues or simplified polyhydroxylated cyclohexenes emerged (fig. 1.12), including compound 25 which reached submicromolar levels of potency.\cite{153,200} To our knowledge, these inhibitors exhibited the most potent activity towards ST6Gal-I but very little is known about their activity against other ST subtypes.
Replacing the native carboxylic acid group of the sialic acid fragment with the phosphonate congener resulted in an increase in the ST inhibitory activity of up to 100-fold. Various aromatic groups were evaluated to replace the carbohydrate moiety, starting from phenyl \(26\) and furyl groups \(27\), substituted benzenic rings \(28\), heterocycles \(29\) and substituted phenyl groups bearing a fluorescent dye \(30\) (fig. 1.13). The fluorescent probe \(30\) was efficiently taken up into cells and translocated to compartments surrounding the cell nucleus without the need for transfection agents.

This strategy offers the advantages of providing potent inhibitors, ranging in the low nanomolar range, while considerably alleviating the synthesis of the sialic mimic. The substitution of the aromatic ring is important and the 3-phenoxy pattern (drawn in orange in fig. 1.14) gave the most potent inhibitor \(31\) of the series, with \(K_i\) values of 0.07-0.3 nM against rat ST6Gal-I and 19 nM against human ST6Gal-I. The compound \(31\)’s structure is reminiscent of the sugar-based compound \(32\) reported by Schwörer (fig. 1.14).
synthesis of the phosphate-based transition-state analogues used the phosphoramidite route activated with $1H$-tetrazole. Compound 31 is the lead structure for the present study.

![Chemical structures](image)

**Figure 1.14.** Optimisation of the transition-state analogues from 25 to the lead compound 31 and their activity against rat ST6Gal-I.

More recently, Li et al. reported potent cyclopentane-based CMP-Neu5Ac analogues. These transition-state inhibitors also bear a CMP fragment and an α-hydroxyphosphonate moiety and the sialic acid fragment was replaced by a five membered ring (fig. 1.15). The design of more elaborate sugar mimic fragments was inspired by the structurally related sialidase inhibitor peramivir 33, which provided the most potent examples (36, $K_i = 28$ nM) towards human ST6Gal-I.208

![Chemical structures](image)

**Figure 1.15.** The neuraminidase inhibitor Peramivir 33 and examples of functionalised cyclopentane derivatives as sialic acid mimics.

As with neuraminidase inhibitors, sialic acid mimics can range from highly functionalised chiral compounds229,230 to much simpler aromatic moieties.231 This was illustrated in Kumar’s work with a series of benzamide derivatives linked to cytidine with a sulfamate group.197 The compounds were tested against the bacterial α-2,3-ST Cst-06 and ranged from 9 to 79 % inhibition at 500 µM. The two best inhibitors 37 and 38 ($K_i = 87$ µM) are shown in fig. 1.16-A.
with their possible binding mode docked as a part of this study in the active site of the bacterial enzyme Cst-I (PDB: 2P2V, fig. 1.16-B).

![Structure of benzamide derivatives](image)

**Figure 1.16.** Structure of benzamide derivatives 37 and 38 and their inhibition at 500 µM against Cst-06 (A) and the possible binding mode of 38 in Cst-I (B, PDB: 2P2V) docked as part of this study.

A number of studies explored various isosteric replacements for the native phosphodiester linker. Historically, the exploration started with deleting the linker, with some of the earliest ST inhibitors bearing no linker and the sugar mimic was tethered directly to the cytidine fragment. Amongst these, the activity of the inhibitor KI-8110 39 (fig. 1.17) was evaluated against colon cancer (NL-17, SW 837, H-29 and MIP-101 cells) and in animal models. When the cells were exposed to KI-8110, they showed significant reduction of cell surface sialylation along with tumour regression and a decrease in metastasis. After further evaluation, other experiments showed that the activity of 39 was not directly related to ST inhibition but rather to blockage of the CMP-Neu5Ac transporter into the Golgi.

Of note, most GTs use sugar-nucleotides that contain diphosphates and this makes CMP-Neu5Ac unique donor, in which cytidine is linked to sialic acid via a monophosphate. The phosphodiester linker is the moiety present in the CMP-Neu5Ac 2 and utilised in the design of a large number of compounds which exhibit moderate to highly potent ST inhibition. A comparative study of ST inhibitors reported in literature showed that the CMP moiety, which
includes the phosphate group, provides the most potent compounds against rat liver ST6Gal-I. As a result, the incorporation of the phosphodiester group played a major role in the development of ST inhibitors.\textsuperscript{237}

In an early study, Amann \textit{et al.} prepared CMP-Neu5Ac derivatives bearing a phosphonate group instead of the native phosphate linker (40, fig. 1.17). The design strategy was to prevent the cleavage between the nucleoside fragment and the sugar mimic by using a non-labile linker. The concept of using a phosphonate group was later used by Izumi \textit{et al.} to produce the compound 41 which displays comparable affinity for ST3Gal-I to the parent compound 2, with a IC\textsubscript{50} value of 47 µM.\textsuperscript{221}

Another phosphorous-based isosteric replacement for the phosphodiester group was evaluated by Skropeta \textit{et al.} by tethering cytidine to an \(\alpha\)-aminophosphonate via a phosphoramidate linker.\textsuperscript{226} The sugar mimic were prepared by using a stereoselective hydroxylation method leading to the compounds 42-43 (fig. 1.17). Both diastereoisomers of the nucleoside conjugate bearing a phenyl group were tested against rat liver ST6Gal-I and showed considerably lower activity than the phosphate counterparts, with the \(R\) isomer being the most potent diastereoisomer. Interestingly, no examples of the phosphoramidate linking a 5’-aminonucleoside to an \(\alpha\)-phosphonate fragment were reported in the literature to our knowledge, and so at least for ST inhibition purpose. A phosphoramidate linker was also explored to attach the cytidine fragment to amino-acids.\textsuperscript{238}
Further examples of moieties used to replace the phosphate linker include the 1,2,3-triazole ring. Lee et al. prepared a series of 5’-triazonucleosides to evaluate their activity on rat liver ST3Gal. Among the cytidine based triazole molecules, only compound 44 showed significant activity ($IC_{50} = 37.5 \mu M$, fig. 1.18). Interestingly, the presence of a phenyl group seems to be a determinant factor, presenting a 2-fold improvement over the analogues cyclopentyl compound 45 (44 % inhibition at 300 μM). Finally, Kumar et al. also investigated different types of linkers involving complex functional arrangements. These include mostly combinations of triazole and amide, and amino acids (fig. 1.18). These functional groups were selected to increase the hydrophobicity of the inhibitors by bearing non-charged linkers. Further modifications on the sialyl moiety also included 2-deoxy-2,3-dehydro-acetylneuraminic acid derivatives or aromatic rings. The inhibitory activity of these compounds was evaluated against the bacteria *C. jejuni* Cst 06, using a HPLC-based technique, and relatively low inhibition was observed (11-80 % at 500 μM). The 5’-triazole nucleoside analogues obtained by click chemistry appeared to be competitive inhibitors. On the other hand, two of the most active compounds (46-47) showed non-competitive inhibition, suggesting the nitro group could play a role in the binding with the active site of the enzyme.
Figure 1.18. Sialyltransfrase inhibitors bearing a triazole linker.

In summary, a variety of functional groups have been evaluated to replace the native phosphodiester linker. The lack of consistency in regard to structures makes any comparison difficult for establishing the SAR. The phosphate moiety remains optimal in terms of potency but it cannot contribute to selective activity towards the different ST subtypes because it belongs to the natural sialyl donor 2, which is used by all ST enzymes. In addition, comparing the functional groups and their influence on the activity of the corresponding inhibitors indicates that the size of the linker matters and a three atom chain is optimal. In Kumar’s compounds, the nucleoside and the sialic acid mimic are spaced by up to five atoms, which keeps the fragment too far from each other and reduces the affinity of the inhibitor with the active site of the enzyme.

1.3.5. Global metabolic inhibitors

A global metabolic inhibitor was investigated in Paulson’s group based on glycoengineering techniques. By feeding the peracetylated 3-fluorosialic acid P-3F\textsubscript{ax}-Neu5Ac 48 into cells as a biogenic precursor, the authors observed successive incorporation of the modified sugar inside the cells, deprotection of the hydroxyl groups and conversion into the inhibitor CMP-
3F-Neu5Ac 7 both in vitro and in vivo conditions (fig. 1.20). CMP-3F-Neu5Ac was first discovered by Burkart et al. in 1999. A fluoro group was added at the 3-position of sialic acid leading to the inhibitor 7 ($K_i$ (ST6Gal-I) = 5.7 µM, fig. 1.5). The presence of a fluorine atom at the 3-position of the sialic acid prevents turnover by inductively destabilizing the oxocarbenium ion-like transition-state of the catalytic process. Although highly effective in vitro, subsequent in vivo studies by Paulson involving systemic blockade of sialylation in mice revealed the limitations of using global sialylation inhibitors such as P-3Fax-Neu5Ac in vivo due to renal failure. Given the crucial role of sialylation in such a myriad of biological processes and in particular the high level of expression in liver and kidney tissue, this is not unexpected. However, as stated by the authors, these results highlight the importance of developing selective, small-molecule ST inhibitors for therapeutic purposes, while metabolic inhibitors will still have a crucial role to play as biological tools in uncovering more about the role of sialylation in disease progression.

In a different study, the metabolic inhibitor was also illustrated by Horstkorte et al. by exploring the activity of 5-N-acyl-modified sialic acids on polysialyltransferases. The CHO-mutant 2A10 cells expressing either ST8Sia-II or ST8Sia-IV were fed with 5-N-acyl-D-mannosamines and the artificial sialic acid precursors bearing longer chains, i.e. propanoyl (49), butanoyl (50) and pentanoyl (51), at the 5-position were found to considerably reduce the occurrence of cell surface $\alpha$-2,8-sialylation (fig. 1.19). Real time RT-PCR demonstrated that these compounds also affected gene expression. Unnatural sialic residues were also found to be incorporated into sialoglycoconjugates. These results indicate the formation of the corresponding CMP-Neu5Ac derivatives (52-54) takes place inside the cells. These non-natural sialic acid derivatives were also prepared and tested in a different study with consistent findings (fig. 2.2-C). The authors suggest that the inhibition may be indirect as
the polymerisation of α-2,8-sialic acids stops after the first incorporation of these 5’-modified analogues as the unnatural conjugates are no longer suitable substrates for the enzyme.

Figure 1.19. Possible mode of action of metabolic inhibitors peracetylated 3F-Neu5Ac 48, and 49-51.²⁴⁻²⁴₃-²⁴₄
1.4. Aims of the project

The scalable synthesis of selective sialyltransferase inhibitors remains as an unmet need. The literature in the field largely highlights the therapeutic potential of ST inhibitors with isolated studies showing tumour regression both in vivo and in vitro. Amongst the wide molecular diversity of known ST inhibitors, the compounds mimicking the sialyl donor CMP-Neu5Ac and, more precisely the transition-state of the sialylation reaction represent the most promising group because of the large data set available and the high potency of its members. This knowledge allowed the elaboration of a SAR model, which could be simplified to 3 key fragments: the nucleoside fragment, the sialic acid mimic and the phosphate linker. This model was the framework leading to the rational design of novel ST inhibitors central to this project.

Therefore, in order to address these issues, the specific aims of this research project were:

- **Synthesis**: To create a concise and cost effective synthetic route in order to further explore the SAR of ST inhibitors and enable a more complete biological assessment of their activity. This requires a versatile strategy to facilitate pharmacomodulation and allow the structural modification of the fragments separately. The most appropriate synthetic strategy for the preparation of donor-analogue ST inhibitors is a convergent approach which allows the coupling of the sugar mimic with the nucleoside fragment via a linker. The type of linker chosen defines the synthetic route because the key fragments must bear the required functional groups suitable for the coupling step.

- **Computational methods**: To utilise the recently released crystal structures of STs in order to gain insight into the different binding modes between subtypes. Molecular docking will support the design of the building blocks.
o **Biological testing**: The ST inhibitory activity of the final target compounds will be evaluated in both enzyme and cell-based assays, along with screening the building blocks for their own inherent activity in other bioassays including anti-inflammatory and antibacterial assays. Cytotoxicity will be evaluated for all building blocks and final compounds, however, the cell toxicity of the ST inhibitors is expected to be low, as inhibiting cell surface sialylation affects cell migration not viability. This low cell toxicity is beneficial and will enable us to better examine the anti-metastatic effects of ST inhibition on cell adhesion and migration.

**Figure 1.20.** The 3 fragments framework used as a guide for the design of new potent sialyltransferase inhibitors.
CHAPTER 2

Sialic acid mimics
Chapter 2 – Sialic acid mimics

The vast majority of reported sialyltransferase (ST) inhibitors are built around the three key components of the natural ST donor CMP-Neu5Ac: the sialic acid moiety, the phosphate linker and the cytidine nucleoside. This gives rise to structures bearing a combination of sialic acid and cytidine mimics coupled together through an array of linkers including sulphonamides and triazole. A review of known inhibitors has revealed that each fragment plays a significant role independently.\(^\text{42}\) A convergent synthetic route is preferable for the preparation of such structures and ensures the flexibility of the method. Consequently, the choice of the linker is a central theme in this study for several reasons. First, modifying the linker is expected to contribute to major changes in the biological activity as the native phosphate linker is often described as critical for the enzyme activity. Second, the charged molecules are known to impart poor cellular permeability, by reducing the affinity for the lipidic nature of cell membranes.\(^\text{197}\) Finally, the type of linker selected directs the synthetic strategy by imposing the connectivity with the two other fragments.

![Figure 2.1](image-url) Figure 2.1. Design of triazole and carbamate-based sialyltransferase inhibitors.
2.1. Design of new building blocks

2.1.1. Car bam ate derivatives

As a part of the study dedicated to the modification of the linker between the nucleoside fragment and the sialic acid mimic of ST inhibitors, the carbamate moiety was selected as a potential candidate. The carbamate moiety meets the criteria for the isosteric replacement of the phosphate linker because of its similarities with the phosphate group. Structurally, the functional group is a linear assembly with a length of three atoms and the central planar sp² carbon at the basis of the carbonyl group provides some rigidity. In medicinal chemistry, the carbamate group has typically been used to replace ester groups, to prevent cleavage of the natural parent scaffold and improve the metabolic stability. This was illustrated in the chemistry of fatty acid amide hydrolyase inhibitors and acetylcholinesterase inhibitors, both of which have various libraries of carbamate-based ligands.

In the context of ST inhibitors, the carbamate linker offers a non-cleavable alternative to the native phosphate group. In addition, carbamate-linked ST inhibitors have not previously been evaluated to the best of our knowledge.

Retrosynthetic scheme: Carbamate derivatives can be prepared from an alcohol, an amine and a coupling reagent responsible for the carbonylation such as phosgene or less hazardous reagents such as carbonyldiimidazole, isocyanates and others. The synthetic strategy proposed in fig. 2.2 utilises an intermediate shared with the preparation of the triazole derivatives (discussed later), that being the 5’-aminonucleoside fragment attained by simple reduction of the azido counterpart. The investigation of a series of α-hydroxyphosphonate derivatives required as precursors to the carbamate-linked ST inhibitors is presented in this chapter along with their biological evaluation.
Figure 2.1. Retrosynthetic scheme for the preparation of carbamate-linked sialyltransferase inhibitors (PG: protecting group).

2.1.2. Triazoles

The triazole ring is readily attained using the 1,3-dipolar cycloaddition reaction between acetylenes and azides, pioneered by Huisgen in the 1960’s,\textsuperscript{253-255} and brought back into focus by Sharpless and others.\textsuperscript{256-262} The concept, nicknamed “click chemistry”, gives rise to reactions that are high yielding, modular, wide in scope, stereospecific, and environmentally friendly.\textsuperscript{263} The reaction between an alkyne and an azido derivative was initially performed using a thermal cycloaddition. However, Medal, and then Sharpless\textsuperscript{264-265} later discovered that copper (I) salts could efficiently catalyse the reaction, allowing the use of milder conditions, such as room temperature and shorter reaction times. Catalysis also brought more control over the regioselectivity in the reaction, favouring the 1,4-regioisomer when copper is used and 1,5-regioisomers with ruthenium. Because of its numerous advantages, the Cu alkyne-azole cycloaddition, also called CuAAC, became extremely popular and it has become almost synonymous with “click chemistry” itself (fig. 2.3). Compared to other metal-catalyzed reactions, the use of a copper catalyst presents the major advantages of being inexpensive and easy to handle. The active catalytic entity is Cu\textsuperscript{I}, which is often generated in situ by reacting a stable source of Cu\textsuperscript{II}, such as copper sulfate or acetate, with a gentle reducing agent.\textsuperscript{266-267}
Within a short time frame, click chemistry has proven a remarkable utility and broad scope, not only in organic synthesis, but also in chemical biology and drug discovery.\textsuperscript{256,268}

![Click chemistry mechanism](image)

**Figure 2.3.** Mechanism of the copper catalysed alkyne/azide cyclocondensation.

Herein, the triazole ring shows promise as an isosteric replacement for the phosphodiester linker,\textsuperscript{269} as demonstrated by Lee \textit{et al.}\textsuperscript{239} (\textit{Cf} section 1.3.4). Incorporating an unnatural linkage into ST inhibitors provides several benefits, which include retaining geometric and spatial characteristics of the native glycoforms,\textsuperscript{31,51,270-271} yet inhibiting the targeted enzyme. Changing the phosphate group into a triazole ring provides an increase in chemical and enzymatic stability.\textsuperscript{272} In CMP-Neu5Ac 2, the phosphate linker is instrumental in the sialic acid transfer because of it being a leaving group. Consequently, the triazole ring offers here a non-labile alternative.

In terms of geometry, the triazole ring appears as a rigid structure opposed to the flexible phosphodiester moiety. The 1,4-disubstituted triazole is a planar and aromatic heterocycle and the orientation of the substituents attached to the 1- and 4-positions is fixed and defined by the
dihedral angle that separates them. Conversely, the phosphate linker tethers the sialic mimic and the nucleoside fragment via 4 rotatable bonds. This difference between the triazole and the phosphate moieties provides a geometrical constraint, which can influence the activity of the proposed inhibitors as the conformational properties of the acceptor substrates have proven to play a role in the specificities of ST reactions.\textsuperscript{273} Two of the nitrogen atoms that are part of the triazole ring bear lone pairs of electrons, which do not participate in the aromaticity. As a result, they are two hydrogen-bond acceptor sites.

**Retrosynthetic scheme:** The preparation of triazole-based ST inhibitors using the CuAAC reaction requires two key building blocks: an azidonucleoside fragment and an alkyne derivative (fig. 2.2). Incorporation of an azido group on the nucleoside fragment allows access to various possible linkers presented in chapter 3.\textsuperscript{252} As the presence of the phosphonate group was previously found to contribute to the potency of inhibitors, the retrosynthetic scheme proposed in fig. 2.4 includes the proposed preparation of 1-arylpargylylphosphonate esters as described in this chapter.

![Retrosynthetic scheme](image)

**Figure 2.4.** Retrosynthetic scheme for the preparation of triazole-based sialyltransferase inhibitors (PG: protecting group).
2.2. Synthetic strategy

2.2.1. α-Hydroxyphosphonate esters as building blocks for the preparation of carbamate-based ST inhibitors

2.2.1.1. Aspects of the phosphonate group in the lead compound

Originally, the α-phosphonate group was introduced into the early ST inhibitors to replicate the CDP 5 moiety (fig. 2.4),200 which itself is a moderate ST inhibitor, \( K_i \) (ST6Gal-I) = 10 µM. At physiological pH, CDP bears two charges. The methyleneephosphonate analogue 55, for which the activity was not communicated, was an intermediate in the design of the transition-state analogue 26, which was 50-fold more potent than CDP (\( K_i \) (ST6Gal-I) = 200 nM).

In the ST8Sia-III crystal structure, co-crystallised with CDP 5 (PDB: 5BO6), the second phosphate unit binds to the threonine residue Thr301. Furthermore, docking CMP-Neu5Ac 2 into the three subtypes, co-crystallised with CMP, the porcine ST3Gal-I, the human ST6Gal-I and the human ST8Sia-III, reveals similar interactions between the carboxylic acid group and the protein. In all cases, the phosphonate functional group forms a hydrogen bond with a serine or a threonine residue, Thr272 in ST3Gal-I, Ser323 in ST6Gal-I and Thr301 in ST8Sia-III (fig. 2.5). This indicates that the diphosphate unit in CDP mimics the interaction with the carboxylic group in CMP-Neu5Ac. In addition, according to the reported crystal structures,
the environment in the active site is very similar for the three subtypes, therefore this region might not be the most favorable spot to achieve selectivity. The carboxylate group binds to the protein via a hydrogen bond, not an ionic interaction. These last observations suggest that the carboxylate group could be replaced, in theory, with neutral isosteric groups.

Figure 2.6. CMP-Neu5Ac 2 docked in ST3Gal-I (A), ST6Gal-I (B) and ST8Sia-III (C) crystal structures using Autodock Vina. The residue interacting with the carboxylate group is highlighted in green and the co-crystallised CMP 3 in A and B is represented in orange sticks.

In order to gain further insight on the binding mode of the linker itself in the active site, the lead compound 31 bearing a 3-phenoxy group was also docked into the human ST6Gal-I crystal structure (fig. 2.7). Amongst the various possible conformations proposed by AutoDock Vina, only the results that best aligned with the co-crystallised CMP 3 molecule were selected for comparison. Analysing the models presented in fig. 2.7 reveals that the phosphonate group binds to the Ser323 which validates the hypothesis that the phosphonate mimics the native carboxylic group of the natural sialyl donor 2. The docking experiment shown in fig. 2.7 was performed by using both the R and S isomers for the position bearing the phosphonate group. Both diastereomers displayed comparable calculated affinity (-10.6 and -10.1 kCal/mol respectively) as determined by AutoDock Vina. This counter-intuitive result indicates that the configuration of the stereogenic center at the benzylic position of the α-hydroxyphosphonate fragment has little importance because both diastereoisomers give superimposable conformations in their binding pose. This result is supported by the biological activity of the
compound 31. The docking of 31 in the human ST6Gal-I also shows significant interactions with the neighbouring residues Asn212, Asn2333 and the catalytic residue His370.

![Figure 2.7](image)

**Figure 2.7.** Docking pose of the R (A) and S (B) isomers of the lead compound bearing a 3-phenoxy group 31 in the human ST6Gal-I (PDB: 4SJ2, CMP 3 is represented in green sticks).

### 2.2.1.2. General procedure for α-hydroxyphosphonate esters

α-Hydroxyphosphonate esters and their analogous phosphonic acids exhibit a wide range of biological activities such as anti-osteoporosis, anticancer, antibacterial, antibiotic, antimalarial, and inhibition of several types of phosphatases. Many procedures have been reported for the preparation of α-hydroxyphosphonate derivatives, including the reaction of aldehydes with dialkyl phosphite. This procedure is the most commonly used and can be promoted by the use of additives such as acids or bases.

Most α-hydroxyphosphonate derivatives used herein were prepared from readily available benzaldehyde. The choice of the starting material was directed by the SAR model proposed and focussed on aromatic rings bearing hydrophobic groups at the 3-position. Inspired by previous aromatic sialic acid mimics, a range of benzaldehyde derivatives were chosen to prepare the requisite α-hydroxyphosphonate esters. These aldehydes bear electron donating
(4-methyl, 4-ethyl, 3-hydroxy, 3-methoxy) or withdrawing groups (3-nitro, 3-bromo, 2-, 3-, and 4-fluoro). Methoxy groups were used to mimic the typical polyhydroxylated six-membered ring moiety found in hexoses (2, 5-, 3, 4- and 3, 5-dimethoxy).

Other starting materials were prepared to increase the molecular diversity of the library. Hydrophobic groups were chosen in order to balance the overall polarity of the coupled compounds, mainly because of the high hydrophilicity of the nucleoside fragment. 3-Hydroxybenzaldehyde 56 was alkylated using 1-iodopropane in the presence of potassium carbonate (fig. 2.8) to give 3-propoxybenzaldehyde 57 as described by Nishikawa et al. A similar procedure was applied to 5-bromosalicylaldehyde 58 and 2-hydroxy-α-naphthaldehyde 59 to prepare the known intermediates 60 and 61 respectively, with spectral data matching those reported.

![Synthesis of the known intermediates 57, 60 and 61 by alkylation of the phenolic aldehydes.](image-url)

Another two derivatives were synthesised from vanillin 62, following reported procedures, by acetylating the 4-hydroxyl group to give 63 (96 % yield) or by successive bromination and acetylation to give 64 (82 %, fig. 2.9). This sequence was also applied to 4-
hydroxybenzaldehyde 66 and yielded successively the brominated derivative 67 (83 % yield) and 68 after acetylation (89 % yield).

![Diagram](image)

**Figure 2.9.** Preparation of benzaldehyde 65 and 68 as precursors of α-hydroxyphosphonate esters.

In the context of this study, the straightforward and reliable methodology reported by Wong *et al.* for the synthesis of α-hydroxyphosphonate esters was preferred because of its practicality and low cost. Benzaldehyde derivatives were treated with 1.1 equiv. of diethyl phosphite and 2.2 equiv. of triethylamine at room temperature until disappearance of the starting material as judged by TLC, with addition of CH₂Cl₂ if required. Unless solubility issues were encountered, the reaction was set up with no solvent. This method did not provide any noticeable side product and as a result, the expected α-hydroxyphosphonate esters were isolated in excellent yields after a simple extraction (table 2.1).

In total, 19 diethyl α-hydrophosphonate esters were prepared in this manner as coupling partners for the preparation of carbamate-based ST inhibitors. At this time in the project, however, a change in focus to the triazole-based inhibitors meant that a second PhD student pursued the development of the carbamate-based inhibitors taking off from this point, while I pursued the triazole counterparts. To round off the investigation of the α-hydrophosphonate esters, a selected set of compounds were subjected to biological evaluation.
Table 2.1. Summary of the α-hydroxyphosphonate esters prepared from aldehyde derivatives.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>cLog P</th>
<th>Ar</th>
<th>Yield (%)</th>
<th>Compound</th>
<th>cLog P</th>
<th>Ar</th>
<th>Yield (%)</th>
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<td></td>
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</tbody>
</table>

* Novel compound.
2.2.1.3. Biological evaluation of α-hydroxyphosphonate esters

The series of 19 α-hydroxyphosphonate esters produced in this study underwent various biological assays, including cytotoxicity, anti-inflammatory and antibacterial activity assays.

**Cytotoxicity:** The MTS cell proliferation assay is widely accepted as a reliable measurement of cell viability.\(^{287}\) This assay was performed by using the MTS CellTiter 96®, which consists of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt \(88\) with an electron coupling reagent (phenazine ethosulfate, PES).\(^{288}\) In contact with viable cells, the MTS reagent gets reduced to red formazan \(89\) and the number of remaining viable cell can be determined through colourimetry (fig. 2.10). The reduction is carried out by mitochondrial dehydrogenase enzymes along with the production of NADH or NADPH. The amount of formazan produced is proportionally related to the number of living cells which justifies the accuracy of the assay as the MTS reagent can only be converted into formazan by metabolically active cells. After seeding overnight in 96 wells plates, the cell cultures were treated with the α-hydroxyphosphonate esters and incubated for 24 to 48 h. The MTS reagent was added to the media and incubated for 3 h before reading the absorbance at 492 nm. DMSO and media in the absence of compound were used as positive and negative controls, respectively.

![Figure 2.10. Reduction of the yellow MTS tetrazolium salt 88 to purple formazan 89 by viable cells.](image-url)

\(^{287}\) Ref. \(^{288}\) Ref.
Chapter 2

The 19 diethyl α-hydroxyphosphonate derivatives 69-87 were evaluated for their cytotoxicity against two different cell lines, Mia-PaCa-2 (human pancreatic cancer) and RAW264.7 (murine macrophages). The cell viability was evaluated in exposure to these samples at 100 µM. At this concentration, the cells exposed showed more than 100% survival with all compounds tested, and are thus considered non-toxic to these cells at concentrations of up to 100 µM.

**Anti-inflammatory activity:** Nitric oxide (NO) is a signalling molecule involved in many physiological and pathophysiological processes in the human body, including cardiovascular, immune, inflammatory and neuronal. NO has thus been the object of intensive research for drug development, appearing as a target for a wide range of pathologies such as cardiogenic shock, cancer, anxiety disorders, neurodegenerative diseases and inflammation. The production of NO and other mediators by macrophages has been determined in many inflammatory tissues following exposure to immune stimulants including bacterial endotoxin lipopolysaccharide (LPS). Amongst the number of synthetic compounds that inhibit NO production, endogenous molecules also contribute to modulate this inflammatory signalling pathway such as melatonin, a tryptamine derivative synthesised in the pineal gland, which regulates circadian rhythms.

In accordance with some recent examples of α-hydroxyphosphonate derivatives showing inhibition of NO production reported by Abdou et al., the activity of our series of diethyl α-hydroxyphosphonate derivatives was also evaluated using the Griess reagent assay.

For these studies, the murine macrophages RAW264.7 were used. The cells were cultured in the appropriate media such as DMEM or RPMI supplemented with fetal bovine serum (FBS) and optional antibiotics to avoid contamination (commonly streptomycin-penicillin). The cells were treated with the samples and incubated for 24 h before adding LPS, at a concentration of 1 µg.mL⁻¹ in order to induce the inflammatory cascade. After 30 min, an aliquot of the cell
supernatants was collected and to it was added the Griess reagent (1 % sulphanilamide 90 and 1 % N-(1-naphthal)ethylenediamine dihydrochloride 91 solution), which initiated a colour change. At this stage, the NO produced reacts with the sulphanilamide in a Sandmeyer reaction fashion and the resulting diazonium salt reacts with the N-(1-naphthal)ethylenediamine (fig. 2.11). The absorbance of the diazo product 92 was measured at 540 nm and the nitrite concentration was determined from a sodium nitrate standard curve.302

![Figure 2.11. Mechanism of action of the Griess reagent.301](image)

Using the Griess reagent assay, 14 diethyl α-hydroxyphosphonate derivative were evaluated for their ability to inhibit the production of NO in LPS-activated RAW264.7 cells at a final concentration of 10 µM (table 2.2). A few compounds were not tested, such as 73, 74 and 79, because of their low solubility in aqueous media, leading to precipitation during the assay.
Table 2.2. Inhibition of NO production in LPS-stimulated RAW264.7 cells by selected diethyl α-hydroxyphosphonate esters, tested at a final concentration of 10 µM and listed by order of potency.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>% Inhibition</th>
<th>Compound</th>
<th>Formula</th>
<th>% Inhibition</th>
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<td>7.7</td>
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Amongst the 14 diethyl α-hydroxyphosphonate derivatives tested, a few compounds exhibited promising activity, showing over 80% inhibition of NO production at 10 µM. The presence of hydrogen bond acceptors is probably not a major requirement given that compounds 80 and 81 are more potent than their methoxylated analogues 69, 82 and 84. The electronic effects of the functional groups present on the aromatic ring do not follow any particular trend. For
example, the 3-nitro (73) and the 4-ethyl (70) derivatives display similar potency (46 % and 47.5 % inhibition respectively). The orientation of the substituents on the ring may not play a critical role in the activity but a bulky hydrophobic group at the 3-position is preferred (compounds 87 and 86). In conclusion, the influence of the substituents attached to the aromatic ring is more steric than electronic.

In previous studies evaluating α-hydroxyphosphonate derivatives, Abdou et al. identified tetrazoloquinoline-based compounds as anti-inflammatory agents. The NO inhibition was not measured by the compounds successfully reduced paw edema in rat. The study reports tricyclic derivatives bearing a hydrophobic moiety at the 3-position of the central benzenic ring. This structural similarity with our most potent α-hydroxyphosphonate derivatives suggests a possible related mode of action eventhough, the biological evaluation is not comparable for both studies.

**Antibacterial activity:** Various recent studies on the activity of α-hydroxyphosphonate esters highlighted the benefits of these derivatives for antibacterial purposes. The 19 diethyl α-hydroxyphosphonate derivatives 69-87 were tested against five Gram negative bacteria *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The assay was performed by WADI (Worldwide Antibiotic Discovery Initiative), now CoADD (Community for Open Antimicrobial Drug Discovery). At concentrations up to 32 µg/mL, the α-hydroxyphosphonate esters tested had no effect on the viability of the five bacteria listed above.
2.2.2. Propargylphosphonate esters for triazole ST studies

Alkynes are important in organic and medicinal chemistry as extremely versatile intermediates or functional groups of natural products and molecules of a pharmacological interest. There has been a renewed interest in alkynes since the advances in Sonogashira reactions, metathesis, click chemistry and the recent development of other novel synthetic methods. Attention has also been paid to transition-metal-catalyzed propargylic substitution reactions of alcohol derivatives with nucleophiles, showing that an acetylenic carbon-carbon triple bond has a high potential for a wide variety of transformations.

In order to obtain ST inhibitors bearing a phosphonate group on the benzylic position (fig. 2.2), an original synthetic pathway using the corresponding propargyl alcohol was explored. Catalytic propargylic substitution is relatively common and a wide range of nucleophiles have been introduced from propargylic alcohols or esters with the help of metal-based catalysts.

Historically, the Nicholas reaction was a pioneering example for propargylic nucleophilic substitutions. The procedure consists of forming a dicobalt hexacarbonyl complex with the propargylic substrate. The complex stabilises the carbocation and thus facilitates S_N_1 type reactions with nucleophiles. This methodology also improves thermodynamic control of the reaction. To an extent, the dicobalt complex can be considered as a protecting group for alkynes. Despite the relatively versatile applications for the Nicholas reaction, no records of this method leading to phosphonate derivatives are available to our knowledge. More recently, a large number of research groups investigated the activation of propargylic alcohol of esters promoted by Lewis acids. These studies described propargyl substitutions using a wide variety of catalyst such as InCl_3, Rh(PPh_3)_3Cl, TiCl_4, SnCl_2, FeCl_3, Au(OTf)_3, BiCl_3, Bi(OTf)_3, ionic liquids, and para-toluenesulfonic acid.
Copper-catalysed amination of propargyl acetate has been inspirational for the development of a new synthetic strategy leading to propargylphosphonate esters. Catalytic propargylic amination emerged about 20 years ago and has attracted renewed interest including the exploration of various catalysts, enantioselective versions and applications to synthesis of natural products. These methods were also found to be particularly attractive here as the use of a chiral ligand in addition to the catalyst would allow us to obtain the desired product as a single enantiomer.

In order to prepare the propargylphosphate derivatives required for this study, a starting material was chosen to screen conditions for the copper-catalysed propargylic substitution reaction. The addition of ethynylmagnesium bromide to $p$-tolualdehyde 93 to give the $\alpha$-arylpropyn-1-ol 94, which was not isolated and directly acetylated as reported by Ghosh et al. to give 95 (fig. 2.12). The 4-methyl group was chosen for the optimisation process to simplify the spectral data and facilitate the identification of the reaction products. In the $^1$H NMR spectrum, two doublets are expected in the aromatic area (6-8 ppm) and a singlet, downfield in the spectrum is expected to be characteristic for the 4-methyl group.

**Figure 2.12.** Synthesis of the alkynyl intermediate 95 used for the propargylic substitution trials.

Despite the number of different catalysts reported for this transformation, it was decided to focus on copper because of it being relatively inexpensive. Moreover, using a catalyst suitable for the next step would enable the development of a one-pot strategy within the optimisation of the sequence. To our knowledge, no procedure to prepare propargylphosphonate esters has been reported to date. Given the previous work using copper catalysts to prepare
propargylamine derivatives, as well as ether and thioethers, screening similar conditions involving diethyl phosphite as a nucleophile was our priority. The first attempts consisted in screening catalysts, with copper catalysts considered a priority as a previous study reported the synthesis of allene derivatives by using a palladium-based catalyst.\textsuperscript{347} The initial conditions that were evaluated for the preparation of the \(\alpha\)-arylpropargylphosphonate 96 are summarised in table 2.3.

**Table 2.3.** Screening catalysts and conditions for the propargylic substitution.

<table>
<thead>
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<th>Entry</th>
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<th>Yield of 97 (%)</th>
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</thead>
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<td>CH(_2)Cl(_2)</td>
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<td>61</td>
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<td>MeOH</td>
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<tr>
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<td>(iPr)(_2)NEt</td>
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<td>THF</td>
<td>6</td>
<td>N.D.*</td>
</tr>
</tbody>
</table>

* Not detected.

Various conditions were screened with the aim of synthesising the propargylphosphonate ester 96. First of all, palladium acetate was used (table 2.3, entry 1) as a reference for the catalyst screening study, in accordance with a method previously reported by Kalek \textit{et al.}\textsuperscript{347,348} The authors described the synthesis of allenylphosphonate esters by using activated propargylic substrates and dialkyl phosphites in a reaction catalysed with a palladium complex.
The following entries 2-6 in table 2.3, show the results of the propargylic substitution when a copper catalyst was used. The major product that was isolated is the same for all these trials, as monitored by TLC and ESI mass spectroscopy giving a \( m/z = 266 \ [\text{M+H}]^+ \), which matches the mass of the expected product 96. These first results were particularly encouraging because the spot on the TLC plate was moving faster than the product from the reference reaction using palladium acetate (table 2.3, entry 1). The \(^1\text{H}\) NMR experiment confirmed the presence of the skeleton, with the \textit{para}-substituted aromatic motif appearing as two doublet (around 7.10-7.17 ppm) and the diethyl ester moiety as two triplets (at 1.28 and 1.3 ppm for the terminal methyl groups) and a multiplet in the 4.07-4.14 ppm area and integrating for 4 H (the two methylene groups). Conversely, the terminal alkyne signal is not clear and instead, a doublet at 5.77 ppm and a doublet of doublet at 6.42 ppm suggesting the formation of an allenic derivative 97. Further investigation on the trial catalysed with palladium (table 2.3, entry 1) revealed that the reaction mixture contained a major product with a mass of \( m/z = 405 \), which suggested the addition of two equivalents of the phosphite as previously described by Milton \textit{et al.}^{349}

One trial using triphenylphosphine as a ligand (table 2.3, entry 6) also provided the allenylphosphonate. Surprisingly, the kinetic of the reaction was slower in this case than the trials using no ligands. The phosphines seem to stabilise an intermediate, not isolated, as shown by the appearance of a faster moving spot TLC, which was not observed in the other trials. No reaction was observed when using copper oxide, which seems to have limited solubility in the solvents evaluated (\( \text{CH}_2\text{Cl}_2 \) and MeOH, table 2.3, entries 2 and 3). To our surprise, Cu\(^I\) and Cu\(^{II}\) gave similar products. In trials using CuCl, CuI or Cu(OAc)\(_2\), only the allene derivative 97 was isolated in good yields (72-79 %). From this set of trials, the base used plays an important role and tertiary amines are preferred. It appears that the use of sodium salts (table 2.3, entries 10 and 11) promoted the precipitation of the phosphite or, at least, reduced its solubility in the
media leading to decrease its reactivity. No reaction occurred when no base was used, indicating that the phosphite may react in the deprotonated form.

Our results were consistent with those reported by Shen et al. at the time of our study, although with noticeable differences. In their 2016 study, the authors also described the isolation of the allene product in excellent yields. As a part of the optimisation, they first screened catalysts and additives, mostly ligands for the copper catalyst. They found that the CuI/TMEDA system was optimal and report considerably lower yields when using other ligands such as 2,2'-bipyridine, 1,10-phenanthroline or dppe (1,2-bis(diphenylphosphanylmethyl)ethane). In the present study, a slight excess of Et₃N (1.2 equiv.) was used for all trials. The base was added to deprotonate the phosphite and to optimise its nucleophilicity but the amount present in the reaction mixture was not enough to coordinate with the metal. It is reasonable to conclude that a ligand is not required for the reaction. Shen performed most of the reactions at 0 °C but only a trace amount, if any, of the side product was observed when the reaction was set up at room temperature. Other comparable results to ours were observed by Trost et al. using C-centered nucleophiles and propargyl bromides. In a more recent study, the allenylphosphoryl derivatives were isolated from the reaction of propargylic alcohols and catalysed with Cu(OTf)₂.
The formation of the propargylphosphonate is not excluded and could possibly occur before rearranging in the allene product. This type of reactivity has been observed with other alkynyl compounds such as propargylsulfinamides.\textsuperscript{353}

Some examples in the literature suggest the possibility to attain propargylphosphonate derivatives, in particular a study reported by the Uemura group showing the preparation of a propargylphosphine oxide using a ruthenium catalyst.\textsuperscript{354} Despite leading to versatile propargylic substitution, this catalyst is not readily available and involves synthesis from expensive starting materials. Therefore, this method does not fulfil the requirements of the present study. Other metals, such as iron,\textsuperscript{327-328} bismuth\textsuperscript{330,331} or indium,\textsuperscript{323} will be considered for further exploration in future work.
2.2.3. Arylpropargyl ester derivatives for triazole ST studies

Encouraged by the successful synthesis of the propargyl acetate derivative 95, a series of 1-arylpropargylic esters were considered for click purposes. This fragment, which aims at mimicking the sialic acid residue in the three key fragment model presented in the introduction, offers an interesting opportunity for exploring SARs. Following the example of 95, which was prepared by addition of ethynylmagnesium bromide on the carbonyl group of tolualdehyde and then acetylated, various benzaldehyde derivatives were prepared and screened as a source of molecular diversity.

2.2.3.1. Benzenic derivatives

In accordance with the retrosynthetic schemes presented in section 2.1.2 of this chapter, the preparation of the sugar-mimic fragment requires aldehyde derivatives as a starting material. Following the SAR model described in chapter 1, the 3-position of the aromatic ring appears to be a privileged scaffold for making a library of analogues. Hence a series of benzaldehyde derivatives was selected for this study, including some of the starting material introduced for the synthesis of the α-hydroxyphosphonate esters (Section 2.3.1).

Further derivatives were prepared in order to mimic the side chain of sialic acid. Amongst the transition-state analogues, the use of arylethers was found to greatly improve the inhibitory activity of the sialyl donor analogue. The resulting inhibitor 31 displays inhibition in the low nanomolar range ($K_i$ (ST6Gal-I) = 19 nM) and 32 ($K_i$ (ST6Gal-I) = 29 nM, fig. 1.14).

$N$-Arylation from aryl halides and amines (the so-called Büchwald-Hartwig reaction) or $O$-arylation resulting from the coupling of an aryl halide and a phenol (the Ullmann reaction) are well-known and suitable pathways. However, since all these reactions usually require an expensive catalyst (albeit in extremely low amount in some cases), the cost of
the metal catalyst may become an issue for their widespread application. Interesting results have been obtained with nickel catalysts, but in the last few years copper catalysts have undoubtedly taken the lead. A few attempts for the synthesis of diarylethers were performed using the Ullmann reaction. The trials involved 3-bromobenzaldehyde and 4-chlorophenol under various conditions and using tertiary amines or NaH as a base. In the best case, only the starting material was recovered. Suspecting that the reactivity of the aldehyde group might compete, the acetal was prepared and led to similar results.

Independently reported by the groups of Chan, Evans and Lam in 1998, this new simple procedure allows for the formation of C(aryl)-N and C(aryl)-O bonds in mild conditions. The aryl fragment is provided by an arylboronic acid in combination with a tertiary amine base (triethylamine or pyridine in most cases) and the coupling is catalysed by copper acetate. Anhydrous conditions are preferred to restrain the amount of side-products formed, molecular sieves are thus often added to the reaction mixture. The reaction is performed at room temperature in air, as exposure to oxygen was found to be advantageous. The solvent used for this reaction does not seem to be of importance. CH₂Cl₂ is the most commonly used solvent, however successful couplings have also been reported using many other solvents such as acetonitrile, dimethylformamide and dioxane. Since the extensive development of the Suzuki reaction, a large number of highly functionalized boronic acids are now readily available. The Evans-Chan-Lam reaction offers a simple and mild alternative to the Ullmann reaction, which usually requires high temperatures.

This elegant method using arylboronic acids and catalysed with copper was applied to the arylation of isovanillin (fig. 2.14). The reaction was performed by using 1.2 equivalents of phenylboronic acid in the presence of copper acetate and triethylamine and led to the 4-methoxy,3-phenoxybenzaldehyde in 79%.
The preparation of further functionalised building blocks was attempted by using the unique physicochemical properties of fluorine. 4-Fluoro-3-phenoxybenzaldehyde is commercially available and was used as received. The Chan-Lam-Evans cross coupling reaction was suitable for the introduction of a fluorine atom on the second aromatic ring, i.e. the phenoxy group (fig. 2.15). Encouraged by the successful results attained when using isovanillin, similar conditions were used for the coupling reaction. 3-Hydroxybenzaldehyde 57 was treated with 2- or 4-fluorophenylboronic acid 100 and 101 in the presence of copper acetate and triethylamine in CH₂Cl₂ at room temperature (fig. 2.14).

According to the SAR model (fig. 1.20), the substituent attached to the 3-position of the aromatic ring aims at mimicking the sialic acid fragment of the natural donor CMP-Neu5Ac 2. The structure of the side chain present in the parent compound 2 is a glyceryl residue and can be described as polar and flexible. Despite giving the best biological results, the physicochemical properties of the phenoxy group present in the lead compound 51, planar aromatic and hydrophobic, and those of a glyceryl residue are not alike. This observation motivated the preparation of derivatives bearing various substituents at the 3-position such as cyclopentoxy and propoxy, which are hydrophobic, but more flexible than the phenoxy
counterpart. For this purpose, the corresponding benzaldehyde derivatives were synthesised by alkylation of 3-hydroxybenzaldehyde \( \text{56} \) (fig. 2.16). The 3-propoxy derivative was used as reported in section 2.2.1.2.

![Synthesis of the intermediates 104 and 105 by alkylation of phenolic benzaldehyde derivatives.](image)

The requisite alkyne fragments were prepared from the starting material (\( \text{57, 94, 99 and 103-105} \)) by using the method described by Ghosh et al.\(^{346} \) that showed positive results for the synthesis of \( \text{57} \). The synthesis proceeds in two step starting with addition of the ethynylmagnesium bromide to the suitable benzaldehyde derivative and followed by acetylation. The acetylation step helps diminishing the polarity of the intermediate, which alleviates the purification of the intermediate. Consequently, the propargyl acetates were isolated by a simple extraction, and the organic layers were washed with sodium bicarbonate to provide the expected alkyne derivative in excellent yields (89-97 %) and the product was used without further purification (table 2.4). In addition, decreasing the polarity of the alkyne fragment results in increasing the difference of polarity with the nucleoside building block, hence promoting a very efficient separation for the purification of the coupling step.

The 1-arylpropargyl alcohol intermediate was usually obtained in acceptable purity (>90 %) as judged by NMR and used in the following step without any further purification. The alkyne derivatives obtained from benzaldehydes presented consistent patterns in the \(^1\)H and \(^{13}\)C NMR spectra allowing their characterisation. The C-H on the benzylic position (doublet at 6.6-6.9 ppm and 61-65 ppm respectively) and the terminal alkyne position (doublet at ~2.6 ppm and ~75 ppm
respectively). A series of 1-arylpropargyl acetates were prepared by using the substituted benzaldehyde derivatives (57, 99 and 103-105) described above. This method perfectly matches the project objectives by providing a series of clickable building blocks in excellent yields.

### Table 2.4. Preparation of the alkyne-based building block from aldehyde and ketone derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>R&lt;sub&gt;3&lt;/sub&gt;</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>106</td>
<td>MeO</td>
<td>H</td>
<td>H</td>
<td>93</td>
</tr>
<tr>
<td>2</td>
<td>107’</td>
<td>MeO</td>
<td>H</td>
<td>MeO</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>108’</td>
<td>PrO</td>
<td>H</td>
<td>H</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>109’</td>
<td>cyPentO</td>
<td>H</td>
<td>H</td>
<td>89</td>
</tr>
<tr>
<td>5</td>
<td>110’</td>
<td>cyPentO</td>
<td>MeO</td>
<td>H</td>
<td>91</td>
</tr>
<tr>
<td>6</td>
<td>111</td>
<td>PhO</td>
<td>H</td>
<td>H</td>
<td>97</td>
</tr>
<tr>
<td>7</td>
<td>112’</td>
<td>PhO</td>
<td>MeO</td>
<td>H</td>
<td>83</td>
</tr>
<tr>
<td>8</td>
<td>113’</td>
<td>PhO</td>
<td>F</td>
<td>H</td>
<td>94</td>
</tr>
<tr>
<td>9</td>
<td>114’</td>
<td>4-F-PhO</td>
<td>F</td>
<td>H</td>
<td>92</td>
</tr>
</tbody>
</table>

* Novel compounds.

### 2.2.3.2. Extension to heterocyclic derivatives.

In order to further extend our library of inhibitors, bicyclic and heterocyclic derivatives were considered as promising targets. Docking the compound 29 (fig. 1.13) bearing a benzothiazole ring into the human ST6Gal-I crystal structure (PDB: 4SJ2, fig. 2.17) provided possible binding modes similar to results attained with the 3-phenoxy counterpart 31 (Cf section 2.3.2.1). In addition, in this case the configuration of the pseudobenzylic position seemed to have little importance as the α-phosphonate group could bind to both catalytic residues, either Tyr354 or His370, as already observed with the lead compound 51.
Figure 2.17. Docking of the benzothiazole 29 in the human ST6Gal-1 crystal structure (PDB: 4SJ2). CMP is present (grey sticks) along with the R (green sticks) and S (orange sticks) isomers of 29.

The first heterocyclic derivative was prepared from 2-benzothiophenecarboxaldehyde 115 (fig. 2.18). Following the sequence used for the initial series in section 2.2.3.1, the 2-benzothienyl 116 derivative was isolated in 86% yield.

Figure 2.18. Preparation of the benzothienyl derivative 116.

When 2-quinolinecarboxaldehyde 117 was used to prepare the corresponding alkyne derivative, a complex mixture was recovered (fig. 2.19). The crude material did not display any evidence of desired alkyne derivative 118, either by NMR or mass spectrometry. The absence of reactivity of the aldehyde group could be explained by a possible chelation of magnesium by the quinoline nitrogen atom decreasing its reactivity.

Figure 2.19. Attempted preparation of the quinoline derivative 118.
Examples of ST inhibitors bearing an indolic moiety motivated us to explore this scaffold.\textsuperscript{238} Amongst these examples, Whalen \textit{et al.} synthesised the CMP-Neu5Ac analogues 119-124 by linking cytidine to amino acids via a phosphoramidate group (fig. 2.20).\textsuperscript{238} The rationale rested on mimicking the carboxylic acid function present in the native sugar. The series exhibited inhibition in the low millimolar range and the tryptophan analogue 124, bearing the indole ring was the most active ($K_i$ (ST3Gal-I) = 0.3 mM and $K_i$ (ST6Gal-I) = 0.73 mM).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.20}
\caption{Phosphoramidate amino-acid derivatives 119-124 as sialyltransferase inhibitors.\textsuperscript{238}}
\end{figure}

The commercially available indole-3-carboxaldehyde 125 was a suitable starting material in order to keep our synthetic methods consistent. Direct reaction of the ethynylmagnesium bromide on indole-3-carboxaldehyde required protection of the $N^1$-position because of the acidity of the proton attached to the nitrogen atom.\textsuperscript{364} \textit{Tert-butoxycarbonyl} represented an attractive option for the entire protecting system to maintain consistency, \textit{i.e.} with the acetonide group on the nucleoside fragment. Indole-3-carboxaldehyde was thus treated with Boc$_2$O in the presence of a catalytic amount of DMAP to give 126 in 76 % yield (fig. 2.21).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.21}
\caption{Preparation of indolic aldehyde derivative 126.}
\end{figure}
The substituent at this position mimics the native carboxylic acid group at the anomeric position of sialic acid. The carboxylate binds to a serine or threonine residue depending on the ST subtype (Cf section 2.3.2.1). Even if modifications at this position may not contribute to the selectivity of the inhibitor toward the different ST subtypes, they might be informative about the importance of interactions taking place with specific residues. As a result, a derivative bearing an additional ester group was also prepared. Following reported procedures, the reaction of indole 127 with oxalyl chloride was quenched with a solution of triethylamine in methanol to give 128 (fig 2.22). The indole \(^{N1}\)-position was then protected with a tert-butoxycarbonyl group for the same reason mentioned for the aldehyde counterpart 125.

![Figure 2.22. Synthesis of indole derivative 129 bearing an ester group.](image)

In the case of these indolic derivatives, the addition of the Grignard reagent required protection of the \(^{N1}\)-position of the heterocycle. The proton attached to this position is relatively acidic and probably reacts with the carbanion, which loses its nucleophilicity. The Boc protected compounds 126 and 129 were suitable for the general sequence and provided the corresponding alkyne building block in 73 % and 59 % respectively (fig. 2.23).
Isatin is another heterocycle of interest in drug design for the development of compounds with a wide range of activity including cancer. As discussed for the preparation of the indolic alkyne derivative 130, using isatin 132 as a starting material would lead to an alkyne derivative bearing a hydrogen bond acceptor at the pseudobenzylic position. In addition, as the carbonyl group is a part of the oxolactam ring, the expected product 133 is attractive because of the geometrical constrain promoted by its cyclic structure. The geometry of this fragment results in restricted conformations for the aromatic ring by preventing its free rotation. Examples of cytidine-based ST inhibitors, such as 134, bearing a lactam moiety reported by Schaub et al. (fig. 2.24) Ethynylmagnesium bromide successfully reacted with isatin to give the alkyne derivative 133 in 58% (fig. 2.24). In this case, the starting material did not require any protection and the spectral data was consistent with those reported by Ghosh et al.
Finally, the trifluoromethyl group was investigated as a substituent at the benzylic position. For this purpose, the method using ethynylmagnesium bromide was applied to 1,1,1-trifluoroacetophenone 135 and, after acetylation, the alkyne derivative 136 was isolated in 89 % yield, as reported by Kourist et al.368 (fig. 2.24).

![Figure 2.25. Preparation of 1,1,1-trifluoroacetophenone derivative 136.](image)

The entire series of alkyne derivatives synthesised in this chapter is summarised in table 2.5. These 15 α-arylpropargylic derivatives will serve as building blocks for the preparation of novel ST inhibitors as described in section 2.1.2. Given the hydrophobicity of the alkyne and their lack of solubility in aqueous media, no biological evaluation was conducted on this series. In addition, no reported biological activity was relevant to this project.
Table 2.5. Summary of 1-arylpropargyl esters from aldehyde derivatives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Ar</th>
<th>Yield (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Compound</th>
<th>R</th>
<th>Ar</th>
<th>Yield (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
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<td>H</td>
<td><img src="image" alt="PhO" /></td>
<td>90</td>
<td>113&lt;sup&gt;a&lt;/sup&gt;</td>
<td>H</td>
<td><img src="image" alt="PhO" /></td>
<td>94</td>
</tr>
<tr>
<td>106</td>
<td>H</td>
<td><img src="image" alt="H_2CO" /></td>
<td>93</td>
<td>114&lt;sup&gt;a&lt;/sup&gt;</td>
<td>H</td>
<td><img src="image" alt="F" /></td>
<td>92</td>
</tr>
<tr>
<td>107&lt;sup&gt;a&lt;/sup&gt;</td>
<td>H</td>
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<td>89</td>
<td>116&lt;sup&gt;a&lt;/sup&gt;</td>
<td>H</td>
<td><img src="image" alt="S" /></td>
<td>86</td>
</tr>
<tr>
<td>108&lt;sup&gt;a&lt;/sup&gt;</td>
<td>H</td>
<td><img src="image" alt="O" /></td>
<td>92</td>
<td>130</td>
<td>H</td>
<td><img src="image" alt="Boc-N" /></td>
<td>73</td>
</tr>
<tr>
<td>109&lt;sup&gt;a&lt;/sup&gt;</td>
<td>H</td>
<td><img src="image" alt="O" /></td>
<td>89</td>
<td>131&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CO_2CH_3</td>
<td><img src="image" alt="Boc-N" /></td>
<td>51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
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<td>H</td>
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<td>91</td>
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<td>CO-NH-</td>
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<td>58&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
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<td>89</td>
</tr>
<tr>
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<td>H</td>
<td><img src="image" alt="PhO" /> <img src="image" alt="H_2CO" /></td>
<td>83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Novel compounds.
<sup>b</sup> Overall yields for the two steps.
<sup>c</sup> The alcohol derivative was isolated and characterised without acetylation.
This chapter describes the synthesis of building blocks suitable for mimicking the sialic acid fragment in the natural ST donor CMP-Neu5Ac. Different synthetic approaches were explored depending on the type of connectivity required for the linker.

First, a series of 19 diethyl α-hydroxyphosphonate esters was prepared from benzaldehyde derivatives in good to excellent yields (56-96 %), including 5 novel compounds. The α-hydroxyphosphonate derivatives were subject to biological testing and were found to exhibit no toxicity and some anti-inflammatory activity, via the inhibition of nitric oxide production in murine macrophages. Secondly, attempts to prepare propargylphosphonate esters using a copper-catalysed coupling reaction led to the corresponding allenic derivatives. Finally, a series of 15 α-arylpropargyl acetates was achieved in good to excellent yields. These alkyne derivatives are suitable for the preparation of the target triazole-based ST inhibitors as will be discussed in chapter 4.
CHAPTER 3

The nucleoside fragment
Chapter 3 – The nucleoside fragment

Nucleosides are a large family of molecules, well represented in the pharmaceutical field. There are currently fifteen nucleoside-based drugs approved as anticancer agents and twenty-five as antiviral agents by the FDA. Nucleosides represent a scaffold of choice for the design of donor-analogue ST inhibitors because of cytidine being a component of the natural sialyl donor CMP-Neu5Ac. Using a nucleoside fragment as a part of the pharmacophore presents a number of advantages. First, incorporating components of the natural donor guarantees to conserve a certain integrity in the mode of binding of the proposed inhibitors. In addition, the most potent inhibitors reported to date contain a cytidine moiety. Finally, as nucleosides constitute key elements of living systems, specific transporters are present on the cell membranes to ensure their uptake and the fulfilment of their function through the body, the blood brain barrier, as well as cancer cells. Retaining the nucleoside fragment will also aid the drug’s bioavailability. The cytidine fragment was identified as essential for the activity of inhibitors towards ST6Gal-I from rat liver but little is known about the SAR for this fragment and its activity towards other ST subtypes. It should be noted however that metabolic weakness may be an issue when using cytidine-based drugs as the pharmacological evaluation of FDA approved compounds have highlighted the possibility of rapid deamination at the 4-position.

3.1. Background

3.1.1. Overview of small cytidine-based inhibitors

Small cytidine derivatives have been the object of extensive studies in the context of anticancer drug development including analogues such as gemcitabine, cytarabine, decitabine and capecitabine (fig. 3.1). However, many of these are rapidly converted...
to their uridine counterpart by action of cytidine deaminase which results, in most cases, in inactive metabolites.\textsuperscript{379,380} In this study, we are planning to use both cytidine and uridine derivatives and comparing their relative activity in enzyme and cell-based assays would be a good indicator of their metabolic stability.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{example_figure}
\caption{Examples of cytidine 137 and FDA approved nucleoside derivatives 138-141.}
\end{figure}

Small cytidine derivatives and cytidine 137 itself display some inhibitory activity against STs. These include the product of the sialylation reaction itself, cytidine monophosphate CMP 3 (\(K_i\) (ST3Gal-I) = 64 \(\mu\)M,\textsuperscript{235} \(K_i\) (rST6Gal-I) = 50 \(\mu\)M,\textsuperscript{235} and \(K_i\) (ST8Sia-I) = 10 \(\mu\)M\textsuperscript{381}). CDP 5 and CTP 6 also are ST inhibitors and are both more potent than CMP (e.g. CDP, \(K_i\) (ST3Gal-I) = 5 \(\mu\)M, \(K_i\) (rST6Gal-I) = 19 \(\mu\)M and CTP, \(K_i\) (ST3Gal-I) = 64 \(\mu\)M,\textsuperscript{235} \(K_i\) (rST6Gal-I) = 16 \(\mu\)M, respectively). These cytidine derivatives were shown to act as competitive inhibitors.\textsuperscript{382,383}

3.1.1.1. Variations on the cytosine ring

A number of natural nucleosides have been evaluated as potential ST inhibitors, mostly uridine and adenosine derivatives. Interestingly, despite being closely related to cytidine in terms of structure, simple uridine-based nucleotides promoted weak but non-competitive inhibition towards ST6Gal-I,\textsuperscript{382} with UTP 142 being the most active inhibitor (\(K_i\) = 350 \(\mu\)M), followed by UDP 143 (\(K_i\) = 2.0 mM), and UMP 144 (\(K_i\) = 5.67 mM, fig. 3.2). In ST6Gal-I-catalysed reactions UMP-Neu5Ac 145 (fig. 3.2) showed about 10% of the activity observed for CMP-
Neu5Ac. The sugar-nucleoside conjugate was also found to be a poor substrate for ST3Gal-II, ST3Gal-III and ST6Gal-I. In comparison to uridine derivatives, adenosine-based nucleotides showed weak, non-competitive inhibition profiles. Of these, the triphosphate derivative is also the most potent, ATP, $K_i = 200 \mu M$, and the monophosphate analogue (not shown) the weakest, AMP, $K_i = 7.0 \text{ mM}$ (fig. 3.2). Other adenosine derivatives bearing a functionalised triazole ring at the 5'-position were evaluated, however they exhibited lower potency than their cytidine-based counterparts. The CMP derivative bearing a methyl group at the 5-position, 5-MeCMP, was tested against a wide range of ST subtypes and showed some activity with a marked preference for the ST8Sia subfamily (fig. 3.2). In the context of ST inhibition, only a few non-nucleoside replacements for cytidine have been reported. For example, the cytosine ring was replaced with a resorcinol moiety (not shown), however the resulting CMP-Neu5Ac analogue showed reduced affinity for ST6Gal-I.

To a larger extent, 5-Aza-dC, also called azacytidine, was found to promote the induction of ST3Gal-VI (fig. 3.2). However, the activity of this modified nucleoside on STs via direct interaction has not been evaluated in ST inhibition assays to our knowledge.

Figure 3.2. Structures of small nucleoside derivatives including UMP-Neu5Ac, 5-MeCMP and azacytidine.
3.1.1.2. Variation on the ribose fragment

There are a limited number of examples of cytidine-based ST inhibitors in the literature bearing a modified ribose fragment. Most studies involved small nucleoside derivatives.

**Variations at the 2’-position:** 2’-Deoxycytidine 150 was evaluated against human serum ST activity and showed very mild inhibition, ranging from 5.9 % inhibition at 100 µM to 32.5 % at 1 mM (fig. 3.3). The phosphorylated analogues of 2’-deoxycytidine were tested later by Kleineidam et al. and exhibited stronger activity, despite being less potent than the CTP parent compound 151 (72 % and 84 % inhibition at 250 mM towards ST3Gal-I and ST6Gal-I respectively, fig. 3.3). According to the same study, the 5-methyl analogue thymidine 152 remains significantly less potent than cytidine. Amongst nucleoside derivatives modified at the 2’-position, arabinonucleosides represent an important family. Both ara-CMP 153 and ara-CTP 154 exhibited weak competitive inhibition against ST6Gal-II with \( K_i \) values of 1.10 mM and 0.50 mM respectively (fig. 3.3).

Modifications of the functional group at the 2’-position have also been explored in terms of ST inhibition. Phosphorylation of cytidine at the 2’-position results in a weak inhibitor, 2’-CMP 155 (fig. 3.3), displaying 40 % inhibition at 250 mM against ST6Gal-I. 2’-Deoxy-2’-fluorocytidine 156 inhibits ST6Gal-I with less potency than cytidine (74 % inhibition at 250 mM, fig. 3.3) and had no effect on ST3Gal-I. The 2’-methylated derivative 2’-O-MeCMP 157 displayed 84 % inhibition at 250 µM against ST3Gal-III but was not active against ST6Gal-I (CMP exhibited 67 % and 49 % inhibition respectively at the same concentration, fig. 3.3). This result suggests that the environment in the active site neighbouring the 2’-hydroxyl group’s binding site is significantly different from one enzyme subtype to another. In addition, this confirms that this pocket provides an avenue for creating selective ligands as observed in the X-ray crystal structures.
Figure 3.3. Examples of cytidine derivatives modified at the 2′-position and thymidine.

To our knowledge, modifications at the 3′-position of the ribose fragment are absent in the literature, with the exception of 3′-CMP which is cytidine-3′-monophosphate.\(^{235}\) The activity of 3′-CMP against ST6Gal-I is weak (39 % inhibition at 250 mM, not shown).

**Variation of the ring:** Ribodialdehyde-CMP 158 prepared by Klohs et al. was about 20 times less potent than CMP against ST6Gal-II, ranging from 13 % inhibition at 250 µM to 50 % at 1 mM.\(^{382}\) 1,3-Dioxolane was also evaluated as a ribose replacement to give compound 159 and 160 but this led to dramatically decreased activity compared to that of the parent cytidine derivative (5 % and 16 % inhibition against ST3Gal-I at 100 µM, fig. 3.4).\(^{239}\)

Figure 3.4. Examples of variations on the ribose ring.
In a different approach, Tanaka et al. explored the replacement of the entire ribose moiety by a peptide scaffold,\textsuperscript{388} however, the resulting compound \textbf{161} exhibited low activity, IC\textsubscript{50} (ST6Gal-I) = 1 mM (fig. 3.5). A few years later, the same research group published the 5-FU analogue \textbf{162} for which the inhibitory activity towards ST6Gal-I doubled, albeit still weak.\textsuperscript{389}

\textbf{Figure 3.5.} Examples and activity of ST inhibitors bearing a 5-FU moiety.

In summary, various nucleoside derivatives have been evaluated for the inhibition of STs. Despite showing more structural integrity than the sialic acid fragment, the nucleoside derivatives with ST inhibitory activity reported in the literature do not lead to an effective SAR model. Comparing inhibitors that bear two or more different parameters is not accurate but provides an intuitive starting point for the design. A selection of representative examples of small nucleoside derivatives and their activity towards the main ST subtypes is summarised in table 3.1.
Table 3.1: Summary of activity of simple nucleoside derivatives against ST3Gal-I, ST6Gal-I and ST8Sia-II displayed as $K_i$, expressed in µM, or as percent inhibition\textsuperscript{235-381-382-385}.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ST3Gal %</th>
<th>$K_i$ (µM)</th>
<th>ST6Gal %</th>
<th>$K_i$ (µM)</th>
<th>ST8Sia %</th>
<th>$K_i$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMP</td>
<td>-</td>
<td>64</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>CDP</td>
<td>-</td>
<td>50</td>
<td>85\textsuperscript{a}</td>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CTP</td>
<td>-</td>
<td>60</td>
<td>90\textsuperscript{a}</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AraCMP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AraCTP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>500</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2'-O-Methyl-CMP</td>
<td>84\textsuperscript{b}</td>
<td>-</td>
<td>0\textsuperscript{a}</td>
<td>-</td>
<td>66-81\textsuperscript{a}</td>
<td>-</td>
</tr>
<tr>
<td>5-Methyl-CMP</td>
<td>31\textsuperscript{b}</td>
<td>-</td>
<td>16\textsuperscript{b}</td>
<td>-</td>
<td>32-53\textsuperscript{a}</td>
<td>-</td>
</tr>
<tr>
<td>UMP</td>
<td>14\textsuperscript{a}</td>
<td>-</td>
<td>24\textsuperscript{a}</td>
<td>5670</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UTP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>350</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cytidine</td>
<td>30\textsuperscript{d}</td>
<td>-</td>
<td>70\textsuperscript{a}</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2'-Deoxycytidine</td>
<td>-</td>
<td>-</td>
<td>32\textsuperscript{e}</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thymidine</td>
<td>-</td>
<td>-</td>
<td>16\textsuperscript{a}</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2'-Fluoro-2'-deoxycytidine</td>
<td>0\textsuperscript{a}</td>
<td>-</td>
<td>74\textsuperscript{a}</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Percentage inhibition at 250 mM. \textsuperscript{b} 250 µM. \textsuperscript{c} 50 µM. \textsuperscript{d} 0.65 mM. \textsuperscript{e} 1.0 mM.

### 3.1.2. Sequence comparison of ST subtypes

Prior to the release of crystallographic data for STs, various studies were performed to analyse and compare the sequences of the ST subtypes, which enabled the identification of the residues involved in the enzyme’s activity.\textsuperscript{171} Mutation studies highlighted the importance of motif L and motif S in the recognition and affinity of the enzyme with the donor CMP-Neu5Ac.\textsuperscript{167-168} Comparing the sequence of the motif L shows a high homology between the different subtypes (table 3.2). The sequence of motif S is highly conserved (47-62 %) within the ST8Sia subfamily but presents significant variations in the ST3Gal and ST6Gal subgroups. The motif S contributes to the recognition of the ribose fragment, in particular the 2’- and 3’-hydroxyl groups, which interact with the beginning of the sequence as well as the binding of the nucleobase, mostly through a hydrogen bond with the 2-position (located at the
end of the sequence). The amino acids located between these two keys sections of the motif S are mostly hydrophobic residues such as leucine, isoleucine, valine and alanine. The sequence and conformation of this region in ST3Gal-I allows the formation of an hydrophobic pocket, which explains the selective activity of 2’-MeCMP.

Table 3.2. Sequence alignment of motif L, motif S and of the 4-amino group binding region of the human sialyltransferase subtypes ST3Gal-I, ST3Gal-III, ST3Gal-IV, ST6Gal-I, ST8Sia-II, ST8Sia-III and ST8Sia-IV. Major hydrogen bond interactions between the protein and the donor CMP-Neu5Ac are highlighted in green.

**Motif L, CLUSTAL W (1.83) multiple sequence alignment**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>hST3Gal-I</td>
<td>RRCAVVGGNLRESSEYGEIDSHDFVLRKAPTAGFEADVGTKTT</td>
</tr>
<tr>
<td>hST3Gal-III</td>
<td>RRCIVGVGVLANKSLGSRIDDDYDIVRLNSAPVKFEDVSKTT</td>
</tr>
<tr>
<td>hST3Gal-IV</td>
<td>RRCVVVGNGHRLRNSSLGDAINKYDVIRLNNAPVAGYEGDVSKTT</td>
</tr>
<tr>
<td>hST6Gal-I</td>
<td>GRCATVGGSGLLDSECGKEIDSHDFVLRKAPTAGFEADVGTKTT</td>
</tr>
<tr>
<td>hST8Sia-II</td>
<td>GTCAIVNVGNSVLLNSGCQEIDAHSFVIRCNLAPVQYARDVGLKTD</td>
</tr>
<tr>
<td>hST8Sia-III</td>
<td>NTCAVVNGSGILTSQGCEIQDKSDVFRFCNFAPTEAFQRDVGKTN</td>
</tr>
<tr>
<td>hST8Sia-IV</td>
<td>KTCAVVGGSGILLDSECGKEIDSHDFVLRKAPTAGFEADVGTKTT</td>
</tr>
</tbody>
</table>

↑ interaction with O-1’ (ribose)
↑ interaction with phosphate group

**Motif S, CLUSTAL W (1.83) multiple sequence alignment**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>hST3Gal-I</td>
<td>PTTGLLVFSMHVCDEVDDLYG</td>
</tr>
<tr>
<td>hST3Gal-III</td>
<td>PTLGSVAVMTALHGCDEVAVAGFGY</td>
</tr>
<tr>
<td>hST3Gal-IV</td>
<td>PTTGGLAAITLALHLCDEVAVAGFGY</td>
</tr>
<tr>
<td>hST6Gal-I</td>
<td>PTTGGLMYTLATRFCKIQLYGFWP</td>
</tr>
<tr>
<td>hST8Sia-II</td>
<td>PTTGLLMYTLASAIIEHLYGFWP</td>
</tr>
<tr>
<td>hST8Sia-III</td>
<td>PTTGLLMYTLATRFCKIQLYGFWP</td>
</tr>
<tr>
<td>hST8Sia-IV</td>
<td>PTTGLLMYTLATRFCKIQLYGFWP</td>
</tr>
</tbody>
</table>

↑ interaction with O-2’ (ribose)
↑ interaction with carboxylate (Neu5Ac)
↑ interaction with O-3’ (ribose)
↑ interaction with C=O-2

**NH₂ binding region, CLUSTAL W (1.83) multiple sequence alignment**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>hST3Gal-I</td>
<td>AYN-KQKTVHYYEQIT</td>
</tr>
<tr>
<td>hST3Gal-III</td>
<td>AYN-KKQTVHYYEQIT</td>
</tr>
<tr>
<td>hST6Gal-I</td>
<td>SKRKTDDVKYQKFF</td>
</tr>
<tr>
<td>hST8Sia-II</td>
<td>QNQ-NPVKTHYDSDLK</td>
</tr>
<tr>
<td>hST8Sia-III</td>
<td>PNTREDLPYHDKKG</td>
</tr>
<tr>
<td>hST8Sia-IV</td>
<td>LNG-KAVKRYYDDLK</td>
</tr>
</tbody>
</table>

↑ interaction with N-4 (cytosine)
3.1.3. Insights on STs from X-ray crystal structures

Based on the range of derivatives described in the literature and their accompanying biological activity, the essential component of the nucleoside structure as expected appears as made of two key components: the ribose fragment and the pyrimidine base. In addition, the binding mode of the phosphate moiety will also be discussed in this section. In all ST subtypes, the cytidine binding pocket is decorated with a number of polar residues, such as serine, threonine and asparagine, which explains the affinity of the ribose and cytosine rings through a network of hydrogen bonding.

The ST crystal structures all show similar binding interactions between the ribose fragment of the donor and the active site of the enzyme for all three subtypes. The endocyclic oxygen atom on the tetrahydrofuran ring forms a hydrogen bond with the protein backbone via the N-H donor of the residue Asn150 in ST3Gal-I, Ser169 in ST6Gal-I and Asn167 in ST8Sia-III (fig. 3.6). The hydroxyl group at the 3’-position of the ribose fragment forms the same type of hydrogen bond with Gly273 in ST3Gal-I, Gly324 in ST6Gal-I and Gly302 in ST8Sia-III, in which the secondary alcohol is an acceptor. Slight differences are observed in the interaction between the hydroxyl group at the 2’-position of the ribose ring and the different ST subtypes. In ST6Gal-I, a hydrogen bond occurs directly between the substituent, this time behaving like a donor, and the OH side chain of the residue Ser322. In contrast, in ST3Gal-I and ST8Sia-III, the secondary alcohol interacts with a water molecule, which creates a bridge between the ligand and the protein.
Figure 3.6. CMP 3 in the active site of ST3Gal-I (A, PDB: 2WNB), ST6Gal-I (B, PDB: 4JS2) and ST8Sia-III (C, PDB: 5BO6). The motif L and S are shown in orange and purple respectively.

In the case of the cytosine ring, more significant differences between the ST subtypes are apparent. First, the carbonyl group at the 2-position of the cytosine moiety forms a hydrogen bond with the N-H of the Lys376 side chain in ST6Gal-I. In ST3Gal-I, this carbonyl oxygen atom binds to the N-H donor of the residue Gly293. In the case of ST8Sia-III, the crystal structure does not show clear evidence of an interaction taking place between the nucleobase and the protein despite the proximity of the hydrogen bond donor part of the residue Trp322.

All the interactions between CMP and the protein are summarised in table 3.3.

Table 3.3. Summary of the interactions between CMP and the crystal structures of sialyltransferase subtypes ST3Gal-I, ST6Gal-I and ST8Sia-III.

<table>
<thead>
<tr>
<th>Fragment</th>
<th>pST3Gal-I (PDB: 2WNB)</th>
<th>hST6Gal-I (PDB: 4JS2)</th>
<th>hST8Sia-III (PDB: 5BO6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytidine</td>
<td>2-C=O Gly293 (via N-H)</td>
<td>Lys376 (via NH$_2$)</td>
<td>Trp322 (via N-H)</td>
</tr>
<tr>
<td></td>
<td>4-NH$_2$ His301 (via C=O)</td>
<td>Cys353 (via C=O)</td>
<td>His337 (via C=O)</td>
</tr>
<tr>
<td></td>
<td>Gly293 (via C=O)</td>
<td>Thr365 (via OH)</td>
<td>Trp322 (via C=O)</td>
</tr>
<tr>
<td>Ribose</td>
<td>1'-O Asn150 (via N-H)</td>
<td>Ser189 (via N-H)</td>
<td>Asn167 (via N-H)</td>
</tr>
<tr>
<td></td>
<td>2'-OH H$_2$O-Ser271 (via OH)</td>
<td>Ser322 (via OH)</td>
<td>H$_2$O-Ser300 (via OH)</td>
</tr>
<tr>
<td></td>
<td>Asn150 (via NH$_2$)</td>
<td>Asn167 (via NH$_2$)</td>
<td>Asn190 (via NH$_2$)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0' Asn212 (via NH$_2$)</td>
<td>Asn212 (via NH$_2$)</td>
<td>Asn337 (via NH$_2$)</td>
</tr>
<tr>
<td></td>
<td>His302 (via NH$^+$)</td>
<td>Tyr354 (via OH)</td>
<td>His337 (via NH$^+$)</td>
</tr>
</tbody>
</table>

* The interaction mentioned is not clearly observed in the crystal structure but considered possible because of the proximity of the residue with a hydrogen bond acceptor/donor.
Kuhn et al. suggested the possibility for key differences in the binding mode of cytidine-based and uridine-based donor mimics: “Of note, the exocyclic amino group of cytosine does not engage in specific contacts with the protein, raising the possibility that porcine ST3Gal-I could also accept the non-natural donor substrate UMP-Neu5Ac, which is impossible for human ST6Gal-I.”¹⁸⁹ Some of these hypotheses were confirmed by biological experiments, for example UMP-Neu5Ac was found to be a poor donor for ST3Gal-II, ST3Gal-III and ST6Gal-I.³⁸⁴ The same study demonstrated the ability of UMP to inhibit ST3Gal-II and to participate in reverse sialylation reactions. This experimental evidence suggests that CMP and UMP may act as substrates for the enzyme to perform the reverse reaction rather than inhibiting the active site by binding.

The change from the imidine moiety present on the cytosine ring to a lactam in the uracil counterpart may promote a considerable shift in the possible binding mode of the ligand. The hypothetical interactions between the enzyme and the uridine moiety were evaluated herein by docking experiments (fig. 3.6). The major changes appearing consistently related to the hydrogen bonds, which are summarised in table 3.4. As expected, the unchanged carbonyl group at the 2-position of the pyrimidine base retains the same type of bonds. The nitrogen atom at the 3-position of uridine, which did not participate in any obvious interaction with the protein in the case of cytidine, is protonated in the uracil ring. In the case of the imide moiety of uridine, the N-H group is now a hydrogen bond donor and the docking experiments suggest it binds with the carbonyl group of the residue Gly293 of ST3Gal-I. No binding seemed to take place with ST6Gal-I and ST8Sia-III at this position. Finally, the major change in replacing cytidine by uridine happens at the 4-position, where an exocyclic carbonyl group takes the place of an amino group. In ST3Gal-I, the crystal structure does not clearly show significant interactions between the cytidine amino group and the protein, even though some hydrogen bonds could take place with the carbonyl group of neighbouring residues (Gly293
and His301). In ST6Gal-I, the hydrogen bond between the amine and the carbonyl group of Cys353 is lost, while a possible interaction of this group with the residue Thr365 seems to be possible. Finally, our docking of UMP into ST8Sia-III did not provide any significant interaction between the pyrimidine base and the ST protein. Although, the proximity of hydrogen bond donors or acceptors in the backbone of the enzyme suggests potential binding.

In summary, both from the literature data and our docking experiments, the exchange of the nucleobase from cytidine to uridine could favour the selectivity of the potential inhibitor towards the ST3Gal subfamily.

**Table 3.4. Summary of the differences in ST binding interactions when replacing CMP with UMP.**

<table>
<thead>
<tr>
<th>Fragment</th>
<th>pST3Gal-I (PDB: 2WNB)</th>
<th>hST6Gal-I (PDB: 4SJ2)</th>
<th>hST8Sia-III (PDB: 5BO6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytidine</td>
<td>His301 (via C=O)*</td>
<td>Cys353 (via C=O)</td>
<td>His337 (via C=O)</td>
</tr>
<tr>
<td></td>
<td>Gly293 (via C=O)</td>
<td>Thr365 (via OH)</td>
<td>Trp322 (via C=O)</td>
</tr>
<tr>
<td>Uridine</td>
<td>3-NH</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gly293 (via C=O)</td>
<td>Cys364 (via C=O)*</td>
<td>Trp322 (via C=O)*</td>
</tr>
<tr>
<td></td>
<td>4-C=O</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ser151 (via OH)*</td>
<td>Thr365 (via OH)</td>
<td>Ser168 (via OH)*</td>
</tr>
</tbody>
</table>

* The interaction mentioned is not clearly observed in the crystal structure but considered possible because of the proximity of the residue with a hydrogen bond acceptor/donor.

The binding mode of the uracil ring that was observed in the docking experiments of UMP into ST3Gal-I is similar to those reported in other GTs using a uridine-based donor. For example, the fucosylgalactoside 3-alpha-galactosyltransferase (EC 2.4.1.37), involved in the biosynthesis of the blood group ABO, exhibits this particular type of interaction with a co-crystallised uridine-based inhibitor (PDB: 4Y62, fig. 3.7D). This provides further support for our proposal that uridine-based ST inhibitors may have selectivity towards the ST3Gal subfamily.
Figure 3.7. UMP 144 docked into the porcine ST3Gal-I (A, PDB: 2WNB), the human ST6Gal-I (B, PDB: 4JS2) and the human ST8Sia-III (C, PDB: 5BO6). Crystal structure of EC 2.4.1.37 co-crystallised with a uridine-based inhibitor (D, PDB: 4Y62).

Of note, the crystal structures of STs, as with many other proteins in the PDB server, are reported with a large number of water molecules present in the crystallographic network. In some cases, the water molecules create a bridge between the ligand and the protein via hydrogen bonds. Some studies on different ligand-protein complexes demonstrated the implication of the water molecules in the affinity, but this has not been clearly elucidated for STs. It is important to remember that the crystal structure represents the complex in the unique and static conformation, which helps to understand the binding mode of the substrates but does not illustrate the protein in a dynamic environment such as an aqueous solution. Molecular dynamics provide hypothetical conformational changes, which simulate the possible behaviour of the enzyme in solution. These more in-depth calculations have been carried out in our group on the human ST6Gal-I subtype by my fellow co-worker Andrew Montgomery as part of a separate project.
Chapter 3

3.1.4. Fragments of Interest

The first azidonucleoside (5’-azido-5’-deoxyuridine) was prepared by Horwitz et al. in 1962 by azidonation reaction of 5’-tosyl nucleoside and lithium azide. In general, azido analogues have been used mostly as intermediates in the preparation of aminonucleosides. In order to investigate the practicality of protecting systems, different strategies for the synthesis of the ST inhibitors were evaluated. The chemistry of nucleosides is well known, as a result, a range of methods of preparation and strategies are available in the literature. Numerous nucleoside derivatives are also commercially available.

Nucleoside-sugars belong to a group of compounds responsible for critical biological functions. Amongst them, different combinations of nucleoside and small carbohydrates are typical donors for GTs, a large family of enzymes widely distributed in mammalian tissues. Nine sugar-nucleotides have been identified in mammals and are UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc, UDP-Xylose, UDP-Glucuronic acid, GDP-Mannose, GDP-Fucose and CMP-Neu5Ac. The structural similarity between CMP-Neu5Ac itself, the present series of inhibitors and the natural donors for other GTs could represent a challenge in the development of potent and selective ST inhibitors due to potential off-target activity.

3.2. Cytidine derivatives

A common synthetic route to nucleosides functionalised at the 5’-position involves a multistep sequence starting with selective protection of this position using TBMSCl or TrtCl followed by acetylation (fig. 3.8). Despite the efficiency of this method in achieving regioselective protections the entire synthetic pathway involves a large number of steps, which can affect the overall yield.
Herein, cytidine 137 was first treated with TBDMScI in the presence of triethylamine and DMAP to give the selectively protected intermediate 163 in 67% yield. The silylated compound 163 was acetylated in excellent yields by using acetic anhydride in pyridine and the resulting intermediate 164 was treated TBAF to provide the primary alcohol derivative 165 in 77%. Of note, the resulting 2’,3’,4-triacetylcytidine 165 has relatively low solubility in organic media, including CH₂Cl₂ and acetonitrile. Consequently, the use of this building block in organic solvents was considerably diminished and led to lower yields and longer reaction times, often associated with the formation of side products which affected the accessibility of the desired coupling products by purification. As a result, the preparation of the tosylate 166 was achieved in dry pyridine. After reaction of the tosylate 166 with sodium azide in DMF, the clickable building block 167 was attained in an overall yield of 36% over five steps.

\[ \text{Figure 3.8. Preparation of protected 5’-azido-5’-deoxycytidine 167.} \]

A different approach was also used in order to improve the overall yield and to evaluate the influence of the protecting system on the reactivity of the building block (fig. 3.9). The protected 5’-azidocytidine 167 was prepared according to a procedure reported by Kumar et al.\(^{197}\) First, \(N^4\)-acetylcytidine 169 was treated with sulfuric acid in acetone at room temperature to install the
acetonide group on the ribose fragment. This was confirmed by $^1$H NMR spectroscopy with the appearance of two peaks at 1.29 and 1.49 ppm. This first intermediate 170 was then reacted with methanesulfonyl chloride and triethylamine to generate the 5' -mesyl derivative 171 in 82 %. Finally, the reaction of mesylate 171 with sodium azide in the presence of benzyltriethylammonium chloride (as a phase transfer catalyst) in DMF provided the ‘clickable’ azido derivative 172 in 71 % yield. In order to protect the cytidine fragment with a consistent protecting system, the N-acetyl group was interchanged with a N-Boc protection. The acetamide group was first cleaved with concentrated ammonia in methanol at room temperature to give the free amino derivative 173 which was not isolated. The amide cleavage was followed by reaction with Boc$_2$O in THF in the presence of a catalytic amount of DMAP. Evidence for the Boc group was provided by the appearance of a singlet at 1.52 ppm in the $^1$H NMR, integrating for 9 protons. These two additional steps led to the key protected azido compound 174 with an overall yield of 72.5 %, and provided the advantage of simultaneous cleavage of all protections on this fragment at once, i.e. the Boc and the acetonide groups, by using acidic conditions.

**Figure 3.9.** Preparation of the protected 5'-azido-5'-deoxyuridine derivatives 172 and 174.
3.3. Modification of the base

3.3.1. Uridine

The preparation of cytidine-based building blocks requires several steps in order to achieve regioselective functionalisation, while protecting the uracil ring is often not required, making uridine a more convenient building block than cytidine. The azidonation of uridine has already been reported and in the context of this study, the synthetic route was adapted from a three-step sequence, starting with the protection of the ribose diol using an acetonide group.\(^{393}\) First, uridine 175 was suspended in dry acetone and treated with concentrated sulfuric acid at room temperature to give the acetonide derivative 176 in 86 % yield. The primary alcohol at the 5’-position was converted into a leaving group by treating 176 with mesyl chloride and triethylamine in 91 % yield. The reaction of the resulting mesylate 177 with sodium azide provided the expected clickable building block 178 in 82 % (fig. 3.10) with the spectral data matching those reported.

![Chemical Diagram](image)

**Figure 3.10.** Preparation of the protected 5’-azido-5’-deoxyuridine 178.
Following the approach described with cytidine (Cf section 3.2), the azidouridine derivative 178 was deprotected to provide the versatile synthon 5’-azido-5’-deoxyuridine 179 (fig. 3.11). The acetonide protection was cleaved using a method reported by Golden et al., using In(OTf)₃ at room temperature. The reaction took place under reflux conditions in a mixture of acetonitrile and water and was completed within 6 h to give the intermediate 179 in 95 % yield. Alternatively, the azido group could be installed in one step with high regioselectivity and high yield (86 %) using the Appel or the Mitsunobu reaction. This was followed by the acetylation of the ribose fragment, using the classical procedure with acetic anhydride in pyridine, and the desired clickable uridine derivative 180 was obtained in 97 % yield.

![Figure 3.11. Preparation of acetyl-protected the 5’-azido-5’-deoxyuridine 180.](image)

3.3.2. 5-Fluorouridine

5-Fluorouracil has been widely used in the treatment of various cancers over the last 30 years. It is the phosphorylated form of the drug, 5-fluoroUMP, generated via metabolic pathways, which promotes the activity by causing DNA and RNA damage.

In the context of ST inhibition, some promising 5-FU derivatives were reported by Nakahara et al., showing that the 5-fluorouracil derivative displaced cytosine. Furthermore, the introduction of a fluoro group at the 5-position of the nucleoside in ST inhibitors may not raise major sterical issues, as the fluorine atom is slightly larger than hydrogen but smaller than a methyl group.
Chapter 3

The azidonation of 5-fluorouridine 181 was similar to the uridine counterpart (fig. 3.12). The protection of the ribose moiety was previously reported by Ajmera et al.\textsuperscript{401} In the case of 5-fluorouridine 181, the reaction was completed in 1 h, about 20 times faster than the reaction with uridine, and the protected intermediate 182 isolated in 80\% yield. Next, reaction with mesyl chloride in the presence of triethylamine gave the resulting O-mesyl intermediate 183, which was treated with sodium azide in DMF to give the clickable building block 184 in 94\% yield. The phase transfer catalyst benzyltriethylammonium chloride was also used in order to improve the azide salt’s reactivity. The spectral data for the 5-FU derivative 184 matched those reported by Ajmera.\textsuperscript{401}

The acetonide protection was cleaved from the ribose fragment by using a catalytic amount of indium triflate (5\% mol) and the reaction was carried out in a mixture of acetonitrile and water [9:1], as described earlier.\textsuperscript{394} 5'-Azido-5-fluorouridine 185 was isolated in 96\% yield. As described for the preparation of the azidouridine derivative, the solubility of the intermediate 185 in organic media is significantly lower than the protected counterpart. For this reason, the hydroxyl groups present on the ribose fragment were acetylated in order to decrease their polarity and aid their solubility. 5'-Azido-5-fluorouridine 185 was dissolved in pyridine and treated with acetic anhydride to give the clickable building block 186 in 92\% yield.
3.4. Modification at the 2’-position of the ribose fragment

2’-Deoxyuridine 187 is a naturally occurring nucleoside, differing to uridine by bearing only one hydroxyl group on the tetrahydrofuran ring of the ribose moiety, at the 3’-position. In the porcine ST3Gal-I crystal structure, the 2’-hydroxyl group of the ribose moiety engages in a hydrogen bond with a water molecule, not the protein (fig. 3.6). This is not the case in human and both rat ST6Gal-I and the human ST8Sia-III in which, this alcohol binds to a serine residue (Ser322, Ser319 and Ser300 respectively).<ref> Thus, the absence of the 2’-hydroxyl group in 187 offers the possibility to decrease the affinity of the inhibitor for the ST6Gal sub-family, while having less impact on the affinity towards both the ST3Gal and ST8Sia subtypes.

Similarly, thymidine 150 lacks a hydroxyl group at the 2’-position but bears an additional methyl group at the 5-position. As no thymidine-based ST inhibitor has been reported to our knowledge, it was interesting to explore the influence of these two features. 5-Methyl-CMP
146 has been shown to weakly inhibit both ST3Gal-III and ST6Gal-I (31 % and 16 % at 250 µM respectively) as well as ST8Sia-IV (30-40% at 500 µM).\textsuperscript{385} This nucleoside analogue \textsuperscript{150} might trigger an active transport through the cancer cell membranes.\textsuperscript{402} Thymidine \textsuperscript{150} on its own remains significantly less potent than cytidine against ST6Gal-I (16 % inhibition at 250 mM) according to the inhibition study reported by Kleineidam \textit{et al.}\textsuperscript{235}

Other moieties worth investigating are 2’-deoxy-2’-fluorocytidine derivatives, which have mostly been evaluated for potential antiviral activity,\textsuperscript{403} against the cytomegalovirus, the herpes simplex virus\textsuperscript{404} and the hepatitis C virus\textsuperscript{405-406} as well as against various cancer cell lines.\textsuperscript{407}

Fluoro groups are highly represented in medicinal chemistry. In addition to providing metabolic stability the fluorine atom rarely engages in hydrogen bonds and allows modulating the hydrophilicity of the compound. The structurally related 2’-deoxy-2’-fluorocytidine \textsuperscript{154} was found to exhibit some inhibition (74 %) against ST6Gal-I at 250 µM, while having no activity on ST3Gal-I at the same concentration.\textsuperscript{235} For this reason, 2’-fluorinated nucleoside derivatives constitutes an interesting scaffold in the context of this study.

In addition to fluoro groups, other compounds bearing a slightly bulkier group at the 2’-position, such as a methoxy group, were synthesised. The methyl ether moiety presents the advantage to accept hydrogen bonds, while promoting significant changes in the hydrophilicity of the substrate. Interestingly, ST3Gal-IV and ST8Sia-III activity were inhibited by 2’-O-methyl CMP \textsuperscript{155}, which had no effect on ST6Gal-I.\textsuperscript{385} For this reason, 2’-O-methyl derivatives constituted an interesting avenue in the aim of achieving selectivity between ST3Gal-I and ST6Gal-I.
3.4.1. 2’-Deoxyuridine, Thymidine and 2’-Deoxy-2’-fluorouridine

The preparation of 5’-azido-2’,5’-dideoxynucleoside derivatives has been reported and the literature provides numerous examples of the regioselective activation of the 5’-position by using sulfonyl chlorides.\textsuperscript{391,408}

Thymidine \textbf{150} is the most common building block utilised and the synthesis of its azido derivatative is usually carried out via a short sequence\textsuperscript{409} starting with a regioselective tosylation (undertaken at low temperature),\textsuperscript{410} followed by the acetylation of the 3’-hydroxyl group to give the intermediate \textbf{188} (fig. 3.13). The tosylate \textbf{188} is then treated with sodium azide to afford the building block \textbf{189}, which is ready for the CuAAC reaction. In our experience, the regioselective first step produced the expected product \textbf{188} in 59\% yield along with regioisomers. On the other hand, the azidonation provided the clickable material \textbf{189} in 93\%.

![Figure 3.13. Preparation of the 3’-acetyl-5’-azido-5’-dideoxythymidine building block \textbf{189}.](image)

Given that the structures of thymidine \textbf{150} and 2’-deoxyuridine \textbf{187} is closely related and only differ by the presence of the unreactive methyl group at the 5-position, similar procedures were applied (fig. 3.14). The novel tosylated intermediate \textbf{190} was isolated in 62\% yield. By using the present sequence, the building block \textbf{191} was achieved in an overall yield of 58\% over three steps. The 3’-acetyl-5’-azido-2’,5’-dideoxyuridine \textbf{191} had already been reported by using a different synthetic approach.\textsuperscript{411}
This successful procedure was then applied to the 2'-fluoro analogue 192. After tosylation and acetylation performed in one pot, the intermediate 193 was treated with sodium azide in DMF to give the clickable nucleoside 194 in 89% yield. The spectral data for these two compounds was very similar to the non-fluorinated analogues (189 and 191), with the exception of the $^{13}$C spectra. The C-2’ position appears as a doublet with a large coupling constant (90.4 ppm, $J = 126.6$ Hz) due to the presence of the fluorine atom, as well as the C-3’ albeit displaying a smaller coupling value (70.2 ppm, $J = 15.3$ Hz). Surprisingly, the C-1’ appeared on the $^{13}$C spectrum as a singlet (91.5 ppm) with no obvious coupling with the neighbouring fluorinated carbon atom.

Figure 3.14. Preparation of the building blocks 191 and 194.

3.4.2. 2’-O-Methyluridine

The synthetic scheme used for the preparation of other nucleosides modified at the 2’-position was attempted (Cf section 3.4.1). 2’-Methyluridine 195 was thus treated with tosyl chloride and acetic anhydride in pyridine at 0 °C. After work up, a product came out of the column as a single spot with LRMS analysis showing a mass matching the expected product 196, $m/z = 477$ [M+Na]$^+$. However, $^1$H NMR experiments showed the presence of a mixture of diastereoisomers.
Figure 3.15. Reaction of 2'-methyluridine 190 using one-pot tosylation/acetylation method.

The longer route that successfully gave the azidocytidine derivative 165 by starting with the selective protection of the primary alcohol at the 5’-position, was evaluated for 2’-methyluridine. The TBDMS protected intermediate 197 was prepared according to Rozner’s procedure, and was acetylated to give 198 in 74% yield. The 1H NMR spectrum clearly displays the tert-butyl (singlet integrating for 9 H at 0.92 ppm) group and the dimethylsilyl moiety (singlet integrating for 6 H at 0.10 ppm). The LRMS experiment gave the expected sodiated molecular ion peak of m/z = 437 [M+Na]+. The silylated protection was cleaved using a TBAF solution in THF in the presence of acetic acid in order to avoid the migration of the 3’-acetyl group and the known compound 199 was isolated in 76% yield. The azido group was installed following the method used for uridine and cytidine, by successive reaction with methanesulfonyl chloride and sodium azide, which provided the mseylate 200 in 72% yield and the building block 201 in 91% yield. In summary the azide derivative 201 was achieved in 37% over five steps.
The present chapter discussed the various aspects of the nucleoside fragment of ST transition-state analogue inhibitors, highlighting the importance of cytidine as a component of the natural sialylation machinery. Different nucleoside derivatives have been tested against a number of ST subtypes along with crystallographic and computational data, which suggests that functionalising this fragment constitutes an avenue for achieving the preparation of selective ST inhibitors. Seven commercially available nucleosides were selected as starting materials for the preparation of new potential ST inhibitors. Cytidine and uridine were considered as key elements of this study. 2’-Deoxyuridine and thymidine as well as synthetic analogues such as 2’-methyluridine, 5-fluorouridine and 2’-deoxy-2’-fluorouridine were also chosen because of their structural modification at the 2’- or 5-position of the nucleoside scaffold. Several synthetic routes were optimised for the synthesis of the required clickable 5’-azidonucleoside derivatives. In total, this chapter reported the synthesis of eleven azido nucleoside derivatives usable for the key coupling step to generate the target ST inhibitors, as presented in the next chapter. In addition to the types of activity pursued in this project, the small azido-nucleoside building blocks presented in this chapter may also find utility in the development of other types of enzyme inhibitors, in particular for antiviral activity.
CHAPTER 4

Triazole-based sialyltransferase inhibitors
In Chapter 2, the design and preparation of sialic acid mimics was presented, along with the synthesis of the nucleoside building blocks in Chapter 3. The current chapter describes the coupling of the building blocks leading to the final target inhibitors. After cleavage of the protecting groups, the library of newly developed compounds was submitted for biological evaluation, and the experimental biological data compared to the earlier calculated enzyme affinities.

4.1. Copper-catalysed azide/alkyne cycloaddition

4.1.1. Optimisation

Once both the sugar mimic and nucleoside fragments were successfully obtained, conditions for the coupling step using the ‘click chemistry’ strategy were screened (table 4.1). The 3-phenoxyalkyne derivative 111 was used for all the preliminary trials and the synthetic approach to the triazoles 202-206 is shown in table 4.1. The synthesis of 1,4-disubstituted triazoles involves the in situ generation of the Cu(I) catalyst from Cu(OAc)$_2$ and sodium ascorbate as a reducing agent to facilitate the CuAAC reaction. The reaction can be carried out in various solvents, at different temperatures and also using additives in some cases.$^{413}$

The first trials were performed using the azidocytidine derivative 172 and catalysed with 10 mol% copper acetate and 20 mol% sodium ascorbate in aqueous $t$-butanol as reported by Lee et al. (table 4.1).$^{239}$ The formation of the triazole ring was monitored by TLC showing the appearance of a slower moving spot, while the starting material was disappearing and the expected coupled product 202 was isolated in 49% yield (table 4.1, entry 1). Replacing $t$-
butanol with acetonitrile increased the reaction yield from 49 % to 57 % (table 4.1, entry 2).

The miscibility of acetonitrile and water is probably responsible for this improvement by facilitating the formation of the complex intermediate. Interestingly, the protecting system proved to have a great influence on the reactivity, in particular for the cytidine-based starting material. While the reaction of 172, bearing a N-acetyl group, takes over 20 h to complete, the 
N-Boc counterpart 174 required only 90 min for completion under otherwise identical conditions (table 4.1, entry 4). When only acetyl groups were used to protect the azidocytidine fragment 167, the reaction completed in 2 h and the product 204 was attained in 91 % yield (table 4.1, entry 5). The reaction of the azidouridine derivatives 178 and 180 proceeded in the same conditions as above, which led to the expected triazoles 205 and 206 in 88 % and 90 % yields respectively (table 4.1, entry 6 and 7).

Table 4.1. Preparation and optimisation of triazole-based inhibitors using click chemistry.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Equiv. alkyn</th>
<th>Equiv. Cu(OAc)₂</th>
<th>Equiv. Ascorbate</th>
<th>Solvent</th>
<th>Reaction time</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NHAc</td>
<td>-C(Me)₂</td>
<td>-C(Me)₂</td>
<td>1.0</td>
<td>0.1</td>
<td>0.2</td>
<td>tBuOH/H₂O [1:1]</td>
<td>24 h</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>NHAc</td>
<td>-C(Me)₂</td>
<td>-C(Me)₂</td>
<td>1.0</td>
<td>0.25</td>
<td>0.5</td>
<td>MeCN/H₂O [4:1]</td>
<td>24 h</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>NHAc</td>
<td>-C(Me)₂</td>
<td>-C(Me)₂</td>
<td>1.2</td>
<td>0.25</td>
<td>0.5</td>
<td>MeCN/H₂O [4:1]</td>
<td>24 h</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>NHBoc</td>
<td>-C(Me)₂</td>
<td>-C(Me)₂</td>
<td>1.2</td>
<td>0.25</td>
<td>0.5</td>
<td>MeCN/H₂O [4:1]</td>
<td>1 h</td>
<td>86</td>
</tr>
<tr>
<td>5</td>
<td>NHAc</td>
<td>Ac</td>
<td>Ac</td>
<td>1.2</td>
<td>0.25</td>
<td>0.5</td>
<td>MeCN/H₂O [4:1]</td>
<td>2 h</td>
<td>91</td>
</tr>
<tr>
<td>6</td>
<td>OH</td>
<td>-C(Me)₂</td>
<td>-C(Me)₂</td>
<td>1.2</td>
<td>0.25</td>
<td>0.5</td>
<td>MeCN/H₂O [4:1]</td>
<td>1.5 h</td>
<td>88</td>
</tr>
<tr>
<td>7</td>
<td>OH</td>
<td>Ac</td>
<td>Ac</td>
<td>1.2</td>
<td>0.25</td>
<td>0.5</td>
<td>MeCN/H₂O [4:1]</td>
<td>1.5 h</td>
<td>90</td>
</tr>
</tbody>
</table>
In summary, the CuAAC reaction led to the expected results, which makes it an appropriate synthetic tool for the preparation of novel ST inhibitors. The azidocytidine building block showed to be susceptible to solubility issue and those were managed by adapting the protecting system. In comparison the uridine analogues proved to be excellent substrates for this reaction. The conditions involving 1.2 equiv. of alkyne, 0.25 mol% of copper acetate and 50 mol% of sodium ascorbate in a mixture of acetonitrile and water [4:1] consistently led to the expected triazole derivative in excellent yields (> 90%) and were used as ‘optimised conditions’ for the rest of this study.

The triazole derivatives isolated during the optimisation process were fully characterised using mass spectrometry and NMR. Given the number of aromatic protons, the appearance of the CH at the 5”-position was confirmed by 2D NMR experiments. Both carbon atoms at 4”- and 5”-positions were identified in the 147 ppm and 123 ppm areas respectively. The gHSQC correlation between the C-5 carbon resonance signal and that of the triazole H-5 resonance signal is also consistent with the 1,4-regioselectivity assignment. The NMR spectral data for 205 were summarised in table 4.2 with focus on the nucleoside and triazole fragments.
Table 4.2. Summary of the main correlations observed for the HSQC, COSY and HMBC NMR spectra of 205 with focus on the nucleoside and triazole fragments.

4.1.2. Scope of the coupling step

This section describes the application of the optimised method for the preparation of 5’-triazolonucleosides by using the alkyne derivative series previously obtained in chapter 2. The coupling step was performed under the optimised conditions achieved in section 4.1, using 1.2 equivalents of alkyne with copper acetate (0.25 equiv.) and sodium ascorbate (0.5 equiv.) in a mixture of water and acetonitrile [1:4] at room temperature. The azide derivative 178 was used as a common starting material for the synthesis of the first series, hence providing the acetonide-protected uridine fragment, which was reacted with the six propargyl acetates 106-109, 111 and 131 as summarised in table 4.3. The yields for the coupling were consistently high (207-211, 88-93 %) with the highly functionalised indole derivative 211 isolated in 81 %. The reaction also proceeds rapidly as the reaction times did not exceed 2.5 h.
Table 4.3. Preparation of the acetonide protected triazole derivatives 207-211.

![Reaction scheme](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar</th>
<th>Yield (%)</th>
<th>Compound</th>
<th>Ar</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>207</td>
<td>H3CO-</td>
<td>93</td>
<td>205</td>
<td>PhO-</td>
<td>90</td>
</tr>
<tr>
<td>208</td>
<td>H3CO-OC(=O)</td>
<td>88</td>
<td>210</td>
<td>O-</td>
<td>91</td>
</tr>
<tr>
<td>209</td>
<td>CH3-OC</td>
<td>90</td>
<td>211</td>
<td>PhO-OC</td>
<td>81</td>
</tr>
</tbody>
</table>

In order to compare the efficiency of the coupling involving the starting materials 178 and 180, and thus the influence of the protecting system of the reaction, the key step was repeated using the azide 180 and the alkynes derivatives 106, 108-114 and 116 (table 4.4). By using the optimised conditions for the CuAAC (table 4.1, entry 6-7), the triazole derivatives 212-218 were attained in excellent yields (84-95 %). The yields were similar to those observed for the reactions with the azide derivative 178.
Table 4.4. Preparation of the acetyl protected triazole derivatives 212-218.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar</th>
<th>Yield (%)</th>
<th>Compound</th>
<th>Ar</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>212</td>
<td></td>
<td>90</td>
<td>216</td>
<td>PhO</td>
<td>87</td>
</tr>
<tr>
<td>213</td>
<td></td>
<td>89</td>
<td>217</td>
<td>F</td>
<td>95</td>
</tr>
<tr>
<td>214</td>
<td></td>
<td>84</td>
<td>218</td>
<td></td>
<td>92</td>
</tr>
<tr>
<td>215</td>
<td>PhO</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The heterocyclic derivative 218 was isolated in 92 %, which shows a consistent reactivity with the benzenic derivatives 106-114. The indolic alkyne derivatives 130 and 131 were used next in the same conditions as above and the resulting triazoles 219 and 220 were isolated in 90 % and 81 % yield respectively (fig. 4.1). The compounds 211 and 220 only differ by the protecting groups attached to the uridine and were both attained in the same yield (81 %), which shows that the functional group chosen as a protection for the nucleoside fragment has little influence on the coupling step.

Figure 4.1. Preparation of the indole-based derivatives 219 and 220.
The reaction of the 5’-azidouridine derivative 180 with 3-ethyl-3-hydroxyindolinone 133 gave the triazole-linked compound 221 in a slightly lower yield (84%), which can be attributed to the solubility of the alkyne derivative 133 in the solvent system CH₃CN/H₂O (fig. 4.2).

![Figure 4.2. Preparation of isatin derivative 221.](image)

Finally, the trifluoromethyl derivative 136 was employed for the CuAAC reaction with the uridine building block 180 and provided the protected inhibitor 222 in 97% yield (fig. 4.3).

![Figure 4.3. Preparation of triazolouridine 222 bearing a trifluoromethyl group at the benzylic position.](image)

In summary, the CuAAC reaction between the azidouridine derivatives 178 and 180 and the series of alkynes 106-114 as well as the heterocyclic derivatives 116, 130, 131 and 133 led to a series of protected ST inhibitors in consistently excellent yields. At this stage, the protecting system present on the azidonucleoside fragment, acetyl esters or acetonide, showed little influence on the reactivity with the alkyne derivatives, if any.

The nucleoside fragment is a key component of our triazole-based ST inhibitors. As mentioned in the previous chapter, cytidine has been largely explored in the field. Not only because the natural donor CMP-Neu5Ac and natural inhibitors such as CMP and CDP are cytidine derivatives but
also because, the large majority of studies focused on the activity of nucleoside-based ST inhibitors. Once the relevant 5' azidonucleoside derivatives were prepared as described in chapter 3, the coupling step was performed using the optimised conditions (fig. 4.4).

![Figure 4.4. Huisgen coupling using the alkyne 111 and the 5'-azidonucleosides modified at the 2' and 5'-positions.]

When using the acetonide protected 5-FU derivative 184 and the 3-phenoxy alkyne derivative 111, the CuAAC provided the triazole derivative 228 in 91% yield. As described at the time of the azidonation reaction described in section 3.3, uridine and its 5-fluoro counterpart have similar properties.

![Figure 4.5. Preparation of the acetonide protected 5-FU derivative 228.]

108
4.2. Deprotection

4.2.1. Cleavage of the acetonide protection

Once the coupling step has been optimised and validated within the scope of azidonucleoside and alkyne derivatives, the various deprotection methods were evaluated. The deprotection of the 5'-triazolonucleoside derivatives described earlier was attempted in various conditions. The acetonide protection on nucleosides is commonly cleaved by using concentrated aqueous trifluoroacetic acid (TFA) solutions. In our experience, this method led to unreliable results. The success of this procedure seems to depend on the substrate and moreover the reaction time. For example, the reaction of the 3-phenoxy derivative 206 in 90% TFA for 4 h led to the expected product 228 in moderate yield (48%, fig. 4.6). A variable amount of degradation products were observed depending on the conditions used and the starting material and, as a result, the crude material attained would require a case to case purification on top of discordant yields. The outcome of using TFA to cleave the acetonide protection on the ribose was unreliable hence did not fulfil the requirement of this project, which focus on developing straight-forward and cost effective synthetic access to new ST inhibitors. As a result, several other acidic conditions were evaluated such as dilute sulfuric acid (1 M to 6 M concentrations in water or methanol), and acetic acid.

![Figure 4.6. Deprotection of the acetonide-protected derivative 206 using TFA.](image-url)
Encouraged by the successful cleavage of the acetonide protection on the building block 178 using indium triflate, to generate the deprotected triazole-based inhibitors prepared using the CuAAC reaction. The coupled compound 210, bearing the isopropylidene protection on the ribose fragment and a cyclopentoxy group at the 3’’’-position, was dissolved in acetonitrile and treated with water and In(OTf)$_3$. The mixture was stirred at room temperature, according to the procedure described by Golden et al.\textsuperscript{394} After 24 h, no reaction had happened and the mixture was brought to reflux. Increasing the temperature resulted in the appearance of a slower moving spot on the TLC suggesting the presence of the expected product. After work up, the crude material was purified by flash chromatography column and two products were isolated. The expected fully deprotected product 229 was recovered in 29\% yield, while the major less polar product 230, represented 48\% of the mixture. Mass spectrometry indicated a slightly higher molecular weight, $m/z = 522$ [M+Na]$^+$, which is 14 units more than expected, and a singlet at 3.25 ppm on the $^1$H NMR spectrum was observed, suggesting the presence of the methoxy group. Comparison of the NMR spectra of both fractions, which remain relatively similar with the exception of the details mentioned above, it seems like the use of In(OTf)$_3$ was affected by the reactivity of the benzylic position. Although, the mechanism involved in installing the methoxy group remains unclear and the methyl group might result from a reaction with the solvent (CH$_3$CN). Identifying the mechanism for the formation of the O-methyl side product requires more experiments, which were not undertaken as this unexpected product does not take part in the focus of the study.

Figure 4.7. Reaction of 210 with In(OTf)$_3$ leading to a mixture of partially and fully deprotected triazoles 229 and 230.
Chapter 4

4.2.2. Cleavage of the acetate protection

The unsuccessful attempts to cleave the acetonide protection of compounds 205 and 210, encourage us to explore a different approach. Consequently, the deprotection of acetate groups proceeded in mild conditions by treating a solution of the protected triazole derivative in methanol with concentrated ammonia. The mixture was stirred at room temperature until disappearance of the starting material and provided the expected target in excellent yields (> 90 %).

Table 4.5. Cleavage of the acetyl protection.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar</th>
<th>Yield (%)</th>
<th>Compound</th>
<th>Ar</th>
<th>Yield (%)</th>
</tr>
</thead>
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<tr>
<td>232</td>
<td></td>
<td>94</td>
<td>234</td>
<td></td>
<td>85</td>
</tr>
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<td>230</td>
<td></td>
<td>92</td>
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<tr>
<td>229</td>
<td></td>
<td>93</td>
<td>237</td>
<td></td>
<td>94</td>
</tr>
</tbody>
</table>

The acetyl group attached to the position mimicking the anomeric center of derivatives 212-218 was cleaved efficiently (table 4.5). In contrast, the methyl ester of 219 and 220 remained protected under these conditions, so as the Boc group attached to the \( N^1 \)-position of the indole fragment (fig. 4.8). The corresponding triazole derivatives 238 and 239 were isolated in 83 % and 79 % yields respectively.
For both 218 and 219, the Boc group at the 1-position of the indole ring was inert to ammonia’s nucleophilicity. This was deducted from the mass spectrum and confirmed by large peaks in the $^1$H NMR spectrum, appearing at 1.62 ppm and integrating for 9 protons. These compounds will be tested in the current form in which the Boc group takes part in the hypothetical interaction with a hydrophobic pocket in the active site, consistently with the phenoxy (as in 229) and cyclopentoxy groups (as in 230) used for this purpose in the rest of the series. The hydrophobic groups provide three different steric and electrostatic features from the flat aromatic phenyl group to the envelope-shaped cyclopenyl and the wider tert-butoxy group. In addition, the three moieties align consistently when the structures of compounds 229, 230 and 238 are overlayed (fig. 4.9).

The deprotection of the isatin derivative 221 using ammonia in methanol provided the expected product 240 in 88 % yield (fig. 4.10). This additional example illustrates the
practicality of the deprotection method. Under such mild conditions, the deprotected product $240$ is recovered in high yield and no side reaction was observed.

![Figure 4.10. Preparation of the isatin-based derivative 240.](image)

The cleavage of the acetyl protections of compound $222$ using concentrated ammonia gave the fluorinated ST inhibitor $241$ in 86 %. The trifluoromethyl group attached to the benzylic position did not promote any noticeable sterical hinderance and had no effect on the reactivity of the benzylic acetate group.

![Figure 4.11. Synthesis of the fluorinated triazole derivative 241.](image)

Using this last procedure with the coupled products bearing exclusively acetate groups as a protecting system also led to excellent results. The expected final cytidine-based compound $242$ was attained in 87 % yield (fig. 4.12).

![Figure 4.12. Deprotection of the cytidine derivative 242.](image)
The synthetic strategy employed to prepare the 5'-azidonucleoside derivatives provided building block protected with acetyl groups. These were cleaved following the optimised method described in chapter 3, by reaction of the ester protected compounds with concentrated ammonia in methanol at room temperature. This procedure provided the expected 5'-triazolenucleoside derivatives 242-246 in excellent yields (fig. 13). As expected the aliphatic fluoro group at the 2'-position of compound 244 was inert to nucleophilic substitution with ammonia.

![Figure 4.13](image-url)

**Figure 4.13.** Deprotection of the ST inhibitors modified on the nucleoside fragment.

There is a lack of evidence in the literature for acetonide protected nucleosides to be an appropriate moiety for a prodrug strategy. Enzymes responsible for the *in vivo* cleavage of acetal groups attached to the ribose fragment of nucleoside derivatives, if they exist, may not have been identified to our knowledge. Although, the presence of this type of functional group on such molecular edifice promotes other features such as increasing the hydrophobicity, and thus elevate the log P, of the compound by masking the diol present on the sugar and creating sterical hindrance, which might affect the affinity of the compound with the active binding site to the enzyme. This second aspect endorses potential for achieving selectivity towards the different ST subtypes as the cytidine binding pocket in ST6Gal-I is geometrically tight, as indicated by the X-ray crystal structure,\(^{188-189}\) opposed to the porcine
ST3Gal-I\textsuperscript{183} and human ST8Sia-III.\textsuperscript{190} This last point is confirmed by the docking of 248 and 249 into the porcine ST3Gal-I (fig. 4.14).

![Image](79x530 to 516x704)

**Figure 4.14.** Cytidine derivative 248 (A) and uridine derivative 249 (B) overlayed with CMP 3 (in orange sticks) in the active site of porcine ST3Gal I (PDB: 2WNB).

### 4.2.3. Separation of the diastereomers

Since the CuAAC reaction was set up using racemic propargyl esters, the corresponding triazole derivatives were isolated as a mixture of diastereoisomers. In theory, the separation of diastereoisomers should be attainable by using chromatographic methods but in our experience, both stereoisomers had similar retention times. As stereoisomers are expected to exhibited significantly different activity,\textsuperscript{414-416} their separation was attempted by using HPLC. The indolic 239 and the 5-FU derivative 247 were subjects to the preliminary trials. All experiments were performed using a 0.1% TFA solution in MilliQ H\textsubscript{2}O for elution in a C18 column. Various flow rates were examined and the optimal conditions only lead to a resolution of a few seconds, which was not satisfying for translating the experiment into preparative HPLC. As a result, the compounds were tested as a mixture of diastereoisomers.
4.3. Computational data

4.3.1. Docking in sialyltransferases

All docking experiments were performed using Autodock Vina and the binding poses used for this study were selected according to their ability to match alignment with the nucleoside fragment which was co-crystalised with the protein. The docking results mostly highlighted differences between the different ST subtypes used.

**Cytidine binding region:** The differences between ST3Gal and ST6Gal in the cytidine binding pocket were consistently observed as suggested by Kuhn/Meng. Moreover, this applies to ST8Sia as well which displays a binding mode comparable to ST6Gal. In ST3Gal, the cytosine moiety binds exclusively through a hydrogen bond between the carbonyl group at the 2-position and the amino group of the residue Gly293. When replaced with uracil, the nucleoside fragment engages by forming an additional hydrogen bond between the NH group at the 3-position and the carbonyl group of the same residue Gly293. In ST6Gal, C2=O forms a hydrogen bond with Lys376. N4 gives a hydrogen bond with C=O from Cys353 and OH from Thr365. Swapping from cytidine to uridine induces the loss of the interaction with Cys353 but the C=O at the 4-position is in theory still able to form a hydrogen bond with OH from Thr365 which is observed during docking experiments. In ST8Sia, The carbonyl group at the 2-position of cytosine binds to the amino group of Trp322. The amino group at the 4-position engages both hydrogen atoms in hydrogen bonds with the carbonyl groups of Trp322 and Tyr336.

**Ribose binding region:** Endocyclic oxygen atom behaves like a hydrogen bond acceptor with Asn150 in ST3Gal, Ser169 in ST6Gal and Asn167 in ST8Sia. Hydroxyl groups: 3’-OH is a hydrogen bond acceptor with Gly273 in ST3Gal, Gly324 in ST6Gal and Gly302 in ST8Sia. The type of bond is identical for the 3 subtypes. In ST3Gal, the crystal structure describes the
2’-OH in CMP bridging with a water molecule. The docking results also showed the possibility to form a hydrogen bond with Ser271 in ST6Gal-I or Ser300 in ST8Sia. In regards to the hydroxyl groups at 2’ and 3’-positions, both OH do not necessarily bind simultaneously. In some results, one of them forms a hydrogen bond while the second one seems to be too far to interact with the neighbouring residues. The rigidity of the linker appears to impose a different binding pose.

**Triazole binding region:** The triazole ring is composed of three nitrogen atoms and two of them bear lone pairs of electrons, which do not participate in the aromaticity of the ring. As a result, they are two hydrogen-bond acceptor sites. According to the docking experiments, the linker shows limited number of interactions with the amino-acid residues in the active site. The rigidity of the triazole ring promotes the alignment of the two other fragments by imposing dihedral angles. In addition, by being rigid, the triazole linker restricts the freedom of rotations allowed by the phosphate counterpart. This, for example, results in a fixed distance between the atoms attached to the 1- and the 4-position of the heterocycle (fig. 4.15). In compounds bearing a phosphate linker, the distance between the carbon atoms at the 5’-position of the nucleoside and the anomeric position of sialic acid is variable because of the flexible nature of the linker. Measuring this distance amongst the docking results showed a range of distances oscillating between 4.38 and 4.99 Å. Interestingly, the range of distances determined is different depending of the ST subtype used for docking, e.g. 3.925-4.949 Å in ST3Gal-I, 4.036-4.932 Å in ST6Gal-I and 4.380-4.892 Å, ST8Sia-III. In the 5’-triazolonucleoside derivatives, the range of distances between the 5’position and the benzylic position is narrower because of the rigidity of the heterocycle and is 4.81 Å. As a result, using triazole as a linker constitutes a geometrical constrain.
Acceptor site: The acceptor site is the part of the active site in which the sequence between subtypes differs the most. This is what contributes to the functional selectivity of each subtype of STs. For all the derivatives bearing a phenoxy group or another bulky hydrophobic group at the 3-position of the benzylic sialic mimic, similar binding poses were observed. The diaryl ether does not mimic the sialic acid fragment as proposed in the first place but binds to the acceptor site. This was particularly obvious in the porcine ST3Gal-I structure because the crystal structure included a dissacharide acceptor. It is to note that for each subtype, the pocket is relatively hydrophobic, particularly for ST8Sia. The interactions of the protein with the phenoxy group include possible stacking with Tyr194 and Phe212 in ST3Gal-I. Hydrophobic interactions with Trp257, Tyr369, the backbone of Gln235 and Pro259 were found in ST6Gal-I, as well as stacking or hydrophobic interactions with Leu304, Trp244, Pro246, Phe249, Phe250 and the backbone of Asn211 in ST8Sia-III. The preferred substituents for binding to this pocket are phenoxy, cyclopentoxy, 2-naphthyl and benzothienyl. In ST8Sia, the hydrophobic pocket in the acceptor site is a lot wider than in the two other crystal structures. As a result, bulky substituents on the phenoxy group might be tolerated and promote a certain degree of selectivity. The diastereoisomers were docked separately in order to evaluated the differences in their active conformation and in most cases, both isomers occupy the same pockets in the ST active site (fig. 4.16).
Figure 4.16. Docking of 229 into the porcine ST3Gal-I (PDB: 2WNB, A), the human ST6Gal-I (PDB: 4SJ2, B) and the human ST8Sia-III (PDB: 5CXY, C). Both diastereoisomers are shown (R in green sticks and S in grey) along with CMP 3 (in orange sticks).

Docking the proposed ST inhibitors modified on the nucleoside fragment (242-247) into the different subtypes highlights the major differences expected depending on the type of linkage. For example, the thymidine derivative 234 and the 2'-O-methyluridine derivative 246 did not provide any satisfying results when docking into the human ST6Gal-I and, as discussed in the section 3.1.3, the methyl group promotes enough steric hinderance to restrict the affinity of such compound in the enzyme’s active site. Conversely, both 234 and 246 fitted in the donor binding pocket of ST3Gal-I and ST8Sia-III (fig. 4.17-A).

The indole derivative 239 bears a carboxylic ester moiety, which aims at mimicking the acid group in the natural donor CMP-Neu5Ac 2. When docking 239 into the STs crystal structures, the carboxylic function was found to engage in hydrogen bonds with Thr272 in ST3Gal-I, Ser323 in ST6Gal-I and Thr301 in ST8Sia-III in a similar fashion to CMP-Neu5Ac. In addition, the ester also interacted with the catalytic residues, as shown in fig. 4.17-B, where the carbonyl group forms a hydrogen bond with the catalytic residue His354 of ST8Sia-III.
4.3.2. Docking into other glycosyltransferases

A number of GT crystal structures has been reported and are available on the RCSB Protein Data Bank website (www.rcsb.org). All these enzymes use a sugar-nucleotide derivative as the donor to perform the glycosylation reaction (Cf section 3.1.4).\textsuperscript{392} In addition, other 5’-triazolonucleoside derivatives (not shown) have been proposed as GT inhibitors,\textsuperscript{418} including galactosyltransferases,\textsuperscript{419-420} as well as chitin synthase.\textsuperscript{421}

Amongst these GTs, two examples using a uridine-sugar conjugate as a natural donor were chosen for docking our triazole-linked nucleosides into their crystal structures. Thus, several compounds of our series of triazolonucleosides were docked into α3GT (PDB: 1GX4). This galactosyltransferase uses (fig. 4.18-A).\textsuperscript{422} This GT enzyme uses UDP-α-D-galactose as a glycosyl donor to catalyse the transfer onto N-acetyllactosamine.\textsuperscript{423} The second enzyme was the O-linked N-acetylglucosamine transferase (EC.2.4.1.255), responsible for the post-translational modification of protein serines/threonines with N-acetylglucosamine and regulating many cellular processes by interfering with protein phosphorylation. Its natural
donor is UDP-N-acetyl-D-glucosamine and the docking experiments were conducted using the PDB: 4N3B (fig. 4.18-B).\textsuperscript{424}

![Docking of 229 into galactosyltransferase (PDB: 1GX4, A) and 230 into N-acetylglucosamine transferase (PDB: 4N3B, B).]

The docking results indicate that the 5’-triazolonucleosides proposed in this thesis have a potential in inhibiting GT enzymes other than STs. As expected, attention must be paid to the activity of the nucleoside derivatives attained using click chemistry towards other GTs.

### 4.4. Biological evaluation

#### 4.4.1. Cytotoxicity

The cytotoxicity of the whole series of 5’-triazolonucleoside derivatives were tested using the MTS assay, previously described in section 2.2.1.3. The compounds were incubated for 24 to 48 h with different cancer cell lines, MiaPaCa2, MCF-7, SKOV3, as well as macrophages RAW264.7. These cell lines were selected because they were previously used in other studies and have been found to express various types of STs. The pancreatic Mia-Paca-2 cells overexpress ST6GalNAc-IV, down-regulates ST3Gal-I.\textsuperscript{425} The breast MCF-7 cells express ST3Gal-III and ST6Gal-I,\textsuperscript{426} ST3Gal-I, III, IV and ST6Gal-I,\textsuperscript{427} as well as ST8Sia I.\textsuperscript{428} The
ovarian SKOV3 cells are known for expressing ST6Gal-I,\textsuperscript{85,429} ST3Gal-IV,\textsuperscript{430} ST3Gal-V,\textsuperscript{431} and ST3Gal-VI.\textsuperscript{432} Finally, the murine macrophages RAW264.7 express ST6Gal-I,\textsuperscript{433} ST6Gal-II\textsuperscript{434} along with various ST subtypes.\textsuperscript{435}

No apparent influence on cell viability for all the cell lines tested at concentrations up to 300 μM was observed. The lack of toxicity of our triazolonucleoside derivatives will enable further cell-based biological assays in the aim of exploring the antimetastatic activity of these compounds.

\section*{4.4.2. Antibacterial activity}

In addition to upregulation of STs (and other GTs) in cancer, sialic acid derivatives also play important roles in a number of other diseases including viruses\textsuperscript{436} such as influenza\textsuperscript{229,437} and Chagas disease.\textsuperscript{231} STs have also been identified in marine bacteria.\textsuperscript{438} Cabral \textit{et al.} observed that α-2,6-ST deficiency improves phagocytosis.\textsuperscript{439} However, decreased sialylation was shown in other cases to enhance bacterial virulence.\textsuperscript{440} STs are often referred to as potential targets for antibacterial purposes, however, the rationale for considering STs as a target for the design of a new antibacterial drug is not well established. In addition, the literature is devoid of examples demonstrating the benefits of ST inhibition against bacteria. To explore whether our compounds did show any potential antibacterial activity, they were screened against a number of Gram-negative bacteria. These include \textit{Escherichia coli}, \textit{Klebsiella pneumoniae}, \textit{Acinetobacter baumannii}, \textit{Pseudomonas aeruginosa} and \textit{Staphylococcus aureus}.

Amongst our series of compounds, the six triazole derivatives 228, 240-243 and 248 were tested and none of these displayed any activity at concentrations of up to 32 μg/mL. The lack of antibiotic activity of this group of compounds does not exclude the possible inhibition of bacteria invasiveness, which is yet to be evaluated.
4.4.3. ST inhibition assay

For this study, 19 of our triazole derivatives were evaluated against ST8Sia-II using a fluorimetric assay developed in the Gerardy-Schahn group, from the Hannover Medical School in Germany. The experiments were carried out by our collaborators on our library of available compounds, however, the fluorinated compound 236 and sati derivative 240 were not tested because they were synthesised at a later date.

Overall, the series of triazole-linked inhibitors tested exhibited varying degrees of inhibition (19-98 %) towards ST8Sia-II at 100 µM (fig. 4.19). DMSO and water were used as negative controls for this assay.

![Figure 4.19](image_url)

**Figure 4.19.** Activity of the triazole-based ST inhibitors towards ST8Sia-II at 100 µM expressed in inhibition rates after 1 h (green bars), 2 h (blue bars) and 4 h (orange bars) of incubation.
**Structure-activity relationship:** Based on the present series of compounds which have been evaluated, a SAR study can be established to compare and predict the activity of the inhibitors. Herein, the SAR is presented in the form of two tables, the first one focuses on comparing the influence of the nucleoside fragment on the activity (table 4.6) and the second one deals with the sialic acid mimic (table 4.7).

First of all, the cytidine moiety led to the most potent inhibition with 91% at 100 µM compared to the rest of the series (table 4.6, entry 1). This was closely followed by the 5-FU derivative 246 in accordance with the results previously reported in this respect. Conversely, the addition of a methyl group at the 5-position of the base was not tolerated by ST8Sia-II and resulted in a decrease in the activity (table 4.6, entry 8). This reduction of the activity is independent to the lack of hydroxyl group at the 2’-position because the 2’-deoxyuridine counterpart retained stronger potency with 80% inhibition at 100 µM (table 4.6, entry 3). Further modifications at the 2’-position resulted in moderate activity. The 2’-fluoro derivative 244 gave slightly more potent inhibition than the uridine counterpart with 53% inhibition (table 4.6, entry 7) compared to 45% inhibition for 228 (table 4.6, entry 9). Sterically hindered groups were tolerated when attached to the ribose fragment, as showed by the 2’-O-methyl derivative 245 with 68% inhibition (table 4.6, entry 5). Similar conclusions can be made for the acetonide protected compounds 247 and 248 (table 4.6, entry 4 and 6) with 62% and 80% inhibition respectively.

Interestingly, the disarylether side chain tethered to the 5’-position of the nucleoside fragment via a tetrazole ring seem to play a significant role in the binding affinity of the inhibitors described here. In comparison to CMP derivatives previously reported by Al-Saraireh and Miyazaki, 5-methylCMP and 2’-O-methylCMP were found to be more potent than CMP itself while in the present series, the cytidine-based inhibitor remains the most potent.
Table 4.6. Structure-Activity Relationship study based on the nucleoside fragment.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>% inhib</th>
<th>Structure</th>
<th>cLog P</th>
</tr>
</thead>
<tbody>
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<td>241</td>
<td>91</td>
<td><img src="image1" alt="Structure" /></td>
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</tr>
<tr>
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<td>246</td>
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<tr>
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<td>80</td>
<td><img src="image3" alt="Structure" /></td>
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</tr>
<tr>
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<td>248</td>
<td>80</td>
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<td>245</td>
<td>68</td>
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<tr>
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<td>9</td>
<td>228</td>
<td>45</td>
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</table>

Table 4.7 reports the activity of the uridine-based inhibitors that differ by the sialic acid mimic. Several trends can be concluded from this set of data, based on the type of aromatic ring used and how it was substituted. First, the 3,4-disubstituted motifs promoted greater potency as shown by the 4-fluoro-3-phenoxy derivative 234 (98 % inhibition, table 4.7, entry 1), the 4-methoxy-3-phenoxy compound 233 (87 % inhibition, table 4.7, entry 3) and its 4-methoxy-3-pentoxy analogue 232 (65 % inhibition, table 4.7, entry 6). Of note, the incorporation of a fluorine atom contributed to the potency of the inhibitor, as described for the nucleoside fragment (table 4.6, entry 2). The short and flexible group 3-propoxy in 231 only led to weak activity (24 % inhibition, table 4.7, entry 9).

The use of heterocyclic scaffold to mimic the sialic acid fragment proved to be beneficial as shown by the benzothienyl derivative 236 (87 % inhibition, table 4.7, entry 4) and the indolic analogues 237 and 238 (87 % and 96 % inhibition respectively, table 4.7, entry 3 and 5). The t-butoxycarbonyl group attached to the 1-position of the indole ring could play a role in the binding, similar to the 3-phenoxy group of 228, as suggested the modelling data.
(Cf section 4.2.2). The ester group present in 238 fulfilled its role in mimicking the carboxylic acid group of the natural donor CMP-Neu5Ac 2 (Cf fig. 4.17-B) and contributed to increase the potency of 238 by about 10 % compared to 237, which is lacking the functional group.

Table 4.7. Structure-Activity Relationship study based on the sialic acid mimic.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
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<th>Structure</th>
<th>cLog P</th>
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</tr>
</tbody>
</table>

Of note, the Log P did not correlate with the potency of the triazolonucleoside derivatives. Nevertheless, the molecular diversity of the library of compounds presented above promoted a range of Log P values (as calculated using chemdraw15, 0.23 < Log P < 2.66 and -1.49 < cLog P < 1.55) allowing to balance the high polarity and hydrophilicity of the nucleoside fragment. In addition, these values are close to those of commonly used anticancer agents in the clinic such as gemcitabine (Log P = -0.9 and cLog P = -0.73).
This chapter presented the preparation of triazolonucleoside derivatives, which was achieved in excellent yields by using the click chemistry approach. The convergent synthetic pathway demonstrated its versatility and the advantage of preparing the building blocks separately. The deprotection step was more challenging than expected and the acetyl protection strategy proved to be the most appropriate for both the coupling step and the cleavage of the ester groups.

The computational study, mostly based on docking experiments highlighted some key features in the possible mode of binding of the proposed inhibitors and these results correlate accurately with the biological evaluation.

In addition to the low toxicity of our series of triazole-linked nucleosides, some compounds exhibited promising inhibition towards ST8Sia-III, up to 98 % inhibition at 100 μM. The most potent compounds of this series 239,243,245 and 248 confirmed the SAR aspects identified earlier, in the design process. This last result gives promise to achieve selective inhibition of STs in the nanomolar level by drugs prepared via cost effective and high yielding synthetic routes.
CHAPTER 5

Conclusions and future directions
Chapter 5 - Conclusion and Future Directions

5.1. General conclusion

Our ever-expanding knowledge of the intricate role of sialylation in cancer, inflammation, immunology, and central nervous system disorders has highlighted the therapeutic potential of this important family of enzymes. Today, a large body of evidence has established the merits of inhibiting specific STs such as ST3Gal I, ST6Gal I and ST8Sia-II as a powerful new antimetastatic strategy, with the potential to be effective on drug-resistant tumors. A small number of pan-ST inhibitors are commercially available; however, for ST inhibitors to proceed to the clinical stage, it is essential that selective inhibitors for the different ST subtypes are developed to specifically target those STs overexpressed in cancers and to reduce off-target effects. A wide variety of inhibitors have been developed over the last two decades by rational design and from natural product and HTS efforts. The recent reports of mammalian crystal structures for pST3Gal I, rST6Gal I and hST6Gal I, and hST8Sia III have enabled the use of structure-based design to drive ST inhibitor design in the future to improve inhibitor selectivity and pharmacokinetic profiles. Increased availability of subtype selective, cell-permeable and synthetically accessible, small-molecule ST inhibitors in the future will enable further exploration and therapeutic exploitation of the role of sialylation in a range of diseases, in particular metastatic cancers.

5.2. Chapter 2

Chapter 2 featured the synthesis of building blocks suitable for mimicking the sialic acid fragment in the natural ST donor CMP-Neu5Ac. Different synthetic approaches were explored depending on the type of connectivity required for the carbamate and triazole linker.
For the carbamate-based inhibitors, a series of nineteen diethyl α-hydroxyphosphonate esters were prepared in excellent yields including 5 novel compounds. Twelve of these derivatives were tested using a well known cell proliferation assay, which revealed no toxicity up to 100 µM. Three of these twelve compounds were also assessed for anti-inflammatory activity and found to reduce nitric oxide production in lipopolysaccharide-activated murine macrophages (>80 % inhibition at 10 µM). The full series of phosphonate esters were also tested against five strains of Gram-negative bacteria, but were found to not inhibit growth. Due to a change in focus to the triazole-based compounds, the α-hydroxyphosphonates were not pursued further in this project.

For the triazole-based inhibitors, the preparation of propargylphosphonate esters from the corresponding propargyl acetates was attempted using a copper catalyst. However, rather than the expected alkyne products, the corresponding allene derivatives were isolated in good yields (47-79 %) in accordance with results reported at the same time as this work by Shen et al. and Hu et al. To synthesise the original propargylic targets via this route, other catalysts and conditions would need to be investigated. In the end, a series of fifteen α-arylpropargyl acetates, which included nine novel compounds, was achieved in good to excellent yields, as the key building blocks for the final targets. Due to their significant lipophilicity, their inherent bioactivity was not assessed.

5.3. Chapter 3

Chapter 3 explored the various aspects of the nucleoside fragment including an overview of the nucleoside-based ST inhibitors and the hypothesis that functionalising this fragment constitutes an avenue for achieving the preparation of selective ST inhibitors. The reported biological data for these types of inhibitors was compared to computational analysis including sequence alignments, to investigate major differences between the different ST subtypes. Docking
experiments provided further insight on the mode of binding of the cytidine fragment in the active site of STs leading to uridine being examined as a replacement for the natural cytidine fragment with the potential to have greater affinity for the ST3Gal family over the other STs.

Seven commercially available nucleosides were selected as starting material for the preparation of new potential ST inhibitors, including cytidine and uridine other nucleosides modified at either the 2’- or 5-position. These included the naturally occurring 2’-deoxyuridine and thymidine as well as synthetic analogues such as 2’-methyluridine, 5-fluorouridine and 2’-deoxy-2’-fluorouridine. Several synthetic routes were optimised to give eleven clickable 5’-azidonucleoside derivatives including three novel compounds. In addition, two different protecting systems were implemented, one acid sensitive and the other one base sensitive.

5.4. Chapter 4

Chapter 4 detailed the preparation of the target triazole-based ST inhibitors by achieving the coupling step between the sialic acid mimic (the α-arylpropargyl acetates from chapter 2) and the nucleoside fragment (5-azidonucleosides from chapter 3). High yielding conditions were obtained using the azidocytidine and azidouridine starting material bearing acetonide, Boc or acetyl protecting groups. However, the best results were attained when using 1.2 equiv. of alkyne and the reaction was catalysed with 25 mol% of copper acetate and 50 mol% of sodium ascorbate in a mixture of acetonitrile and water [4:1]. The expected products were isolated in excellent yields (90 %) and the protection system had little impact on the reaction outcome, expect for the cytidine series for which the $N^\alpha$-Boc group gave the most soluble reagent. In total, 27 novel highly functionalised compounds were synthesised.

The deprotection of the acetonide group attached to the nucleoside fragment was more challenging than expected and inconsistent results were observed. Conversely, the cleavage of
the acetyl groups proceeded successfully and an acetyl protection strategy proved to be the most appropriate for both the coupling step and the cleavage of the ester groups. The series of triazolonucleoside prepared using click chemistry was deprotected to give a further 20 novel compounds in excellent yields (83-98 %).

Molecular docking methodologies were developed and standardised by examination of reported phosphate-based inhibitor CMP. These docking studies indicated that compounds containing triazole as a linker could potentially bind to various ST subtypes. Interestingly, the inhibitors bearing a diarylether moiety, originally designed as a sialic acid mimic, may interact with the acceptor site. The binding mode of compounds prepared from a nucleoside different from cytidine confirmed that this fragment may facilitate selective discrimination of ST subtypes.

The cytotoxicity of the triazolonucleoside derivatives was evaluated against breast, ovarian and pancreatic cancer cell lines (MCF-7, SKOV3 and MiaPaCa-2 respectively) as well as the murine macrophages RAW264.7 and none was detected at concentration of up to 300 µM. The series of triazole derivatives was also subject to antibacterial activity evaluation against five bacteria strains with no observed effect. The evaluation of ST3Gal-I inhibition is currently in progress and the testing of our library of triazole-based derivatives towards ST8Sia-II gave some promising results with inhibition ranging from 14 to 98 % when tested at a concentration of 100 µM. Heterocyclic sialic acid mimics showed high affinity for ST8Sia-II, in particular the indole derivative 239 with 96 % inhibition at 100 µM. The nucleoside fragment also influenced the inhibitory activity, and a bulky group attached to the ribose fragment was tolerated with only a slight decrease in the potency. 5-Fluorouridine is a good candidate for the nucleoside fragment as demonstrated by the inhibitor 247 with 86 % inhibition and far easier synthetic access than the cytidine counterpart.
5.5. Future directions

Although the complete biological evaluation of our library of ST inhibitors is in progress, some promising results already suggest directions for further development.

**Synthesis:** Further optimisation of our triazole-linked inhibitors should start with the incorporation of the phosphonate group at the benzylic position of the sialic acid mimic, in order to validate the SAR model previously described. This requires the preparation of propargylphosphonate esters and which could be achieved via two different approaches. First, the unsuccessful trials for the propargylic substitution described in chapter 2 need to be addressed by screening a number of metal-based catalysts such as Fe, Bi or Ru, in accordance with reported procedures. Another approach, inspired by the reactions achieved in this project involving α-ketoesters, could utilise the addition of ethynylmagnesium bromide to benzoylephosphonate derivatives to synthesise the corresponding α-ethynyl-α-hydroxyphosphonate esters (fig. 5.1). This type of intermediate would be suitable for the CuAAC reaction strategy described in chapter 4.

![Retrosynthetic scheme](image_url)

**Figure 5.6.** Retrosynthetic scheme for the preparation of triazole-based sialytransferase inhibitors bearing a phosphonate group (PG: protecting group).
Amongst the most encouraging results presented in this thesis, the activity of the indole-based inhibitor 239 gives promise to enable the scalable preparation of potent selective ST inhibitors. The relatively complex structure of this sialic acid mimic actually has a straightforward retrosynthetic scheme, starting with inexpensive and readily building blocks (fig. 5.2). The method should involve a protecting system that favours the fragments’ solubility and that is cleavable in mild conditions and high yields, such as the benzyl group. The convergent approach presented in fig 5.2 enables the versatile functionalisation of the targets via cost effective routes.

![Figure 5.2. Retrosynthetic scheme for the preparation of highly functionalised indole-based sialyltransferase inhibitors.](image)

Within the indolic series, the issue of isolating the product as a diastereoisomeric mixture remains. To address this challenge, the carboxylic acid function on the indolic fragment could be protected with a chiral auxiliary in order to increase the physicochemical differences between the diastereoisomers. The readily available natural product menthol has proven to fulfil this role efficiently and could be easily incorporated into our synthetic sequence (fig. 5.3). At this stage, the experiment should confirm the possibility to separate the stereoisomers before or after the coupling step.
In addition, the development of carbamate-based inhibitors using α-hydroxyphosphonate esters is currently under optimisation in our group.

**Computational studies:** The use of molecular dynamics employing one crystal structure of each ST subtype should bring some insights about the mode of binding of our inhibitors and facilitate the elaboration of new compounds with improved selectivity. The ST subtypes that have not been crystalised yet could be the object of homology modelling in order to expand the study.
CHAPTER 6

Experimental data
Chapter 6: Experimental data

6.1. Chemical procedures

6.1.1. General

All chemicals were purchased from Sigma Aldrich Chemical Co. and used as received. All solvents were either AR or HPLC grade for synthesis and purification respectively. Solvents were purified by short path distillation, while solvents for HPLC were filtered through nylon membrane filters (#7404-009, Whatman®) and degassed by sonication using a Soniclean ultrasonic bath (Soniclean 250HT, Thebarton, Australia). Evaporation of solvents was performed using a Büchi R-114 or R200 rotary evaporator, under vaccum and below 40 °C to avoid decomposition of the samples. Melting points were determined using a Reichert melting points apparatus and are uncorrected. The reaction yields were attained after drying under high vacuum.

Chromatography: Thin layer chromatography (TLC) was performed on Merck thin layer aluminium sheets. Most reactions were monitored under an ultra-violet lamp at 254 nm as the compounds prepared were visible under this wave length. Column chromatography was performed on silica gel 60 (230-400 mesh) under “flash” conditions. The solvent used in individual chromatographic experiments is indicated and all solvent proportions are given as vol/vol ratios.

Analytical HPLC was performed using a Shimadzu system Prominence-i LC-2030C with a PDA detector. The column used was a Phenomenex Kinetex C18 with a size of 2.10 x 100 mm.

Nuclear Magnetic Resonance (NMR) spectroscopy: NMR spectra were attained for all samples either on a Varian Unity 500 MHz Inova, where $^1$H and $^{13}$C spectra were attained at 500 MHz and 125 MHz respectively, or on a Varian Unity 300 MHz spectrometer, where $^1$H
and $^{13}$C spectra were attained at 300 MHz and 75 MHz respectively, or on a Bruker Ascend 400, where $^1$H and $^{13}$C spectra were attained at 400 MHz and 125 MHz respectively.

The samples were dissolved in deuterated solvents as indicated in brackets. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. Coupling constants ($J$) are given in Hertz. Hydrogen and carbon assignments were performed using gradient correlation spectroscopy (gCOSY), gradient heteronuclear single quantum correlation (gHSQC), gradient heteronuclear multiple bond correlation (gHMBC) and nuclear Overhauser effect spectroscopy (NOESY) techniques. Signals are reported as singlet (s), doublet (d), doublet of doublet (dd), doublet of triplets (dt), triplet (t) and multiplet (m). Diastereomeric peaks are indicated with +. Samples were subjected to high vacuum overnight before performing NMR spectroscopy.

**Mass Spectrometry:** Low resolution direct insertion electron impact mass spectrometry (EI-MS) was performed on a Shimadzu QP-5050A system. The probe was heated from 40 °C to 200 °C. Electrospray ionisation low resolution mass spectrometry (LRMS-ESI) was performed on Waters Platfrom LCZ spectrometer. High resolution electrospray ionisation mass spectra HRESI-MS were attained using a Waters Q-Tof Ultima spectrometer using leucine encephalin (LeuEnk) as the internal standard.
6.1.2. General synthetic procedures

General Procedure A: Preparation of dialkyl α-hydroxyphosphonate esters: Substituted benzaldehyde (1.0 equiv.), diethyl phosphite (1.1 equiv.) and Et₃N (2.2 equiv.) were stirred at room temperature until disappearance of the starting material as monitored by TLC (18 to 26 h). At this time, the reaction mixture was concentrated under reduced pressure, poured into water and extracted with CH₂Cl₂. The organic layers were pooled, washed with a saturated solution of NaHCO₃ and then brine, dried over MgSO₄, filtered and evaporated under reduced pressure. The oily residue was crystallised from Et₂O or purified by flash chromatography if required.

General Procedure B: Preparation of the alkyne building block: The aldehyde (or ketone) derivative (1.0 equiv.) was dissolved in dry THF (20 mL) and added at 0 °C to a solution of ethynylmagnesium bromide in THF (0.5 N in THF, 1.1-1.5 equiv.). After 3 h the reaction mixture was quenched in a mixture of saturated NH₄Cl solution (50 mL) and ice (50 mL). After the evaporation of THF, diethyl ether (50 mL) was added. The organic and water layers were separated and the organic layer was washed with brine (50 mL). After separation of phases the organic layer was dried over anhydrous MgSO₄ and concentrated to dryness. The crude product was used without further purification in the next step.

A solution of the propargylic alcohol derivative (max. 7.3 mmol, 1.0 equiv.), Ac₂O (1.2 equiv.) and Et₃N (1.5 equiv.) in dry CH₂Cl₂ (20 mL) was stirred overnight at room temperature. If necessary a catalytic amount of DMAP was added to achieve total conversion. The CH₂Cl₂ fraction was evaporated with a laboratory evaporator. The resulting crude mixture was purified by silica gel column chromatography.
**General procedure C: Synthesis of 5'-azido-5'-deoxynucleosides:** The 5'-mesyl or 5'-tosyl nucleoside derivative (1.0 equiv.) was dissolved in dry DMF and added NaN₃ (1.5 equiv.) and benzyltriethylammonium chloride (0.1 equiv.) and the solution was stirred at 80 °C until disappearance of the starting material as monitored by TLC. The reaction mixture was poured into crushed ice and extracted with CH₂Cl₂ or EtOAc. The organic layer was washed several times with water and brine, dried over MgSO₄, filtered and evaporated. The residue was purified by flash chromatography.

**General procedure D: Huisgen cycloaddition:** The protected 5'-azidonucleoside (1 equiv.), the alkyne derivative (1.2 equiv.), Cu(OAc)₂ (0.25 equiv.) and sodium ascorbate (0.5 equiv.) were suspended in a mixture of water and CH₃CN (1:4) and stirred at room temperature. After disappearance of the starting material, as indicated by TLC, the reaction mixture was concentrated under reduced pressure and extracted with EtOAc. The organic layer was washed with water and brine, dried over MgSO₄, filtered and evaporated to dryness. The residue was purified by flash chromatography and eluted with CH₂Cl₂/EtOAc (1:2) to EtOAc/Me₂CO (4:1). The expected compounds were attained as a 1:1 diastereomeric mixture which could not be readily separated in these conditions and reported as such.

**General procedure E: Deprotection of the acetate-protected triazolonucleosides:** The protected 5’-triazolonucleoside derivative was taken up in CH₃OH (2-5 mL) and to this solution was added concentrated ammonia (2-3 mL). Stirring was continued until disappearance of the starting material as monitored by TLC. The solvent was evaporated under reduced pressure and coevaporated twice with CH₃OH to ensure the complete removal of water. The residue was purified by flash chromatography.
6.1.3. Experimental for chapter 2

6.1.3.1. Preparation of benzaldehyde derivatives

3-Propoxybenzaldehyde 57

To a solution of commercially available 3-hydroxybenzaldehyde 56 (3.00 g, 24.58 mmol, 1.0 equiv.) and K₂CO₃ (8.68 g, 24.58 mmol, 2.5 equiv.) in CH₃CN (40 mL) was added 1-iodopropane (2.4 mL, 24.58 mmol, 1.0 equiv.). The mixture was stirred for 50 h at room temperature and poured on crushed ice, and was extracted with EtOAc. The organic layers were pooled and washed with water several times and then dried over MgSO₄ to be evaporated under reduced pressure. The residue was purified by flash chromatography column eluted with CH₂Cl₂/hexane [1:1]. 3-Propoxybenzaldehyde 56 was attained as a colourless oil (2.50 g, 62 %). The spectral data matched those reported.²⁸¹ CAS: 67698-61-7.

¹H NMR (300 MHz, CDCl₃): δ 1.05 (t, J = 7.6 Hz, 3H, H-3’), 1.84 (m, 2H, H-2’), 3.99 (t, J = 6.7 Hz, 2H, H-1’), 7.16-7.20 (m, 1H, ArH), 7.39 (m, 1H, ArH), 7.44-7.45 (m, 2H, ArH), 9.97 (s, 1H, CHO). LRMS-ESI: m/z = 187 [M+Na]⁺

5-Bromo-2-methoxybenzaldehyde 60

To a solution of commercially available 5-bromo-2-hydroxybenzaldehyde 58 (1.95 g, 9.68 mmol, 1.0 equiv.) and K₂CO₃ (2.68 g, 19.40 mmol, 2.0 equiv.) in DMF (50 mL) was added CH₃I (0.9 mL, 14.5 mmol, 1.5 equiv.). The mixture was stirred for 42 h at room temperature and then poured on crushed ice. The white solid that formed was removed by filtration, washed with water and air dried. Recrystallisation from Et₂O gave pure 5-bromo-2-
methoxybenzaldehyde 60 as a beige solid (2.04 g, 98 %). m.p. 111-112 °C (lit. 107-109 °C). The spectral data matched those reported. CAS: 25016-01-7. \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 3.39 (s, 3H, OCH\(_3\)), 7.01 (d, \(J = 8.7\) Hz, 1H, ArH), 7.63 (dd, \(J = 2.3\) Hz, \(J = 2.8\) Hz, 1H, ArH), 7.91 (d, \(J = 2.8\) Hz, 1H, ArH), 10.36 (s, 1H, CHO). LRMS-ESI: \(m/z = 213, 215\) [M+H]\(^+\)\(^{79}\)Br; \(^{81}\)Br

2-Methoxynaphthaldehyde 61

CH\(_3\)I (1.1 mL, 17.42 mmol, 1.0 equiv.) was added to a solution of commercially available 2-hydroxy-1-naphthaldehyde 59 (3.00 g, 17.42 mmol, 1.0 equiv.) and K\(_2\)CO\(_3\) (2.41 g, 17.42 mmol, 1.0 equiv.) in DMF (10 mL). Stirring was followed for 30 h at room temperature and the mixture was poured on crushed ice. The dark green solid formed was removed by filtration, washed with water and air dried (2.40 g, 74 %). m.p. 80 °C (lit. 82 °C). The spectral data matched those reported. CAS: 5392-12-1. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 4.05 (s, 3H, OCH\(_3\)), 7.29 (d, \(J = 9.3\) Hz, 1H, ArH), 7.39-7.43 (m, 1H, ArH), 7.59-7.64 (m, 1H, ArH), 7.76-7.78 (m, 1H, ArH), 8.05 (d, \(J = 9.3\) Hz, 1H, ArH), 9.27 (dd, \(J = 8.8\) Hz, \(J = 1.0\) Hz, 1H, ArH), 10.89 (s, 1H, CHO). LRMS-ESI: \(m/z = 209\) [M+Na]\(^+\)

4-Acetoxy-3-methoxybenzaldehyde 63

Commercially available vanillin 62 (2.00 g, 13.14 mmol, 1.0 equiv.) was suspended in CH\(_2\)Cl\(_2\) (20 mL), and Ac\(_2\)O (1.50 mL, 15.77 mmol, 1.2 equiv.) and pyridine (1.30 mL, 15.77 mmol, 1.2 equiv.) were added. The resulting solution was stirred at room temperature for 18 h. The reaction mixture was evaporated and taken up in EtOAc. The organic layer was washed with water, a saturated NaHCO\(_3\) solution, a CuSO\(_4\) solution and brine, and then dried over anhydrous
MgSO₄. The organic layer was filtered, and concentrated under reduced pressure to give pure 4-acetoxy-3-methoxybenzaldehyde 63 as white crystals (2.45 g, 96%). m.p. 75-76 °C (lit. 77-78 °C). The spectral data matched those reported. 

**CAS:** 881-68-5.

**1H NMR** (300 MHz, CDCl₃): δ 2.35 (s, 3H, CH₃), 3.91 (s, 3H, OCH₃), 7.21-7.24 (m, 1H, ArH), 7.47-7.50 (m, 2H, ArH), 9.95 (s, 1H, CHO). 

**LRMS-ESI:** m/z = 195 [M+H]+

### 3-Bromo-4-hydroxy-5-methoxybenzaldehyde 64

A solution of bromine (3.40 mL, 65.72 mmol, 1.0 equiv.) in glacial AcOH (10 mL) was added dropwise to commercially available vanillin 62 (10.00 g, 65.72 mmol, 1.0 equiv.) dissolved in glacial AcOH (60 mL). After stirring for 2.5 h, the reaction mixture was poured on crushed ice and a creamy white solid 64 formed, which was removed by filtration, washed with water and air dried. (14.12 g, 93%). m.p. 162-164 °C (lit. 163-164 °C). The spectral data matched those reported. 

**CAS:** 2973-76-4.

**1H NMR** (300 MHz, DMSO-d₆): δ 3.91 (s, 3H, OCH₃), 7.42 (m, 1H, ArH), 7.72 (m, 1H, ArH), 9.77 (s, 1H, CHO), 10.74 (br s, 1H, OH).

**LRMS-ESI:** m/z = 229, 231 [M]-Br; ²⁷⁹Br, ²⁸¹Br

### 4-Acetoxy-3-bromo-5-methoxybenzaldehyde 65

5-Bromovanillin 64 (2.30 g, 9.95 mmol, 1.0 equiv.) was suspended in CH₂Cl₂ (15 mL), and then Ac₂O (1.1 mL, 11.95 mmol, 1.2 equiv.) and pyridine (1.0 mL, 11.95 mmol, 1.2 equiv.) were added. The resultant solution was stirred at room temperature for 2 days. Water was added to the reaction mixture, which was then extracted with EtOAc. The organic layer was washed with a saturated NaHCO₃ solution, a CuSO₄ solution and brine, and was dried over MgSO₄. The
organic layer was filtered, and concentrated under reduced pressure to give a pale amber oil. Crystallisation from ethanol gave pure 4-acetoxy-3-bromo-5-methoxybenzaldehyde 65 as clear crystals (2.23 g, 82 %). **m.p.** 83-84 °C (lit. 84 °C). The spectral data matched those reported.**284** CAS: 308088-29-1. **1H NMR (500 MHz, CDCl₃):** δ 2.39 (s, 3H, CH₃), 3.91 (s, 3H, OCH₃), 7.42 (d, *J* = 1.9 Hz, 1H, ArH), 7.69 (d, *J* = 1.9 Hz, 1H, ArH), 9.89 (s, 1H, CHO). **LRMS-ESI:** *m/z* = 271, 273 [M+H]+ 79Br; 81Br

3-Bromo-4-hydroxybenzaldehyde 67

To a solution of commercially available 4-hydroxybenzaldehyde 66 (10.00 g, 81.89 mmol, 1.0 equiv.) in a mixture of CHCl₃ (100 mL) and CH₃OH (10 mL) was added a solution of bromine (4.61 mL, 90.07 mmol, 1.1 equiv.) in CHCl₃ (10 mL) dropwise at room temperature. The mixture was stirred for 2 h and washed with water until at neutral pH. The organic phase was dried over MgSO₄ and the solvent was evaporated. Recrystallization from CHCl₃ afforded 3-bromo-4-hydroxybenzaldehyde 67 as a white solid (13.66 g, 83 %). **m.p.** 123 °C (lit. 118-120 °C). The spectral data matched those reported.**447** CAS: 2973-78-6. **1H NMR (300 MHz, DMSO-d₆):** δ 6.31 (s, 1H, OH), 7.08 (d, *J* = 8.3 Hz, 1H, ArH), 7.70 (dd, *J* = 8.3 Hz, *J* = 1.9 Hz, 1H, ArH), 7.97 (d, *J* = 1.9 Hz, 1H, ArH), 9.74 (s, 1H, CHO). **LRMS-ESI:** *m/z* = 199, 201 [M+H]+ 79Br; 81Br

4-Acetoxy-3-bromobenzaldehyde 68

3-Bromo-4-hydroxybenzaldehyde 67 (2.50 g, 12.43 mmol, 1.0 equiv.) was suspended in CH₂Cl₂ (50 mL), and then Ac₂O (1.4 mL, 14.92 mmol, 1.2 equiv.) and pyridine (1.2 mL, 14.92 mmol, 1.2 equiv.) were added. The resultant solution was stirred at room temperature
for 3 days. Water was added to the reaction mixture, which was extracted with EtOAc. The organic layer was washed with a saturated NaHCO₃ aqueous solution, a CuSO₄ solution and brine, and was dried over MgSO₄. The organic layer was filtered, and concentrated under reduced pressure to give pure 4-acetoxy-3-bromobenzaldehyde 68 as amber crystals (2.69 g, 89%). m.p. 57 °C (lit. 58-60 °C). The spectral data matched those reported. CAS: 74849-11-9. ^1H NMR (300 MHz, CDCl₃): δ 2.39 (s, 3H, CH₃), 7.32 (d, J = 8.3 Hz, 1H, ArH), 7.86 (dd, J = 8.3 Hz, J = 1.5 Hz, 1H, ArH), 8.14 (d, J = 1.5 Hz, 1H, ArH), 9.95 (s, 1H, CHO). LRMS-ESI: m/z = 241, 243 [M+H]^+ 79Br, 81Br

4-Methoxy-3-phenoxybenzaldehyde 99

A suspension of commercially available 3-hydroxy-4-methoxybenzaldehyde 98 (300 mg, 1.97 mmol, 1.0 equiv.), phenylboronic acid (265 mg, 2.17 mmol, 1.1 equiv.), Et₃N (997 mg, 9.85 mmol, 5.0 equiv.), anhydrous Cu(OAc)₂ (394 mg, 1.97 mmol, 1.0 equiv.) and 4 Å molecular sieves in CH₂Cl₂ were stirred at room temperature until disappearance of the starting material as monitored by TLC (14 h). At this time, the reaction mixture was concentrated under reduced pressure, poured on water and extracted with EtOAc. The organic layers were pooled, washed with brine, dried over MgSO₄ and evaporated under reduced pressure. The oily residue was purified using a chromatography column and eluted with CH₂Cl₂ to give 4-methoxy-3-phenoxybenzaldehyde 99 as a beige solid (355 mg, 79%). m.p. 48 °C (lit. 49-51 °C). The spectral data matched those reported. CAS: 4664-64-6. ^1H NMR (300 MHz, CDCl₃): δ 3.58 (s, 3H, OCH₃), 6.60-6.62 (m, 2H, ArH), 6.73-6.76 (m, 2H, ArH), 6.94-6.99 (m, 2H, ArH), 7.07 (m, 1H, ArH), 7.27-7.30 (m, 1H, ArH), 10.62 (s, 1H, CHO). LRMS-ESI: m/z = 251 [M+Na]^+
3-(4'-Fluorophenoxy)benzaldehyde 103

A slurry of commercially available 3-hydroxybenzaldehyde 57 (500 mg, 4.09 mmol, 1.0 equiv.), 4-fluorophenylboronic acid (687 mg, 4.91 mmol, 1.2 equiv.), anhydrous Cu(OAc)_2 (163 mg, 0.82 mmol, 0.2 equiv.), and Et_3N (1.7 mL, 12.28 mmol, 3 equiv.) in CH_2Cl_2 (15 mL) was stirred at room temperature for 22 h. At this time, the solution was washed with water, 1 N aqueous HCl and brine before being dried over MgSO_4, filtered and evaporated under reduced pressure. The residue was purified by silica gel chromatography column eluted with Hexane/CH_2Cl_2 [1:2] to give the title compound 103 as a clear oil (690 mg, 78 %). The spectral data matched those reported. CAS: 65295-61-6. ^1H NMR (500 MHz, CDCl_3): δ 7.00-7.09 (m, 4H, ArH), 7.26 (d, J = 8.1 Hz, 1H, ArH), 7.41 (s, 1H, ArH), 7.50 (t, J = 7.8 Hz, 1H, ArH), 7.59 (d, J = 7.3 Hz, 1H, ArH), 9.96 (s, 1H, CHO). LRMS-ESI: m/z = 239 [M+Na]^+

3-Cyclopentoxybenzaldehyde 104

A solution of commercially available 3-hydroxybenzaldehyde 56 (1.50 g, 12.28 mmol, 1.0 equiv.) in anhydrous DMF was added K_2CO_3 (2.54 g, 18.42 mmol, 1.5 equiv.) and the suspension was stirred at room temperature for 1 h before adding cyclopentyl bromide (15 mL, 13.51 mmol, 1.1 equiv.). After 20 h, TLC indicated the disappearance of the starting material and the reaction mixture was poured on crushed ice and the resulting solution was extracted with CH_2Cl_2. The organic layer was then washed with saturated NaHCO_3 and brine and dried over MgSO_4, filtered and evaporated. The residue was purified by flash chromatography and eluted with CH_2Cl_2/Hexane [1:1] to give the title compound 104 as a
pale yellow oil (1.82 g, 78%). The compound is known but the spectral data has not been reported to our knowledge. CAS: 273722-75-1. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 1.64-1.66 (m, 2H, H-3’\(/H-4’_a\)), 1.78-1.88 (m, 4H, H-2’\(/H-3’_a/H-4’_b/H-5’_a\)), 1.94-1.99 (m, 2H, H-2’\(/H-5’_b\)), 4.82-4.85 (m, 1H, H-1’), 6.96 (dd, \(J = 7.3\) Hz, \(J = 2.9\) Hz, 1H, ArH), 7.37 (d, \(J = 1.5\) Hz, 1H, ArH), 7.42-7.43 (m, 2H, ArH), 9.97 (s, 1H, CHO). \textbf{LRMS-ESI:} \(m/z = 191\) [M+H]⁺

3-(Cyclopentoxy)-4-methoxybenzaldehyde - 105

To a solution of commercially available 3-hydroxy-4-methoxybenzaldehyde 98 (800 mg, 5.26 mmol, 1.0 equiv.) in anhydrous DMF was added K\(_2\)CO\(_3\) (1.45 g, 10.52 mmol, 2.0 equiv.) and the suspension was stirred at room temperature for 1 h before adding cyclopentyl bromide (0.68 mL, 6.31 mmol, 1.2 equiv.). After stirring for 20 h, TLC indicated the disappearance of the starting material and the reaction mixture was poured on crushed ice and the resulting solution was extracted with CH\(_2\)Cl\(_2\). The organic layer was then washed with saturated NaHCO\(_3\) and brine and dried over MgSO\(_4\) and evaporated. The residue was purified by flash chromatography eluted with CH\(_2\)Cl\(_2\)/Hexane [1:1] to give the title compound 105 as a pale yellow oil (940 mg, 81 %). The spectral data matched those reported.\(^{451}\) CAS: 67387-76-2. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 1.52-1.64 (m, 2H, H-3’\(/H-4’_a\)), 1.80-2.14 (m, 6H, H-2’\(/H-3’_a/H-4’_b/H-5’_b\)), 3.90 (s, 3H, OCH\(_3\)), 4.85-4.74 (m, 1H, H-1’), 6.96 (d, \(J = 8.1\) Hz, 1H, ArH), 7.33 (d, \(J = 1.7\) Hz, 1H, ArH), 7.39 (dd, \(J = 8.1\) Hz, \(J = 1.7\) Hz, 1H, ArH), 9.80 (s, 1H, CHO). \textbf{LRMS-ESI:} \(m/z = 221\) [M+H]⁺
**tert-Butyl 3-formyl-1H-indole-1-carboxylate 126**

![Chemical Structure](image)

A solution of commercially available indole-3-carboxaldehyde 125 (500 mg, 3.44 mmol, 1.0 equiv.), di-tert-butyl dicarbonate (1.50 g, 6.89 mmol, 2.0 equiv.), and DMAP (84 mg, 0.69 mmol, 0.5 equiv.) in CH$_2$Cl$_2$ (50 mL) was stirred at room temperature for 16 h. A saturated NH$_4$Cl solution (5 mL) was added and the layers were separated. The organic phase was washed with brine (30 mL), dried over MgSO$_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography eluted with EtOAc/Hexane [1:4] to give the 126 as a white powder (642 mg, 76 %); m.p. 120-121°C (lit. 124-126°C). The spectral data matched those reported. CAS: 57476-50-3.  

**Methyl 2-(1H-indol-3-yl)-2-oxoacetate 128**

![Chemical Structure](image)

Oxalyl chloride (0.86 mL, 10 mmol, 1.0 equiv.) was added dropwise to a solution of commercially available 1H-indole 127 (1.17 g, 10 mmol, 1.0 equiv.) in Et$_2$O (12 mL) cooled in an ice bath. The reaction mixture was kept stirring under the same conditions for 30 min. CH$_3$OH (4.7 mL, 23 mmol, 2.3 equiv.) was added dropwise followed by Et$_3$N (1.68 mL, 12 mmol, 1.2 equiv.). The reaction mixture was allowed to warm to room temperature, stirred for an additional 30 min and quenched by adding water (18 mL). The product was isolated by filtration, washed with CH$_2$Cl$_2$ and dried to give 128 as a yellow solid (1.73 g, 87 %). m.p. 232-234 °C (lit. 228 °C). The spectral data matched those reported. CAS: 18372-22-0.
$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 3.97 (s, 3H, OCH$_3$), 7.35-7.38 (m, 2H, ArH), 7.45-7.48 (m, 1H, ArH), 8.45-8.48 (m, 1H, ArH), 8.52 (d, $J = 3.2$ Hz, 1H, ArH), 8.84 (br s, 1H, NH).

LRMS-ESI: $m/z = 226$ [M+Na]$^+$

Methyl 2-(1H-indol-3-yl)-2-oxoacetate – 129

To a solution of methyl 2-(indol-3-yl)-2-oxoacetate 128 (1.35 g, 6.64 mmol, 1.0 equiv.) in THF (12 mL) was added Boc$_2$O (2.90 g, 13.29 mmol, 2.0 equiv.) and DMAP (81 mg, 0.66 mmol, 0.1 equiv.) and stirred at room temperature for 18 h. The mixture was treated with CH$_3$OH (5 mL) and evaporated under reduced pressure. The residue was purified by flash chromatography and eluted with CH$_2$Cl$_2$/Hexane [2:1] to give the title compound 129 as a white solid (1.45 g, 72 %). m.p. 127-128°C (lit. 129-130°C).$^{364}$ The spectral data matched those reported.$^{364}$ CAS: 473927-91-2. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 1.72 (s, 9H, 3 x CH$_3$), 3.99 (s, 3H, CH$_3$), 7.38-7.45 (m, 2H, H-5/H-6), 8.16-8.19 (m, 1H, ArH), 8.39-8.42 (m, 1H, ArH), 8.82 (s, 1H, H-2). LRMS-ESI: $m/z = 294$ [M+H]$^+$
6.1.3.2. Preparation of α-hydroxyphosphonate esters

**Diethyl (4-methyl-α-hydroxybenzyl)phosphonate 69**

![Chemical Structure](image)

This compound was prepared following procedure A, described p. 142, using commercially available 4-tolualdehyde (1.00 g, 8.32 mmol, 1.0 equiv.), diethyl phosphite (1.18 mL, 9.15 mmol, 1.1 equiv.) and Et₃N (2.54 mL, 18.31 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 69 was attained as colourless needles (1.36 g, 78 %). **m.p.** 100-102 °C (lit. 98-100 °C). The spectral data matched those reported.\(^{454}\) CAS: 79158-40-0. \(^1\)H NMR (300 MHz, CDCl₃): δ 1.23 (t, \(J = 6.9\) Hz, 3H, CH₃), 1.29 (t, \(J = 6.9\) Hz, 3H, CH₃), 2.36 (s, 3H, CH₃), 3.19 (br s, 1H, OH), 3.95-4.10 (m, 4H, 2 x CH₂), 4.98 (d, \(J = 10.3\) Hz, 1H, CHOH), 7.17-7.39 (m, 4H, ArH). **LRMS-ESI:** \(m/z = 281 [M+Na]^+\)

**Diethyl (4-ethyl-α-hydroxybenzyl)phosphonate 70**

![Chemical Structure](image)

This compound was prepared following procedure A using commercially available 4-ethylbenzaldehyde (500 g, 3.73 mmol, 1.0 equiv.), diethyl phosphite (4.80 mL, 4.10 mmol, 1.1 equiv.) and Et₃N (1.15 mL, 8.20 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 70 was attained as white needles (964 mg, 95 %). **m.p.** 46-47 °C (lit. oil).\(^{455}\) The spectral data matched those reported.\(^{455}\) CAS: 1397716-66-3. \(^1\)H NMR (500 MHz, CDCl₃): δ 1.22 (t, \(J = 7.5\) Hz, 3H, CH₃), 1.23 (t, \(J = 7.5\) Hz, 3H, CH₃), 1.29 (t, \(J = 7.0\) Hz, 3H, H-2'), 2.65 (q, \(J = 7.0\) Hz, 2H, H-1'), 3.94-4.09 (m, 4H, 2 x CH₂), 4.98 (d, \(J = 10.0\) Hz, 1H, CHOH), 7.19-7.40 (m, 4H, ArH). **LRMS-ESI:** \(m/z = 295 [M+Na]^+\)
Diethyl (α,3-dihydroxybenzyl)phosphonate 71

This compound was prepared following procedure A using commercially available 3-hydroxybenzaldehyde 56 (0.50 g, 4.09 mmol, 1.0 equiv.), diethyl phosphite (1.05 mL, 8.18 mmol, 2.0 equiv.) and Et₃N (2.27 mL, 16.4 mmol, 4.0 equiv.) at room temperature for 18 h. After work up, the title compound 71 was attained as white needles (0.97 g, 92%). m.p. 96-97 °C (lit. 96-97 °C). The spectral data matched those reported. CAS: 50652-89-6.

\(^1\)H NMR (500 MHz, CDCl₃): δ 1.16 (t, J = 7.5 Hz, 3H, CH₃), 1.26 (t, J = 7.5 Hz, 3H, CH₃), 3.81-4.12 (m, 4H, 2 x CH₂), 4.94 (d, J = 11.5 Hz, 1H, CHOH), 6.70-7.25 (m, 4H, ArH). LRMS-ESI: m/z = 283 [M+Na]^+ 

Diethyl (3-methoxy-α-hydroxybenzyl)phosphonate 72

This compound was prepared following procedure A using commercially available 3-methoxybenzaldehyde (500 mg, 3.67 mmol, 1.0 equiv.), diethyl phosphite (1.06 g, 4.04 mmol, 1.1 equiv.) and Et₃N (1.14 mL, 8.08 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 72 was attained as a white solid (967 mg, 96%). m.p. 91-93 °C. The spectral data matched those reported. CAS: 49640-97-3. \(^1\)H NMR (500 MHz, CDCl₃): δ 1.22 (t, J = 7.2 Hz, 3H, CH₃); 1.26 (t, J = 7.2 Hz, 3H, CH₃), 3.83 (s, 3H, OCH₃), 4.12-3.93 (m, 5H, OH & 2 x CH₂), 5.01 (d, J = 10.7 Hz, 1H, CHOH), 6.82-6.88 (m, 1H, ArH), 7.01-7.08 (m, 2H, ArH), 7.24-7.29 (m, 1H, ArH). LRMS-ESI: m/z = 297 [M+Na]^+
Diethyl (3-propoxy-α-hydroxybenzyl)phosphonate 73

This compound was prepared following procedure A using 3-propoxybenzaldehyde (630 mg, 3.84 mmol, 1.0 equiv.), diethyl phosphite (0.54 mL, 4.22 mmol, 1.1 equiv.) and Et₃N (1.19 mL, 8.44 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 73 was attained as a white solid (1.078 g, 93 %). m.p. 103-104 °C. ¹H NMR (400 MHz, DMSO-d₆): δ 1.02 (t, J = 7.3 Hz, 3H, H-3′), 1.22 (t, J = 7.13 Hz, 3H, CH₃), 1.27 (t, J = 7.13 Hz, 3H, CH₃), 1.79 (sxt, J = 6.6 Hz, 2H, H-2′), 3.91 (t, J = 6.6 Hz, 2H, H-1′), 3.96-4.11 (m, 4H, 2xCH₂), 4.99 (d, J = 11.0 Hz, 1H, CHOH), 5.15 (br s, 1H, OH), 6.81-6.85 (m, 1H, ArH), 7.00-7.03 (m, 1H, ArH), 705-7.06 (m, 1H, ArH), 7.23 (dt, J = 8.3 Hz, J = 0.7 Hz, 1H, ArH). ¹³C NMR (125 MHz, CDCl₃): δ 10.2 (C-3′), 16.1 (2xCH₃), 22.3 (C-2′), 62.7 (d, J = 7.7 Hz, CH₂), 63.0 (d, J = 7.7 Hz, CH₂), 69.2 (C-1′), 70.4 (d, J = 159.2 Hz, CHOH), 112.8 (C-2), 114.1 (C-4), 119.1 (C-6), 128.7 (C-5), 138.3 (C-1), 158.8 (C-3). LRMS-ESI: m/z = 325 [M+Na]⁺. HRESI-MS: m/z calcd for C₁₄H₂₃O₅PNa [M+Na]⁺: 325.1181; found 325.1203; Δ = 6.77 ppm

Diethyl (3-phenoxy-α-hydroxybenzyl)phosphonate 74

This compound was prepared following procedure A using commercially available 3-phenoxybenzaldehyde (0.40 g, 2.20 mmol, 1.0 equiv.), diethyl phosphite (0.29 mL, 2.22 mmol, 1.1 equiv.) and Et₃N (0.62 mL, 4.44 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 74 was attained a thick oil (624 mg, 92 %). The compound is known but the spectral data has not been reported to our knowledge. CAS: 85374-19-2. ¹H NMR (400 MHz, CDCl₃): δ 1.22 (t, J = 7.1 Hz, 3H, CH₃), 1.26 (t, J = 7.1 Hz, 3H, CH₃), 3.98-4.11
(m, 4H, 2 x CH₂), 4.76 (br s, 1H, OH), 4.99 (d, J = 11.0 Hz, 1H, CHOH), 6.93-6.96 (m, 1H, ArH), 6.98-7.02 (m, 2H, ArH), 7.07-7.11 (m, 1H, ArH), 7.16-7.17 (m, 1H, ArH), 7.21-7.24 (m, 1H, ArH), 7.29-7.35 (m, 3H, ArH). LRMS-ESI: m/z = 359 [M+Na]⁺

Diethyl (α-hydroxy-3-nitrobenzyl)phosphonate 75

![Diagram of diethyl (α-hydroxy-3-nitrobenzyl)phosphonate 75]

This compound was prepared following procedure A using commercially available 3-nitrobenzaldehyde (500 mg, 3.31 mmol, 1.0 equiv.), diethyl phosphite (0.47 mL, 3.64 mmol, 1.1 equiv.) and Et₃N (0.94 mL, 7.28 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 75 was attained as white needles (880 mg, 94 %). m.p. 89-90 °C (lit. 94-95 °C).⁴⁵⁷ The spectral data matched those reported.⁴⁵⁷ CAS: 50652-91-0. ¹H NMR (500 MHz, CDCl₃): δ = 1.27 (t, J = 7.5 Hz, 3H, CH₃), 1.29 (t, J = 7.5 Hz, 3H, CH₃), 4.09-4.15 (m, 4H, 2 x CH₂), 5.15 (d, J = 11.5 Hz, 1H, CHOH), 7.53-8.38 (m, 4H, ArH). LRMS-ESI: m/z = 312 [M+Na]⁺

Diethyl (2-fluoro-α-hydroxybenzyl)phosphonate 76

![Diagram of diethyl (2-fluoro-α-hydroxybenzyl)phosphonate 76]

This compound was prepared following procedure A using commercially available 2-fluorobenzaldehyde (500 mg, 4.03 mmol, 1.0 equiv.), diethyl phosphite (0.57 mL, 4.43 mmol, 1.1 equiv.) and Et₃N (1.24 mL, 8.86 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 76 was attained as white needles (972 mg, 92 %). m.p. 62-63 °C. ¹H NMR (500 MHz, CDCl₃): δ = 1.21 (t, J = 7.0 Hz, 3H, CH₃), 1.31 (t, J = 7.0 Hz, 3H, CH₃), 4.04-4.17 (m, 4H, 2 x CH₂), 5.38 (d, J = 11.5 Hz, 1H, CHOH), 7.02-7.68 (m, 4H, ArH). ¹³C NMR (125 MHz, CDCl₃): δ = 16.1 (CH₃), 16.2 (CH₃), 63.1 (d, J = 7.6 Hz, CH₂), 63.4 (d, J = 7.6 Hz, CH₂), 64.1 (d, J = 164.4 Hz, CHOH), 114.9 (d, J = 22.0 Hz, ArCH), 124.1 (3 d,
$J = 2.5 \text{ Hz, ArCH}$, 128.9, 129.5, 124.6 (d, $J = 13.4 \text{ Hz, C-1}$), 159.8 (d, $J = 245.6 \text{ Hz, C-2}$).

**LRMS-ESI:** $m/z = 285 [\text{M+Na}]^+$. **HRESI-MS:** $m/z$ calcd for C$_{11}$H$_{18}$FO$_4$P [M+H]$^+$: 263.0849; found 263.0847; $\Delta = 0.76 \text{ ppm}$

**Diethyl (3-fluoro-α-hydroxybenzyl)phosphonate 77**

![Chemical Structure Image]

This compound was prepared following procedure A using commercially available 4-fluorobenzaldehyde (500 mg, 4.03 mmol, 1.0 equiv.), diethyl phosphite (0.57 mL, 4.43 mmol, 1.1 equiv.) and Et$_3$N (1.24 mL, 8.86 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 77 was attained as white needles (1.003 g, 95 %). **m.p.** 58-59 °C (lit. oil).$^{455}$ The spectral data matched those reported.$^{455}$ **CAS:** 96258-60-5. **$^1$H NMR (500 MHz, CDCl$_3$):** $\delta$ 1.25 (t, $J = 7.5 \text{ Hz, 3H, CH}_3$), 1.28 (t, $J = 7.5 \text{ Hz, 3H, CH}_3$), 4.02-4.12 (m, 4H, 2x CH$_2$), 5.02 (d, $J = 11 \text{ Hz, 1H, CHOH}$), 6.98-7.35 (m, 4H, ArH). **LRMS-ESI:** $m/z = 285 [\text{M+Na}]^+$

**Diethyl (4-fluoro-α-hydroxybenzyl)phosphonate 78**

![Chemical Structure Image]

This compound was prepared following procedure A using commercially available 4-fluorobenzaldehyde (500 mg, 4.03 mmol, 1.0 equiv.), diethyl phosphite (0.57 mL, 4.43 mmol, 1.1 equiv.) and Et$_3$N (1.24 mL, 8.86 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 78 was attained as a white solid (993 mg, 94 %). **m.p.** 56-57 °C (lit. 55-57 °C).$^{455}$ The spectral data matched those reported.$^{455}$ **CAS:** 21386-00-5. **$^1$H NMR (500 MHz, CDCl$_3$):** $\delta$ 1.24 (t, $J = 7.5 \text{ Hz, 3H, CH}_3$), 1.27 (t, $J = 7.5 \text{ Hz, 3H, CH}_3$), 4.02-4.07 (m, 4H, 2x CH$_2$), 5.02 (d, $J = 10.5 \text{ Hz, 1H, CHOH}$), 7.05-7.48 (m, 4H, ArH). **LRMS-ESI:** $m/z = 285 [\text{M+Na}]^+$
Diethyl (3-bromo-α-hydroxybenzyl)phosphonate 79

![Chemical structure of Diethyl (3-bromo-α-hydroxybenzyl)phosphonate 79]

This compound was prepared following procedure A using commercially available 3-bromo-benzaldehyde (800 mg, 4.32 mmol, 1.0 equiv.), diethyl phosphite (0.61 mL, 4.76 mmol, 1.1 equiv.) and Et₃N (1.34 mL, 9.51 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 79 was attained as a colourless oil (1.271 g, 91 %). The spectral data matched those reported. CAS: 73584-08-4. ¹H NMR (300 MHz, CDCl₃): δ 1.26 (t, J = 7.0 Hz, 3H, CH₃), 1.29 (t, J = 7.0 Hz, 3H, CH₃), 3.93 (br s, 1H, OH), 4.03-4.11 (m, 4H, 2 x CH₂), 5.00 (dd, J = 11.1 Hz, J = 5.0 Hz, 1H, CHOH), 7.24 (t, J = 7.7 Hz, 1H, ArH), 7.41-7.46 (m, 2H, ArH), 7.67 (s, 1H, ArH). LRMS-ESI: m/z = 321, 323 [M+Na]+ ⁷⁹Br; ⁸¹Br

Diethyl (hydroxy(naphthalen-2-yl)methyl)phosphonate 80

![Chemical structure of Diethyl (hydroxy(naphthalen-2-yl)methyl)phosphonate 80]

This compound was prepared following procedure A using commercially available naphthaldehyde (600 mg, 3.84 mmol, 1.0 equiv.), diethyl phosphite (0.54 mL, 4.22 mmol, 1.1 equiv.) and Et₃N (1.17 mL, 8.45 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 80 was attained as white needles (972 mg, 86 %). m.p. 83-84 °C (lit. 88-89 °C). The spectral data matched those reported. CAS: 1090-99-9. ¹H NMR (400 MHz, CDCl₃): δ 1.21 (t, J = 7.1 Hz, 3H, CH₃), 1.26 (t, J = 7.1 Hz, 3H, CH₃), 3.97-4.11 (m, 4H, 2 x CH₂), 5.21 (d, J = 13.9 Hz, 1H, CHOH), 7.46-7.50 (m, 2H, ArH), 7.61 (dt, J = 8.6 Hz, J = 1.7 Hz, 1H, ArH), 7.82-7.85 (m, 3H, ArH), 7.96-7.97 (m, 1H, ArH). LRMS-ESI: m/z = 317 [M+Na]+
Diethyl (hydroxy(2-methoxynaphthalen-1-yl)methyl)phosphonate 81

This compound was prepared following procedure A using 2-methoxynaphthaldehyde 61 (1.00 g, 5.37 mmol, 1.0 equiv.), diethyl phosphite (0.76 mL, 5.91 mmol, 1.1 equiv.) and Et₃N (1.66 mL, 11.81 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 81 was attained as a beige solid (975 mg, 56%). m.p. 98-99 °C. ¹H NMR (300 MHz, DMSO-d₆): δ 0.96 (t, J = 7.3 Hz, 3H, CH₃), 1.22 (t, J = 7.3 Hz, 3H, CH₃), 3.64-3.81 (m, 2H, CH₂), 3.92 (s, 3H, OCH₃), 4.00-4.06 (m, 2H, CH₂), 5.99 (dd, J = 18.1 Hz, J = 5.4 Hz, 1H, CHOH), 6.21 (dd, J = 17.1 Hz, J = 5.9 Hz, 1H, OH), 7.30-7.34 (m, 1H, ArH), 7.37-7.43 (m, 2H, ArH), 7.80 (d, J = 8.3 Hz, 1H, ArH), 7.89 (dd, J = 9.3 Hz, J = 1.9 Hz, 1H, ArH), 8.72 (d, J = 8.8 Hz, 1H, ArH). ¹³C NMR (125 MHz, DMSO-d₆): δ 16.0 (d, J = 5.8 Hz, CH₃), 16.3 (d, J = 0.9 Hz, CH₃), 56.7 (OCH₃), 61.5 (d, J = 6.6 Hz, CH₂), 61.8 (d, J = 6.6 Hz, CH₂), 63.2 (d, J = 168.7 Hz, CHOH), 113.4 (d, J = 2.2 Hz, ArCH), 119.0 (ArC), 123.3 (ArCH), 125.0 (ArCH), 127.6 (ArCH), 127.8 (ArCH), 129.1 (d, J = 2.2 Hz, ArC), 129.9 (d, J = 3.7 Hz, C-3), 132.5 (d, J = 2.9 Hz, ArC), 154.2 (d, J = 8.8 Hz, ArC). LRMS-ESI: m/z = 325 [M+H]⁺. HRESI-MS: m/z calcd for C₁₆H₂₁O₅PNa [M+Na]⁺: 347.1024; found 347.1033; Δ = 2.59 ppm

Diethyl (3,4-dimethoxy-α-hydroxybenzyl)phosphonate 82

This compound was prepared following procedure A using commercially available 3,4-dimethoxybenzaldehyde (300 mg, 1.80 mmol, 1.0 equiv.), diethyl phosphite (0.26 mL, 1.98 mmol, 1.1 equiv.) and Et₃N (0.56 mL, 3.97 mmol, 2.2 equiv.) at room temperature for
18 h. After work up, the title compound 82 was attained as white needles (505 mg, 86 %). m.p. 100 °C (lit. 101-103 °C). The spectral data matched those reported.\(^{458}\) CAS: 62002-64-6. 

\(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 1.14 (t, \(J = 7.2\) Hz, 3H, CH\(_3\)); 1.18 (t, \(J = 7.2\) Hz, 3H, CH\(_3\)), 3.73 (s, 3H, OCH\(_3\)), 3.74 (s, 3H, OCH\(_3\)), 3.81-4.02 (m, 4H, 2 x CH\(_2\)), 4.84 (dd, \(J = 12.4\) Hz, \(J = 5.5\) Hz, 1H, OH), 6.09 (d, \(J = 5.8\) Hz, 1H, CHO), 6.89-6.96 (m, 2H, ArH), 7.03 (m, 1H, ArH). LRMS-ESI: \(m/z = 327\) [M+Na]\(^+\)

**Diethyl (3,5-dimethoxy-\(\alpha\)-hydroxybenzyl)phosphonate 83**

![Diagram of the compound 83](image)

This compound was prepared following procedure A using commercially available 3,5-dimethoxybenzaldehyde (600 mg, 3.61 mmol, 1.0 equiv.), diethyl phosphite (0.51 mL, 3.96 mmol, 1.1 equiv.) and Et\(_3\)N (1.12 mL, 7.94 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 83 was attained as a white solid (923 mg, 84 %). m.p. 99-100 °C (lit. 101-103 °C).\(^{459}\) The spectral data matched those reported.\(^{459}\) CAS: 266000-71-9. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 1.24 (t, \(J = 6.9\) Hz, 3H, CH\(_3\)); 1.28 (t, \(J = 6.9\) Hz, 3H, CH\(_3\)), 3.78 (s, 6H, 2 x OCH\(_3\)), 4.00 (q, \(J = 7.6\) Hz, 2H, CH\(_2\)), 4.08 (q, \(J = 7.6\) Hz, 2H, CH\(_2\)), 4.95 (d, \(J = 11.2\) Hz, 1H, CHO), 6.40-6.67 (m, 3H, ArH). LRMS-ESI: \(m/z = 327\) [M+Na]\(^+\)

**Diethyl (2,5-dimethoxy-\(\alpha\)-hydroxybenzyl)phosphonate 84**

![Diagram of the compound 84](image)

This compound was prepared following procedure A using commercially available 2,5-dimethoxybenzaldehyde (800 mg, 4.71 mmol, 1.0 equiv.), diethyl phosphite (0.68 mL, 5.29 mmol, 1.1 equiv.) and Et\(_3\)N (1.49 mL, 10.60 mmol, 2.2 equiv.) at room temperature for
18 h. After work up, the title compound 84 was attained as white needles (1.392 g, 95 %). m.p. 74-75 °C. The compound is commercially available but the spectral data has not been reported to our knowledge. CAS: 430471-09-3. 1H NMR (500 MHz, CDCl3): δ 1.20 (t, J = 6.9 Hz, 3H, CH3), 1.32 (t, J = 6.9 Hz, 3H, CH3), 3.59 (br s, 1H, OH), 3.78 (s, 3H, OCH3), 3.82 (s, 3H, OCH3), 3.91-4.07 (m, 2H, CH2), 4.12-4.17 (m, 2H, CH2), 5.37 (d, J = 12.1 Hz, 1H, CHOH), 6.83 (m, 2H, ArH), 7.11 (m, 1H, ArH). LRMS-ESI: m/z = 327 [M+Na]+

Diethyl (4-acetoxy-3-methoxy-α-hydroxybenzyl)phosphonate 85

This compound was prepared following procedure A using 4-acetoxy-3-methoxy-benzaldehyde 63 (400 mg, 2.06 mmol, 1.0 equiv.), diethyl phosphite (0.3 mL, 2.26 mmol, 1.1 equiv.) and Et3N (0.6 mL, 4.53 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 85 was attained as a beige solid (685 mg, 66 %). m.p. 84 °C. 1H NMR (300 MHz, CDCl3): δ 1.23 (t, J = 7.1 Hz, 3H, CH3), 1.30 (t, J = 7.1 Hz, 3H, CH3), 2.31 (s, 3H, Ac), 3.50 (br s, 1H, OH), 3.90 (s, 3H, OCH3), 3.93-4.12 (m, 4H, 2 x CH2), 4.92 (d, J = 9.9 Hz, 1H, CHOH), 6.88-6.90 (m, 1H, ArH), 6.93-6.96 (m, 1H, ArH), 7.09 (t, J = 2.0 Hz, 1H, ArH). 13C NMR (75 MHz, CDCl3): δ 16.4 (2 x CH3), 56.0 (OCH3), 63.1 (d, J = 7.4 Hz, CH2), 63.2 (d, J = 7.4 Hz, CH2), 70.7 (d, J = 160.2 Hz, CHOH), 109.8 (d, J = 5.4 Hz, C-2), 114.2 (C-5), 120.4 (d, J = 7.4 Hz, C-6), 128.1 (C-1), 145.8 (C-4), 146.6 (C-3), 169.8 (C=O). LRMS-ESI: m/z = 355 [M+Na]+. HRESI-MS: m/z calcd for C14H21O7PNa [M+Na]+: 355.0923; found 355.0935; Δ = 3.38 ppm
Diethyl 4-acetoxy-3-bromo-5-methoxy-α-hydroxybenzylphosphonate 86

This compound was prepared following procedure A using 4-acetoxy-3-bromo-5-methoxybenzaldehyde 65 (500 mg, 1.83 mmol, 1.0 equiv.), diethyl phosphite (0.26 mL, 2.01 mmol, 1.1 equiv.) and Et$_3$N (0.57 mL, 4.03 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 86 was attained as a white solid (708 mg, 94 %). m.p. 141 °C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.27 (t, $J = 6.9$ Hz, 3H, CH$_3$), 1.30 (t, $J = 6.9$ Hz, 3H, CH$_3$), 2.36 (s, 3H, Ac), 3.84 (s, 3H, OCH$_3$), 4.05-4.12 (m, 4H, 2 x CH$_2$), 4.96 (d, $J = 11.0$ Hz, 1H, CHOH), 7.11 (s, 1H, H-2), 7.30 (s, 1H, H-6). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 16.3 (CH$_3$), 16.4 (CH$_3$), 20.4 (Ac-CH$_3$), 56.3 (OCH$_3$), 63.3 (d, $J = 6.9$ Hz, CH$_2$), 63.6 (d, $J = 6.9$ Hz, CH$_2$), 69.9 (d, $J = 160.1$ Hz, CHOH), 110.1 (d, $J = 4.6$ Hz, C-2), 116.8 (d, $J = 3.4$ Hz, C-5), 122.7 (d, $J = 6.9$ Hz, C-6), 136.3 (C-1), 146.0 (C-4), 152.2 (d, $J = 2.3$ Hz, C-3), 167.9 (C=O).

LRMS-ESI: $m/z$ = 433, 435 [M+Na]$^+$; $^{81}$Br, HRESI-MS: $m/z$ calcd for C$_{14}$H$_{20}$BrO$_7$PNa [M+H]$^+$: 433.0028; found 433.0026; $\Delta = 0.46$ ppm

Diethyl (2,5-dimethoxy-α-hydroxybenzyl)phosphonate 87

This compound was prepared following procedure A using 5-bromo-2-methoxybenzaldehyde 60 (600 mg, 2.79 mmol, 1.0 equiv.), diethyl phosphite (0.4 mL, 3.07 mmol, 1.1 equiv.) and Et$_3$N (0.86 mL, 6.14 mmol, 2.2 equiv.) at room temperature for 18 h. After work up, the title compound 87 was attained as white needles (670 mg, 68 %). m.p. 94-95 °C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 1.23 (t, $J = 7.0$ Hz, 3H, CH$_3$), 1.29 (t, $J = 7.0$ Hz, 3H, CH$_3$), 3.83 (s, 3H, OCH$_3$), 3.98-4.15 (m, 4H, 2 x CH$_2$), 5.14 (br s., 1H, OH), 5.39 ($J = 11.7$ Hz, 1H, CHOH), 5.79 (s, 3H, OCH$_3$).
6.75 (d, J = 8.8 Hz, 1H, H-3), 7.37 (dt, J = 8.8 Hz, J = 2.2 Hz, 1H, H-6), 7.66 (t, J = 2.2 Hz, 1H, H-4).  $^{13}$C NMR (125 MHz, CDCl$_3$): δ 16.2 (d, J = 5.9 Hz, CH$_3$), 16.3 (d, J = 5.9 Hz, CH$_3$), 55.8 (OCH$_3$), 63.0 (d, J = 7.3 Hz, CH$_2$), 63.3 (d, J = 7.3 Hz, CH$_2$), 64.8 (d, J = 160.7 Hz, C-CHOH), 112.2 (C-3), 113.1 (d, J = 3.7 Hz, C-5), 127.6 (d, J = 1.5 Hz, C-1), 131.3 (d, J = 4.4 Hz, C-4), 131.7 (d, J = 3.7 Hz, C-6), 155.7 (d, J = 5.9 Hz, C-2). LRMS-ESI: m/z = 375, 377 [M+Na]$^+$; $^{79}$Br, $^{81}$Br. HRESI-MS: m/z calcd for C$_{12}$H$_{18}$BrO$_5$PNa [M+Na]$^+$: 374.9973; found 374.9991; Δ = 4.80 ppm

6.1.3.3. Preparation of alkyne derivatives

1-(4-Methylphenyl)prop-2-yn-1-yl acetate 95

This compound was prepared following procedure B, described p. 142, using commercially available 4-methylbenzaldehyde (250 mg, 2.08 mmol, 1.0 equiv.) and ethynylmagnesium bromide (5.00 mL of a 0.5 M solution in THF, 2.50 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH$_2$Cl$_2$ and treated with Et$_3$N (0.44 mL, 3.12 mmol, 1.5 equiv.) and Ac$_2$O (0.24 mL, 2.50 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 95 was attained as an amber oil (352 mg, 90%). The spectral data matched those reported.$^{346}$ CAS: 179248-90-9. $^1$H NMR (300 MHz, CDCl$_3$): δ 2.10 (s, 3H, Ac), 2.36 (s, 3H, CH$_3$), 2.68 (s, 1H, H-3), 6.42 (s, 1H, H-1), 7.21-7.24 (m, 4H, ArH).
Diethyl (3-(4′-methylphenyl)-propa-1,2-dien-1-yl)phosphonate 97

To a solution of 1-(4-methylphenyl)propargyl acetate 95 (60 mg, 319 µmol, 1.0 equiv.), diethyl phosphite (48 mg, 350 µmol, 1.1 equiv.) and Et₃N (50 µL, 382 µmol, 1.2 equiv.) in CH₂Cl₂ (5 mL) was added CuI (6.1 mg, 32 µmol, 10 mol%) and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was diluted with CH₂Cl₂ (20 mL) and washed with water, saturated NaHCO₃ and brine before being dried over MgSO₄ and evaporated under reduced pressure. The residue was purified by flash chromatography and eluted with CH₂Cl₂/Hexane [1:2] to give the title compound 97 as a pale yellow oil (66 mg, 78%). The spectral data matched those reported.³⁵⁰ CAS: 201932-99-2. ¹H NMR (400 MHz, CDCl₃): δ 1.28 (t, J = 7.1 Hz, 3H, CH₃), 1.31 (t, J = 7.1 Hz, 3H, CH₃), 2.30 (s, 3H, CH₃), 4.07-4.16 (m, 4H, CH₂), 5.77 (d, J = 6.8 Hz, 1H, H-3), 6.42 (dd, J = 13.6 Hz, J = 6.8 Hz, 1H, H-1), 7.11-7.17 (m, 4H, ArH). ¹³C NMR (125 MHz, CDCl₃): δ 16.2 (d, J = 5.8 Hz, CH₃). 21.2 (CH₃), 62.3 (d, J = 4.6 Hz, CH₂), 62.5 (d, J = 4.1 Hz, CH₂), 84.1 (d, J = 192.4 Hz, C-1), 95.3 (d, J = 16.3 Hz, C-3), 127.0 (d, J = 2.3 Hz, C-2′/C-6′), 128.2 (d, J = 8.6 Hz, C-3′/C-5′), 129.2 (d, J = 1.3 Hz, C-1′), 136.9 (d, J = 2.4 Hz, C-4′), 212.6 (d, J = 1.2 Hz, C-2). LRMS-ESI: m/z = 266 [M+H]⁺

1-(3′-Methoxyphenyl)prop-2-yn-1-ol 106

This compound was prepared following procedure B using commercially available 3-methoxybenzaldehyde (450 mg, 3.30 mmol, 1.0 equiv.) and ethynylmagnesium bromide (7.93 mL of a 0.5 M solution in THF, 3.97 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH₂Cl₂ and treated with Et₃N (0.70 mL,
496 mmol, 1.5 equiv.) and Ac₂O (0.37 mL, 3.97 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 106 was attained as an amber oil (628 mg, 93 %). The spectral data matched those reported.³⁴₆ CAS: 179249-08-2. ¹H NMR (500 MHz, CDCl₃): δ 2.11 (s, 3H, Ac), 2.66 (s, 1H, H-3), 3.81 (s, 3H, OCH₃), 6.42 (s, 1H, H-1), 6.89 (d, J = 8.3 Hz, 1H, ArH), 7.06-7.13 (m, 2H, ArH), 7.30 (t, J = 7.9 Hz, 1H, ArH).

1-(2’,5’-Dimethoxyphenyl)prop-2-yn-1-ol 107

![Structure of 1-(2’,5’-Dimethoxyphenyl)prop-2-yn-1-ol](image)

This compound was prepared following procedure B using commercially available 2,5-dimethoxybenzaldehyde (580 mg, 3.49 mmol, 1.0 equiv.) and ethynylmagnesium bromide (8.4 mL of a 0.5 M solution in THF, 4.19 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH₂Cl₂ and treated with Et₃N (0.7 mL, 5.23 mmol, 1.5 equiv.) and Ac₂O (0.4 mL, 4.19 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 107 was attained as an amber oil (728 mg, 89 %). ¹H NMR (500 MHz, CDCl₃): δ 2.23 (s, 3H, Ac), 2.60 (d, J = 2.0 Hz, 1H, H-3), 3.81 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃), 6.76 (d, J = 2.0 Hz, 1H, H-1), 6.83-6.85 (m, 1H, ArH), 6.88-6.90 (m, 1H, ArH), 7.25 (d, J = 3.2 Hz, 1H, ArH). ¹³C NMR (125 MHz, CDCl₃): δ 22.1 (CH₃), 55.8 (OCH₃), 56.3 (OCH₃), 63.5 (C-1), 74.6 (C-3), 80.3 (C-2), 112.2 (C-3’), 114.6 (C-4’), 115.1 (C-6’), 125.7 (C-1’), 150.9 (C-2’), 153.7 (C-5’), 169.5 (C=O). IR (Neat) νmax 3286, 2948, 1739, 1499, 1214, 967, 711 cm⁻¹
1-(3’-Propoxyphenyl)prop-2-yn-1-ol 108

This compound was prepared following procedure B using 3-propoxybenzaldehyde 56 (400 mg, 2.43 mmol, 1.0 equiv.) and ethynylmagnesium bromide (5.8 mL of a 0.5 M solution in THF, 2.92 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH₂Cl₂ and treated with Et₃N (0.5 mL, 3.65 mmol, 1.5 equiv.) and Ac₂O (0.3 mL, 2.92 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 108 was attained as an amber oil (520 mg, 92 %). ¹H NMR (500 MHz, CDCl₃): δ 1.23 (t, J = 7.3 Hz, 3H, H-3”), 2.00 (sxt, J = 7.3 Hz, 2H, H-2”), 2.30 (s, 3H, Ac), 2.85 (s, 1H, H-3), 4.12 (t, J = 6.8 Hz, 2H, H-1”), 6.61 (s, 1H, H-1), 7.08 (d, J = 7.8 Hz, 1H, H-2’), 7.27-7.29 (m, 2H, H-4'/H-6’), 7.47 (t, J = 7.8 Hz, 1H, H-5”). ¹³C NMR (125 MHz, CDCl₃): δ 10.4 (C-3”), 20.9 (C-2”), 22.4 (CH₃), 65.1 (C-1”), 69.4 (C-1), 75.3 (C-3), 80.1 (C-2), 113.7 (C-2’), 115.0 (C-4”), 119.6 (C-6’), 129.6 (C-5”), 137.7 (C-1’), 159.2 (C-3’), 169.6 (C=O).

1-(3’-Cyclopentoxyphenyl)prop-2-yn-1-ol 109

This compound was prepared following procedure B using 3-cyclopentoxybenzaldehyde 102 (600 mg, 3.15 mmol, 1.0 equiv.) and ethynylmagnesium bromide (7.6 mL of a 0.5 M solution in THF, 3.78 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH₂Cl₂ and treated with Et₃N (0.7 mL, 4.73 mmol, 1.5 equiv.) and Ac₂O (0.4 mL, 3.78 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 109 was attained as a clear oil (730 mg, 89 %). ¹H NMR (500 MHz, CDCl₃): δ 1.61-1.64 (m, 2H, H-3”/H-4”a), 1.78-1.94 (m, 6H, H-2”/H-3”a/H-4”b/H-5”), 2.12 (s, 3H, Ac), 2.64 (d, J = 1.9 Hz, 1H, H-3), 4.76-4.79 (m, 1H, H-1”), 6.40 (d, J = 1.9 Hz, 1H, H-1),
6.86 (dd, J = 8.3 Hz, J = 2.4 Hz, 1H, ArH), 7.05-7.07 (m, 2H, ArH), 7.26-7.29 (m, 1H, ArH).

\[ ^{13}C\text{ NMR (125 MHz, CDCl}_3\text{): } \delta 21.0\ (\text{CH}_3), 24.0\ (\text{C-3''/C-4''}), 32.8\ (\text{C-2''/C-5''}), 65.2\ (\text{C-1}), 75.2\ (\text{C-3}), 79.3\ (\text{C-1'''}, 80.2\ (\text{C-2}), 114.9\ (\text{C-2'}), 116.0\ (\text{C-4'}), 119.4\ (\text{C-6'}), 129.6\ (\text{C-5'}), 137.7\ (\text{C-1'}), 158.3\ (\text{C-3'}), 169.7\ (\text{C=O}). \]

\[ ^{1}H\text{ NMR (400 MHz, CDCl}_3\text{): } \delta 1.77-1.97\ (m, 8H, \text{H-2''/H-3''/H-4''/H-5''}), 2.10\ (s, 3H, \text{Ac}), 2.65\ (d, J = 2.3 Hz, 1H, H-3), 3.85\ (s, 3H, \text{OCH}_3), 4.78-4.81\ (m, 1H, \text{CH-1''}), 6.38\ (d, J = 2.3 Hz, 1H, H-1), 6.85-6.87\ (m, 1H, ArH), 7.06-7.08\ (m, 2H, ArH). \]

\[ ^{13}C\text{ NMR (125 MHz, CDCl}_3\text{): } \delta 21.0\ (\text{Ac-CH}_3), 24.0\ (\text{C-3''/C-4''}), 32.8\ (\text{C-2''/C-5''}), 56.1\ (\text{OCH}_3), 63.4\ (\text{C-1}), 65.3\ (\text{C-1'''}, 75.0\ (\text{C-3}), 80.5\ (\text{C-1'}), 80.6\ (\text{C-2}), 111.7\ (\text{C-2'}), 114.6\ (\text{C-5'}), 120.4\ (\text{C-6'}), 128.9\ (\text{C-1''}, 147.8\ (\text{C-3'}), 150.7\ (\text{C-4'}), 169.7\ (\text{C=O}). \]

**1-(3'-Cyclopentoxy-4'-methoxyphenyl)prop-2-yn-1-ol 110**

This compound was prepared following procedure **B** using 3-cyclopentoxy-4-methoxybenzaldehyde 103 (500 mg, 2.27 mmol, 1.0 equiv.) and ethynylmagnesium bromide (5.5 mL of a 0.5 M solution in THF, 2.72 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH$_2$Cl$_2$ and treated with Et$_3$N (0.5 mL, 3.40 mmol, 1.5 equiv.) and Ac$_2$O (0.26 mL, 2.72 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 110 was attained as an orange oil (595 mg, 91 %). \[ ^{1}H\text{ NMR (400 MHz, CDCl}_3\text{): } \delta 1.77-1.97\ (m, 8H, \text{H-2''/H-3''/H-4''/H-5''}), 2.10\ (s, 3H, \text{Ac}), 2.65\ (d, J = 2.3 Hz, 1H, H-3), 3.85\ (s, 3H, \text{OCH}_3), 4.78-4.81\ (m, 1H, \text{CH-1''}), 6.38\ (d, J = 2.3 Hz, 1H, H-1), 6.85-6.87\ (m, 1H, ArH), 7.06-7.08\ (m, 2H, ArH). \]

\[ ^{13}C\text{ NMR (125 MHz, CDCl}_3\text{): } \delta 21.0\ (\text{Ac-CH}_3), 24.0\ (\text{C-3''/C-4''}), 32.8\ (\text{C-2''/C-5''}), 56.1\ (\text{OCH}_3), 63.4\ (\text{C-1}), 65.3\ (\text{C-1'''}, 75.0\ (\text{C-3}), 80.5\ (\text{C-1'}), 80.6\ (\text{C-2}), 111.7\ (\text{C-2'}), 114.6\ (\text{C-5'}), 120.4\ (\text{C-6'}), 128.9\ (\text{C-1''}, 147.8\ (\text{C-3'}), 150.7\ (\text{C-4'}), 169.7\ (\text{C=O}). \]

**1-(3'-Phenoxyphenyl)prop-2-yn-1-yl acetate 111**

This compound was prepared following procedure **B** using commercially available 3-phenoxybenzaldehyde (500 mg, 2.52 mmol, 1.0 equiv.) and ethynylmagnesium bromide.
(6.05 mL of a 0.5 M solution in THF, 3.03 mmol, 1.2 equiv.) at room temperature for 4 h. After extraction, the residue was resuspended in CH$_2$Cl$_2$ and treated with Et$_3$N (0.53 mL, 3.78 mmol, 1.5 equiv.) and Ac$_2$O (0.29 mL, 3.03 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 111 was attained as an amber oil (598 mg, 89%). The spectral data matched those reported.\textsuperscript{346} CAS: 83807-99-2. \textsuperscript{1}H NMR (500 MHz, CDCl$_3$): $\delta$ 2.11 (s, 3H, Ac), 2.63 (d, $J$ = 2.2 Hz, 1H, H-3), 6.41 (d, $J$ = 2.2 Hz, 1H, H-1), 6.97 (dd, $J$ = 11.3 Hz, $J$ = 5.7 Hz, 1H, ArH), 7.02 (d, $J$ = 7.7 Hz, 2H, ArH), 7.12 (t, $J$ = 7.4 Hz, 1H, ArH), 7.21-7.20 (m, 1H, ArH), 7.25 (d, $J$ = 8.2 Hz, 1H, ArH), 7.34 (dd, $J$ = 15.4 Hz, $J$ = 7.6 Hz, 3H, ArH).

1-(4′-Methoxy-3′-phenoxyphenyl)prop-2-yn-1-yl acetate 112

This compound was prepared following procedure B using 4-methoxy-3-phenoxybenzaldehyde 99 (500 mg, 2.19 mmol, 1.0 equiv.) and ethynylmagnesium bromide (5.3 mL of a 0.5 M solution in THF, 2.63 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH$_2$Cl$_2$ and treated with Et$_3$N (0.35 mL, 2.66 mmol, 1.5 equiv.) and Ac$_2$O (0.25 mL, 2.63 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 112 was attained as an amber oil (539 g, 83%). \textsuperscript{1}H NMR (500 MHz, CDCl$_3$): $\delta$ 2.09 (s, 3H, Ac), 2.63 (d, $J$ = 2.4 Hz, 1H, H-3), 3.86 (s, 3H, OCH$_3$), 6.35 (d, $J$ = 2.4 Hz, 1H, H-1), 6.95-6.97 (m, 2H, ArH), 7.00 (d, $J$ = 8.4 Hz, 1H, ArH), 7.07 (m, 1H, ArH), 7.18 (d, $J$ = 8.4 Hz, 1H, ArH), 7.30-7.33 (m, 3H, ArH). \textsuperscript{13}C NMR (125 MHz, CDCl$_3$): $\delta$ 21.0 (CH$_3$), 56.1 (OCH$_3$), 64.7 (C-1), 75.3 (C-3), 80.1 (C-2), 112.5 (C-5), 117.2 (C-2” & C-6”), 120.5 (C-6’), 122.7 (C-4”), 124.3 (ArCH), 129.3 (C-1’), 129.6 (C3” & C-5”), 144.9 (C-4”), 152.0 (C-3’), 157.5 (C-1”), 169.7 (C=O).
1-(4'-Fluoro-3'-phenoxyphenyl)prop-2-yn-1-yl acetate 113

This compound was prepared following procedure B using commercially available 4-fluoro-3-phenoxybenzaldehyde (400 mg, 1.75 mmol, 1.0 equiv.) and ethynylmagnesium bromide (4.2 mL of a 0.5 M solution in THF, 5.25 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH₂Cl₂ and treated with Et₃N (1.2 mL, 8.75 mmol, 1.5 equiv.) and Ac₂O (0.5 mL, 5.25 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 113 was attained as an amber oil (494 mg, 94 %). ¹H NMR (300 MHz, CDCl₃): δ 2.09 (s, 3H, Ac), 2.63 (d, J = 2.2 Hz, 1H, H-3), 6.36 (d, J = 2.2 Hz, 1H, H-1), 6.98-7.00 (m, 2H, ArH), 7.09-7.24 (m, 3H, ArH), 7.27-7.37 (m, 3H, ArH). ¹³C NMR (125 MHz, CDCl₃): δ 20.9 (CH₃), 64.7 (C-1), 75.3 (C-3), 80.1 (C-2), 112.6 (C-2’), 117.2 (C-3” & C-5”), 120.6 (C-6’), 122.7 (C-4”), 124.3 (ArCH), 129.4 (ArCH), 129.6 (C-2” & C-6”), 137.4 (C-1’), 146.4 (d, J = 278.7 Hz, C-4’), 152.1 (C-3”), 157.6 (C-1”), 169.6 (C=O).

1-(3’-(4”-Fluorophenoxy)phenyl)prop-2-yn-1-yl acetate 114

This compound was prepared following procedure B using 4-fluoro-3-phenoxybenzaldehyde 103 (400 mg, 1.75 mmol, 1.0 equiv.) and ethynylmagnesium bromide (4.2 mL of a 0.5 M solution in THF, 5.25 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH₂Cl₂ and treated with Et₃N (1.2 mL, 8.75 mmol, 1.5 equiv.) and Ac₂O (0.5 mL, 5.25 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 114 was attained as an amber oil (484 mg, 92 %). ¹H NMR (500 MHz, CDCl₃): δ 2.07 (s, 3H, Ac), 2.65 (d, J = 2.4 Hz, 1H, H-3), 6.41 (d, J = 2.4 Hz, 1H, H-1), 6.94 (dd, J = 8.3 Hz, J = 1.9 Hz, 1H, ArH), 6.99-7.02 (m, 2H, ArH), 7.04-7.08 (m, 2H, ArH), 7.17
(m, 1H, ArH), 7.24-7.25 (m, 1H, ArH), 7.32-7.36 (m, 1H, ArH). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}): δ 20.9 (CH\textsubscript{3}), 63.5 (C-1), 64.8 (C-3), 75.5 (C-2), 116.3 (C-3\’ & C-5\’), 116.5 (C-2\’), 117.4 (C-4\’), 118.4 (C-6\’), 120.7 (C-2\’ & C-6\’), 122.7 (ArCH), 124.3 (ArCH), 130.0 (ArCH), 138.4 (C-1\’), 152.4 (d, \textit{J} = 2.7 Hz, ArC-O), 158.0 (ArC-O), 169.6 (C=O).

**Benzothien-2-ylpropargyl acetate 116**

![Chemical Structure](image)

This compound was prepared following procedure B using commercially available 2-benzothiophenecarboxaldehyde 115 (400 mg, 2.47 mmol, 1.0 equiv.) and ethynylmagnesium bromide (5.9 mL of a 0.5 M solution in THF, 2.96 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH\textsubscript{2}Cl\textsubscript{2} and treated with Et\textsubscript{3}N (5.2 mL, 3.70 mmol, 1.5 equiv.) and Ac\textsubscript{2}O (0.28 mL, 2.96 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 116 was attained as a dark amber oil (488 mg, 86 %). \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ 2.14 (s, 3H, Ac), 2.73 (d, \textit{J} = 2.3 Hz, 1H, H-3), 6.75 (d, \textit{J} = 2.3 Hz, 1H, H-1), 7.34-7.37 (m, 2H, H-5\’/H-6\’), 7.50 (s, 1H, H-3\’), 7.75-7.83 (m, 2H, H-4\’/H-7\’). \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ 22.1 (CH\textsubscript{3}), 61.1 (C-1), 75.6 (C-3), 79.1 (C-2), 122.4 (C-3\’), 124.2 (C-7\’), 124.5 (C-4\’), 125.6 (C-5\’), 125.1 (C-6\’), 138.8 (ArC), 139.6 (ArC), 140.3 (C-2\’), 166.4 (C=O). IR (Neat) \textit{v}_{\text{max}} 3283, 3063, 2119, 1739, 1458, 1370, 1218, 1207, 1014, 945, 843, 755, 730, 720 cm\textsuperscript{-1}. 
**tert-Butyl 3-(1-acetoxyprop-2-yn-1-yl)-1H-indole-1-carboxylate 130**

This compound was prepared following procedure B using 1-tert-butoxycarbonylindole-3-carboxaldehyde 126 (400 mg, 1.63 mmol, 1.0 equiv.) and ethynylmagnesium bromide (4 mL of a 0.5 M solution in THF, 2.00 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in CH$_2$Cl$_2$ and treated with Et$_3$N (3.4 mL, 2.45 mmol, 1.5 equiv.) and Ac$_2$O (0.2 mL, 1.96 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound was attained as an amber oil (306 mg, 73 %). The spectral data matched those reported.$^{460}$ CAS: 1378870-85-9. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.67 (s, 9H, Boc), 2.11 (s, 3H, Ac), 2.65 (d, $J$ = 1.9 Hz, 1H, H-3), 6.72 (d, $J$ = 2.4 Hz, 1H, H-1), 7.26-7.29 (m, 1H, ArH), 7.34-7.37 (m, 1H, ArH), 7.71-7.72 (m, 1H, ArH), 7.80 (s, 1H, ArH), 8.15-8.17 (m, 1H, ArH).

**tert-Butyl 3-(2-hydroxy-1-methoxy-1-oxobut-3-yn-2-yl)-1H-indole-1-carboxylate 131**

This compound was prepared following procedure B using tert-butyl 3-(2-methoxy-2-oxoacetyl)-1H-indole-1-carboxylate 129 (700 mg, 2.31 mmol, 1.0 equiv.) and ethynylmagnesium bromide (5.5 mL of a 0.5 M solution in THF, 2.77 mmol, 1.2 equiv.) at room temperature for 2 h. After work up, the title compound 131 was attained as an amber oil (388 mg, 51 %). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.68 (s, 9H, 3 x CH$_3$), 2.86 (s, 1H, H-4), 3.82 (s, 3H, OCH$_3$), 5.62 (br s, 1H, OH), 7.29-7.32 (m, 1H, ArH), 7.61-7.65 (m, 1H, ArH), 7.57-7.62 (m, 2H, ArH), 8.12-8.14 (m, 1H, ArH). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 28.1
This compound was prepared following procedure B using commercially available isatin 132 (580 mg, 3.49 mmol, 1.0 equiv.) and ethynylmagnesium bromide (8.4 mL of a 0.5 M solution in THF, 4.19 mmol, 1.2 equiv.) at room temperature for 8 h. After extraction, the residue was purified by flash chromatography and the title compound 133 was attained as an orange solid (728 mg, 58 %). m.p. 176 °C (lit. 185-190 °C).\(^{367}\) The spectral data matched those reported.\(^{367}\) CAS: 1403336-39-9. \(^1\)H NMR (300 MHz, DMSO-\(\text{d}_6\)): \(\delta\) 2.57 (br s, 1H, OH), 6.49 (s, 1H, H-4), 6.81 (d, \(J = 7.7\) Hz, ArH), 6.94 (td, \(J = 7.6\) Hz, \(J = 0.8\) Hz, 1H, ArH), 7.14 (td, \(J = 7.7\) Hz, \(J = 1.2\) Hz, 1H, ArH), 7.39 (d, \(J = 7.3\) Hz, 1H, ArH), 10.12 (br s, 1H, NH).

This compound was prepared following procedure B using commercially available \(1',1',1'\)-trifluoroacetophenone 135 (500 mg, 2.87 mmol, 1.0 equiv.) and ethynylmagnesium bromide (6.9 mL of a 0.5 M solution in THF, 3.44 mmol, 1.2 equiv.) at room temperature for 2 h. After extraction, the residue was resuspended in \(\text{CH}_2\text{Cl}_2\) and treated with \(\text{Et}_3\text{N}\) (0.7 mL, 5.23 mmol, 1.5 equiv.) and \(\text{Ac}_2\text{O}\) (0.4 mL, 4.19 mmol, 1.2 equiv.) at room temperature overnight. After work up, the title compound 136 was attained as an amber oil (728 mg, 89 %). The spectral
data matched those reported.\textsuperscript{368} CAS: 141946-97-6. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ 2.21 (Ac), 2.94 (s, 1H, H-4), 7.41-7.43 (m, 3H, ArH), 7.63-7.65 (m, 2H, ArH).

6.1.4. Experimental for chapter 3

6.1.4.1. Cytidine

5’-(\textit{tert}-Butyldimethylsilyl)cytidine 163

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{cytidine}
\caption{Structure of 5’-(\textit{tert}-Butyldimethylsilyl)cytidine 163}
\end{figure}

\textit{t}-Butyldimethylsilyl chloride (1.00 g, 6.63 mmol, 1.1 eq.), Et\textsubscript{3}N (0.9 mL, 6.63 mmol, 1.1 eq.) and DMAP (75.0 mg, 603 µmol, 0.1 eq.) were added to a solution of commercially available cytidine 137 (1.47 g, 6.03 mmol, 1.0 eq.) in anhydrous DMF (15 mL). The mixture was stirred at room temperature until disappearance of the starting material as monitored by TLC (about 24 h). At this time, the reaction mixture was poured into cold water. A white precipitate was formed and filtered, washed with water and air dried to give the title compound 163 as a white solid (985 mg, 67 %). m.p. 160-161 °C (lit. 162-164 °C). The spectral data matched those reported.\textsuperscript{461} CAS: 72409-16-6. \textsuperscript{1}H NMR (300 MHz, CD\textsubscript{3}OD): δ 0.08 (s, 6H, 2 x CH\textsubscript{3}), 0.88 (s, 9H, Boc), 3.79 (dd, \textit{J} = 10.5 Hz, \textit{J} = 4.5 Hz, 1H, H-5’\textsubscript{a}), 3.92 (dd, \textit{J} = 10.5 Hz, \textit{J} = 4.5 Hz, 1H, H-5’\textsubscript{b}), 4.14-4.27 (m, 3H, H-2’/H-3’/H-4’), 5.85 (d, \textit{J} = 7.5 Hz, 1H, H-5), 5.95 (d, \textit{J} = 3.7 Hz, 1H, H-1’), 8.01 (d, \textit{J} = 7.5 Hz, 1H, H-6). LRMS-ESI: \textit{m/z} = 358 [M+H]\textsuperscript{+}
5′-(tert-Butyldimethylsilyl)-N⁴,O⁴',O⁴''-triacetylcytidine 164

Ac₂O (4.00 mL, 41.96 mmol, 10.0 eq.) was added dropwise to a solution of 163 (1.50 g, 4.19 mmol, 1.0 eq.) in anhydrous pyridine (12.0 mL) cooled to 0 °C. The mixture was stirred at room temperature until disappearance of the starting material as monitored by TLC (18 h). At this time, the reaction mixture was neutralised with saturated NaHCO₃ and extracted with CH₂Cl₂, then EtOAc. The organic layers were pooled, washed with saturated CuSO₄, water and then brine and dried over MgSO₄ to be finally concentrated under reduced pressure to give 164 as a beige solid (1.84 g, 91 %). m.p. 230-231 °C (lit. 229-231 °C). The spectral data matched those reported.⁴⁶¹ CAS: 1257796-15-8. ¹H NMR (300 MHz, CD₃OD): δ 0.13 (s, 6H, 2 x CH₃), 0.93 (s, 9H, Boc), 2.06 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.27 (s, 3H, Ac), 3.82 (dd, J = 10.5 Hz, J = 4.5 Hz, 1H, H-5′ₐ), 3.98 (dd, J = 10.5 Hz, J = 4.5 Hz, 1H, H-5′ₐ), 4.27 (m, 1H, H-4′), 5.35-5.38 (m, 2H, H-2'/H-3′), 6.35 (d, J = 4.5 Hz, 1H, H-1′), 7.38 (d, J = 7.5 Hz, 1H, H-5), 8.31 (d, J = 7.5 Hz, 1H, H-6). LRMS-ESI: m/z = 506 [M+Na]⁺

N⁴,O⁴',O⁴''-Triacetylcytidine 165

A 1.0 M solution of tetrabutylammonium fluoride in THF (1.5 mL, 1.478 mmol, 1.1 eq.) was added to a solution of 163 (650 mg, 1.344 mmol, 1.0 eq.) in anhydrous THF (5 mL). The mixture was stirred at room temperature until disappearance of the starting material as monitored by TLC (1 h). At this time, the reaction mixture was concentrated under reduced pressure. The oily residue was taken up in EtOAc, washed with water then brine, dried over
MgSO₄ and filtered to be evaporated under reduced pressure and purified by flash chromatography and eluted with CH₂Cl₂/CH₃CN [6/4] to give the title compound 165 as a beige solid (382 mg, 77%). m.p. 172-173 °C (lit. 173-175 °C). The spectral data matched those reported.⁴⁶¹ CAS: 40632-06-2. ¹H NMR (500 MHz, CDCl₃): δ 2.07 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.27 (s, 3H, Ac), 3.84 (dd, J = 10.5 Hz, J = 2.9 Hz, 1H, H-5’ₐ), 4.00 (dd, J = 10.5 Hz, J = 4.5 Hz, 1H, H-5’ₐ), 4.26 (m, 1H, H-4’), 5.50 (dd, J = 5.7 Hz, J = 5.5 Hz, 1H, H-3’), 5.59 (dd, J = 5.7 Hz, J = 4.5 Hz, 1H, H-2’), 6.03 (d, J = 4.5 Hz, 1H, H-1’), 7.47 (d, J = 7.5 Hz, 1H, H-5), 8.26 (d, J = 7.5 Hz, 1H, H-6). LRMS-ESI: m/z = 392 [M+Na]⁺

5’-Deoxy-5’-tosyl-2’,3’,4-triacetylcytidine 166

A solution of N₄,O₂’,O₃’-triacylcytidine 165 (105 mg, 284 mmol, 1.0 equiv.) and dimethylaminopyridine (35 mg, 427 mmol) in dichloromethane (4 mL) was added 4-toluenesulfonyl chloride (139 mg, 427 mmol) and stirred at room temperature for 16 h. Triethylamine (200 mL) was added before the solution was concentrated. The residue was purified over silica gel (toluene/acetone 3:1) to give the title compound 166 as a colourless syrup (114 mg, 77%). The spectral data matched those reported.²⁰⁰ CAS: 34245-75-5. ¹H NMR (400 MHz, DMSO-d₆): δ 2.03 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.24 (s, 3H, NHAc), 2.43 (s, 3H, Ts-CH₃), 4.26 (dd, J = 10.9 Hz, J = 3.3 Hz, 1H, H-5’ₐ), 4.32-4.35 (m, 1H, H-4’), 4.41 (m, 1H, H-5’ₐ), 4.17-4.22 (m, 1H, H-4’), 4.24 (dd, J = 6.3 Hz, J = 5.1 Hz, 1H, H-3’), 4.36 (dd, J = 6.3 Hz, J = 3.9 Hz, 1H, H-2’), 6.07 (d, J = 3.9 Hz, 1H, H-1’), 7.32 (d, J = 7.6 Hz, 1H, H-5), 7.35 (d, 2H, H-2”/H-6”), 7.68 (d, J = 7.6 Hz, 1H, H-6), 7.79 (d, 2H, H-3’/H-5”), 9.98 (br s, 1H, NH). LRMS-ESI: m/z = 269 [M+H]⁺
This compound was prepared following procedure C, described p. 143, using 5'-Deoxy-5'-tosyl-2',3',4-triacyctytyidine 166 (680 mg, 1.30 mmol, 1.0 equiv.), NaN₃ (170 mg, 2.60 mmol, 2.0 equiv.) and benzyltriethylammonium chloride (60 mg, 260 µmol, 0.2 equiv.) in dry DMF (15 mL). The suspension was stirred overnight and after work up, the title compound 167 was attained as a pale yellow syrup (484 mg, 94 %). \([\alpha]_{D}^{20} = 68.62 \text{ (c = 5 mg.mL}^{-1}, \text{ CH}_3\text{OH})\).

**1H NMR (500 MHz, CDCl₃):** \(\delta 2.09 (s, 3\text{H, Ac}), 2.10 (s, 3\text{H, Ac}), 2.27 (s, 3\text{H, Ac}), 3.71 (dd, J = 13.4 \text{ Hz}, J = 3.8 \text{ Hz}, 1\text{H, H-5'}_{a}), 3.80 (dd, J = 13.4 \text{ Hz}, J = 2.9 \text{ Hz}, 1\text{H, H-5'}_{b}), 4.27-4.30 (m, 1\text{H, H-4'}), 5.33-5.36 (dd, J = 5.7 \text{ Hz}, J = 5.5 \text{ Hz}, 1\text{H, H-3'}), 5.40-5.42 (dd, J = 5.9 \text{ Hz}, J = 4.8 \text{ Hz}, 1\text{H, H-2'}), 6.11 (d, J = 4.8 \text{ Hz}, 1\text{H, H-1'}), 7.51 (d, J = 7.6 \text{ Hz}, 1\text{H, H-5}), 7.95 (d, J = 7.6 \text{ Hz}, 1\text{H, H-6}), 9.90 (br s, 1\text{H, NH}).

**13C NMR (125 MHz, CDCl₃):** \(\delta 20.4 \text{ (CH}_3\text{)}, 20.5 \text{ (CH}_3\text{)}, 24.9 \text{ (CH}_3\text{)}, 51.6 \text{ (C-5')}, 70.5 \text{ (C-3')}, 73.6 \text{ (C-2')}, 80.6 \text{ (C-4')}, 89.0 \text{ (C-5)}, 97.5 \text{ (C-1')}, 144.6 \text{ (C-6)}, 154.8 \text{ (C-2)}, 163.1 \text{ (C-4)}, 169.5 \text{ (C=O)}, 169.7 \text{ (C=O)}, 170.8 \text{ (C=O)}. \text{ LRMS-ESI: } m/z = 395 [M+H]^{+}, \text{ HRESI-MS: } m/z \text{ calcd for C}_{15}\text{H}_{20}\text{N}_{6}\text{O}_{7} [M+H]^{+}: 395.3520; \text{ found 395.3523; } \Delta = 0.76 \text{ ppm}

**N⁴-Acetyl-2',3'-O-isopropylidenylcytidine 170**

Commercially available \(N⁴\)-acetycytidine 169 (6.70 g, 23.50 mmol) was dissolved in dry \(\text{Me}_2\text{CO} (300 \text{ mL})\). To this suspension was added \(\text{HClO}_4\) drop-wise until the reaction mixture
just turned clear. Excess 2,2-dimethoxypropane (75 mL) was added, and the solution was stirred for 4 h at room temperature. After concentration under reduced pressure to about one third of the original reaction volume, the product solidified. Et₂O was added to induce further precipitation, and the resulting solid was collected by filtration, washed with ether and dried to give the title compound 170 as a white solid (6.30 g, 83 %). \textbf{m.p.} 166 °C (lit. 167-169 °C). The spectral data matched those reported.\textsuperscript{462} CAS: 16667-80-4. \textsuperscript{1}H NMR (300 MHz, CD\textsubscript{3}OD): \(\delta\) 1.29 (s, 3H, CH\textsubscript{3}), 1.49 (s, 3H, CH\textsubscript{3}), 2.10 (s, 3H, Ac), 3.54–3.59 (m, 1H, H-5′\textsubscript{a}), 3.62–3.67 (m, 1H, H-5′\textsubscript{b}), 4.20 (dt, \(J = 7.8\) Hz, \(J = 3.8\) Hz, 1H, H-4′), 4.76 (dd, \(J = 6.2\) Hz, \(J = 3.8\) Hz, 1H, H-3′), 4.87 (dd, \(J = 6.2\) Hz, \(J = 2.1\) Hz, 1H, H-2′), 5.09 (t, \(J = 5.1\) Hz, 1H, OH-5′), 5.85 (d, \(J = 2.1\) Hz, 1H, H-1′), 7.19 (d, \(J = 7.5\) Hz, 1H, H-5), 8.22 (d, \(J = 7.5\) Hz, 1H, H-6), 10.89 (s, 1H, NH). \textbf{LRMS-ESI:} \(m/z = 348\) [M+Na]\textsuperscript{+}

\(N^4\)-Acetyl-2′,3′-O-isopropylidenyl-5′-methanesulfonylcytidine 171

MsCl (0.14 mL, 1.84 mmol, 1.2 equiv.) was added dropwise to a solution of 170 (500 mg, 1.54 mmol, 1 equiv.), Et\textsubscript{3}N (0.32 mL, 2.30 mmol, 1.5 equiv.) in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h. The mixture was diluted with CH\textsubscript{2}Cl\textsubscript{2} (25 mL), washed with saturated aqueous NaHCO\textsubscript{3} and brine, dried over MgSO\textsubscript{4} and filtered. The solvent was evaporated under reduced pressure to give the crude mesylate 171 as a white solid (508 mg, 82 %). \textbf{m.p.} 168-171 °C. The compound is known but the spectral data has not been reported to our knowledge. CAS: 16667-81-5. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) 1.29 (s, 3H, CH\textsubscript{3}), 1.50 (s, 3H, CH\textsubscript{3}), 2.21 (s, 3H, Ac), 2.68 (m, 2H, H-5′), 3.01 (s, 3H, Ms), 4.40-4.45 (m, 1H, H-4′), 4.88 (m, 1H, H-3′), 5.06 (m, 1H, H-2′), 5.62 (s, 1H, H-1′), 7.40 (d, \(J = 7.3\) Hz, 1H, H-5), 7.69
(d, J = 7.3 Hz, 1H, H-6), 10.16 (s, 1H, NH). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 25.1 (Ac-CH$_3$), 25.2 (CH$_3$), 27.0 (CH$_3$), 37.6 (Ms-CH$_3$), 69.2 (C-5’), 81.7 (C-3’), 84.7 (C-2’), 86.9 (C-4’), 96.7 (C-5), 98.3 (C-1’), 114.4 (C), 147.5 (C-6), 154.7 (C-2), 163.1 (C-4), 170.1 (C-8).

LRMS-ESI: $m/\ell$ = 426 [M+Na]$^+$, HRESI-MS: $m/\ell$ calcd for C$_{15}$H$_{21}$N$_3$O$_8$SNa [M+Na]$^+$: 426.0947; found 426.0961; Δ = 3.28 ppm

$^{1}$H NMR (300 MHz, CDCl$_3$): δ 1.30 (s, 3H, CH$_3$), 1.57 (s, 3H, CH$_3$), 2.25 (s, 3H, Ac), 3.59 (dd, 1H, J = 13.0 Hz, J = 6.7 Hz, H-5’), 3.70 (dd, 1H, J = 13.0 Hz, J = 4.4 Hz, H-3’), 4.31 (dt, J = 6.7 Hz, J = 4.1 Hz, 1H, H-4’), 4.85 (dd, J = 6.4 Hz, J = 4.1 Hz 1H, H-3’), 5.07 (dd, J = 6.4 Hz, J = 1.5 Hz, 1H, H-2’), 5.66 (d, J = 1.0 Hz, 1H, H-1’), 7.46 (d, J = 7.3 Hz, 1H, H-5), 7.72 (d, J = 7.3 Hz, 1H, H-6), 9.82 (br s, 1H, NH). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 25.0 (Ac-CH$_3$), 25.2 (CH$_3$), 27.1 (CH$_3$), 52.5 (C-5’), 82.0 (C-3’), 84.9 (C-2’), 87.2 (C-4’), 96.8 (C-5), 97.2 (C-1’), 114.4 (Cq), 147.1 (C-6), 154.7 (C-2), 163.3 (C-4), 170.4 (C=O). LRMS-ESI: $m/\ell$ = 351 [M+H]$^+$, 373 [M+Na]$^+$

$^{N^4}$-Acetyl-5’-azido-5’-deoxy-2’,3’-O-isopropylidenyctydine 172

This compound was prepared following procedure C, described p. 143, using $^{N^4}$-acetyl-2’,3’-O-isopropylidenyctydine 171 (500 mg, 1.240 mmol, 1.0 equiv.) and NaN$_3$ (161 mg, 2.479 mmol, 2.0 equiv.) in DMF (5 mL). The suspension was stirred overnight and after work up, the title compound 172 was attained as a white solid (308 mg, 71%). m.p. 188.5-191.5 °C. The spectral data matched those reported. CAS: 1452840-34-4.
A solution of 172 (600 mg, 1.94 mmol) and Boc₂O (850 mg, 3.89 mmol) in dry THF was heated at 60 °C overnight. To the mixture was added CH₃OH (5 mL), then stirred for 20 min and evaporated to dryness. The residue was purified by flash chromatography eluting with CH₂Cl₂/EtOAc [2:1] to give the title compound 174 as a white solid (750 mg, 94 %). m.p. 109-111 °C. The spectral data matched those reported.¹⁹⁷ CAS: 1452840-35-5. ¹H NMR (300 MHz, CDCl₃): δ 1.35 (s, 3H, CH₃), 1.52 (s, 9H, Boc), 1.57 (s, 3H, CH₃), 3.60 (dd, J = 12.7 Hz, J = 4.4 Hz, 1H, H-5’a), 3.74 (dd, J = 12.7 Hz, J = 7.3 Hz, 1H, H-5’b), 4.28-4.34 (m, 1H, H-4’), 4.90 (dd, J = 6.1 Hz, J = 4.1 Hz, 1H, H-3’), 5.13-5.14 (m, 1H, H-2’), 5.62 (s, 1H, H-1’), 7.23 (d, J = 7.6 Hz, 1H, H-5), 7.41 (br s, 1H, NH), 7.63 (d, J = 7.6 Hz, 1H, H-6). ¹³C NMR (75 MHz, CDCl₃): δ 25.2 (CH₃), 27.1 (CH₃), 28.0 (Boc-C(CH₃)₃), 52.6 (C-5’), 82.2 (C-3’), 83.1 (Boc-C(CH₃)₃), 84.8 (C-2’), 87.5 (C4’), 95.1 (C-5), 97.6 (C-1’), 114.2 (C), 146.7 (C-6), 151.0 (Boc-C=O), 154.6 (C-2), 163.3 (C-4). LRMS-ESI: m/z = 431 [M+Na]⁺
6.1.4.2. Uridine

2',3'-O-Isopropylidenyluridine 176

To a suspension of commercially available uridine 175 (3.00 g, 12.28 mmol) in dry Me₂CO (120 mL) was added concentrated sulfuric acid (12 drops) dropwise and the resulting mixture was stirred for 6 h. The solution was neutralised with Et₃N (1 mL) and the solvent evaporated under vacuum. The residue was resuspended in a minimum amount of Me₂CO and added to Et₂O (100 mL). The supernatant was discarded and the residue was dried under vacuum to give the title product 176 as a white solid (3.00 g, 86 %), which was used in the following step without further purification. m.p. 162-164 ºC (lit. 165-168 ºC). The spectral data matched those reported.⁴⁶³ CAS: 362-43-6. ¹H NMR (300 MHz, DMSO-d₆): δ 1.28 (s, 3H, CH₃), 1.48 (s, 3H, CH₃), 3.54-3.59 (m, 2H, H-5’), 4.06 (dt, J = 8.2 Hz, J = 4.3 Hz, 1H, H-4’), 4.73 (dd, J = 6.3 Hz, J = 3.6 Hz, 1H, H-3’), 4.89 (dd, J = 6.3 Hz, J = 2.5 Hz, 1H, H-2’), 5.09 (t, J = 5.2 Hz, 1H, OH), 5.63 (d, J = 8.2 Hz, 1H, H-5), 5.82 (d, J = 2.5 Hz, 1H, H-1’), 7.79 (d, J = 8.2 Hz, 1H, H-6), 11.38 (br s, 1H, NH). LRMS-ESI: m/z = 285 [M+H]⁺

2',3'-O-Isopropylidenyl-5'-mesyluridine 177

A solution of 2’,3’-O-isopropylidenyluridine 176 (2.00 g, 7.03 mmol) and pyridine (1.7 mL, 21.11 mmol) in 50 mL dry DCM was cooled with an external ice bath and added dropwise a
solution of MsCl (0.8 mL, 10.55 mmol) in 5 mL CH₂Cl₂. The mixture was stirred at 0 °C for 2 h and purified by silica gel chromatography and eluted with CH₂Cl₂/MeOH [95:5] to give the title compound 177 as a beige solid (2.32 g, 91 %). Beige solid. m.p. 107 °C (lit. 108-110 °C). The spectral data matched those reported.\(^{464}\) CAS: 15083-09-7. \(^1\)H NMR (300 MHz, CD₃OD): δ 1.36 (s, 3H, CH₃), 1.57 (s, 3H, CH₃), 3.09 (s, 3H, Ms), 4.39 (dt, J = 9.3 Hz, J = 4.9 Hz, 1H, H-4’), 4.45-4.47 (m, 2H, H-5’), 4.88 (dd, J = 6.4 Hz, J = 3.9 Hz, 1H, H-2’), 5.05 (dd, J = 6.4 Hz, J = 1.9 Hz, 1H, H-3’), 5.62 (m, 1H, H-1’), 5.75 (d, J = 7.5 Hz, 1H, H-5), 7.26 (d, J = 7.5 Hz, 1H, H-6), 8.85 (br s, 1H, NH). LRMS-ESI: m/z = 385 [M+Na]⁺

5’-Azido-5’-deoxy-2’,3’-O-isopropylidenuirimidine 178

![Diagram of 5’-Azido-5’-deoxy-2’,3’-O-isopropylidenuirimidine 178](image)

This compound was prepared following procedure C using 2’,3’-O-isopropylidenuirimidine 177 (2.00 g, 5.52 mmol, 1.0 equiv.), NaN₃ (717 mg, 11.04 mmol, 2.0 equiv.) and benzyltriethylammonium chloride (250 mg, 1.10 mmol, 0.2 equiv.) in dry DMF (15 mL). The suspension was stirred at 80 °C overnight and after work up, the title compound 178 was attained as a white solid (1.47 g, 86 %). m.p. 127-129 °C (lit. 129-132 °C).\(^{465}\) The spectral data matched those reported.\(^{466}\) CAS: 15083-05-3. \(^1\)H NMR (300 MHz, CDCl₃): δ 1.28 (s, 3H, CH₃), 1.48 (s, 3H, CH₃), 3.57-3.59 (m, 2H, H-5’), 4.13 (dt, J = 5.6 Hz, J = 4.4 Hz, 1H, H-4’), 4.75 (dd, J = 6.2 Hz, J = 4.4 Hz, 1H, H-3’), 5.07 (dd, J = 6.2 Hz, J = 2.0 Hz, 1H, H-2’), 5.65 (d, J = 7.9 Hz, 1H, H-5), 5.80 (d, J = 1.8 Hz, 1H, H-1’), 7.73 (d, J = 7.9 Hz, 1H, H-6), 11.46 (br s, 1H, NH). LRMS-ESI: m/z = 310 [M+H]⁺
5’-Azido-5’-deoxyuridine 179

5’-Azido-2’,3’-O-isopropylidenyluridine 178 (2.50 g, 8.08 mmol, 1.0 equiv.) was dissolved in a mixture of CH$_3$CN/H$_2$O [9:1] (15 mL) and to this was added In(OTf)$_3$ (227 mg, 404 µmol, 5 mol%) and the solution was refluxed for 6 h. The solvents were then evaporated under reduced pressure and the residue was purified by silica gel chromatography eluted with CH$_2$Cl$_2$/CH$_3$OH [9:1] to afford the title product 179 as a white foam (2.07 g, 95 %). The spectral data matched those reported.\textsuperscript{284} CAS: 39483-48-2. \textsuperscript{1}H NMR (300 MHz, CDCl$_3$): δ 3.68 (dd, $J = 13.7$ Hz, $J = 4.9$ Hz, 1H, H-5’$_a$), 3.81 (dd, $J = 13.7$ Hz, $J = 3.1$ Hz, 1H, H-5’$_b$), 4.21 (dt, $J = 8.6$ Hz, $J = 5.0$ Hz, 1H, H-4’), 4.27 (m, 1H, H-3’), 4.43 (m, 1H, H-2’), 5.93-5.89 (m, 2H, H-5, H-1’), 7.81 (d, $J = 8.1$ Hz, 1H, H-6). LRMS-ESI: $m/z = 292$ [M+Na]$^+$

5’-Azido-5’-deoxy-2’,3’-O-diacetyluridine 180

A solution of 5’-azidouridine 179 (1.50 g, 5.57 mmol, 1 equiv.) in pyridine (6 mL) was treated with Ac$_2$O (1.3 mL, 13.93 mmol, 2.5 equiv.) and the mixture was stirred at room temperature overnight. The reaction mixture was then poured on crushed ice and extrated with EtOAc. The organic layers were pooled together and washed with water, NaHCO$_3$ and brine before being dried over MgSO$_4$, filtered and evaporated. The residue was purified by silica gel chromatography eluted with CH$_2$Cl$_2$/MeOH [9:1] to afford the title compound 180 as a white foam (1.91 g, 97 %). The spectral data matched those reported.\textsuperscript{467} CAS: 96535-66-9.
**6.1.3.6. 5-Fluorouridine**

**5-Fluoro-2',3'-O-isopropylidenyluridine 182**

![Structural formula of 5-Fluoro-2',3'-O-isopropylidenyluridine](image)

To a suspension of commercially available 5-fluorouridine 181 (1.50 g, 5.72 mmol, 1.0 equiv.) and 2,2-dimethoxypropane (1.4 mL, 11.44 mmol, 2.0 equiv.) in Me₂CO (60 mL) was added 4 drops of concentrated sulphuric acid. After stirring for 1 h at room temperature, the starting material was no longer detectable by TLC (CH₂Cl₂/CH₃OH [9:1]) and 1.0 mL was added to the mixture concentrated ammonia and stirred for an additional hour. The solvent was evaporated under reduced pressure and the residue was washed with Et₂O to give the title compound 182 as a white powder (1.38 g, 80 %). **m.p.** 197-198 °C (lit. 196-197 °C). The spectral data matched those reported.⁴⁶⁸ **CAS: 2797-17-3.** ¹H NMR (300 MHz, CDCl₃): δ 2.10 (s, 3H, Ac), 2.13 (s, 3H, Ac), 3.70 (dd, J = 13.5 Hz, J = 3.7 Hz, 1H, H-5’a), 3.78 (dd, J = 13.5 Hz, J = 2.9 Hz, 1H, H-5’b), 4.22-4.23 (m, 1H, H-2’), 5.31-5.35 (m, 2H, H-3’/H-4’), 5.84 (d, J = 8.1 Hz, 1H, H-5), 6.08-6.09 (m, 1H, H-1’), 7.49 (d, J = 8.1 Hz, 1H, H-6), 9.39 (br s, 1H, NH). **LRMS-ESI:** m/z = 376 [M+Na]⁺
Methanesulfonyl chloride (0.37 mL, 4.73 mmol, 1.1 equiv.) was added dropwise to a solution of 182 (1.30 g, 4.30 mmol, 1.0 equiv.), Et₃N (0.90 mL, 6.45 mmol, 1.5 equiv.) in CH₂Cl₂ (30 mL) at 0 °C. The mixture was stirred at 0 °C during 1 h. The mixture was diluted with CH₂Cl₂ (25 mL), washed with saturated aqueous NaHCO₃ and brine, dried over MgSO₄ and filtered. The solvent was evaporated in vacuo to give the crude mesylate 183 as a pale amber syrup (1.41 g, 86 %). The spectral data matched those reported.⁴⁰¹ CAS: 82207-42-9.

**¹H NMR (300 MHz, CDCl₃):** δ 1.35 (s, 3H, CH₃), 1.56 (s, 3H, CH₃), 3.06 (s, 3H, Ms-CH₃), 4.38-441 (m, 1H, H-4’), 4.46 (m, 1H, H-5’), 4.86 (dd, J = 6.3 Hz, J = 3.9 Hz, 1H, H-3’), 5.02 (m, 1H, H-2’), 5.66 (m, 1H, H-1’), 7.44 (d, J = 5.9 Hz, 1H, H-6), 9.80 (br s, 1H, NH). **LRMS-ESI:** m/z = 403 [M+Na]⁺

5’-Azido-5’-deoxy-5-fluoro-2’-3’-O-isopropylidenyluridine 184

This compound was prepared following procedure C using 5-Fluoro-2’,3’-O-isopropylidenyl-5’-methanesulfonyluridine 183 (1.00 g, 2.63 mmol, 1.0 equiv.), NaN₃ (341 mg, 5.26 mmol, 2.0 equiv) and benzyltriethylammonium chloride (120 mg, 526 µmol, 0.2 equiv) in dry 10 mL DMF. The suspension was stirred at 80 °C overnight and after work up, the title compound 184 was attained as a white solid (793 mg, 94 %). **m.p.** 158-160 °C (lit. 160-163 °C).⁴⁶⁹ The spectral data matched those reported.⁴⁰¹ CAS: 82207-45-2.
$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.28 (s, 3H, CH$_3$), 1.48 (s, 3H, CH$_3$), 3.59-3.61 (m, 2H, H-5'), 4.13 (dt, $J = 10.4$ Hz, $J = 5.4$ Hz, 1H, H-4'), 4.75 (dd, $J = 6.5$ Hz, $J = 4.2$ Hz, 1H, H-3'), 5.06 (dd, $J = 6.5$ Hz, $J = 2.3$ Hz, 1H, H-2'), 5.77 (d, $J = 2.3$ Hz, 1H, H-1'), 8.12 (d, $J = 6.9$ Hz, 1H, H-6), 11.98 (br s, 1H, NH). LRMS-ESI: $m/z$ = 326 [M+H]$^+$

$^5'$-Azido-$5'$-deoxy-$5$-fluorouridine 185

![5'-Azido-5'-deoxy-5-fluorouridine](image)

$^5'$-Azido-$5'$-deoxy-$5$-fluoro-$2' ,3'$-$O$-isopropylidenyluridine 184 (600 mg, 1.83 mmol, 1.0 equiv.) was dissolved in CH$_3$CN/H$_2$O [8:2] (20 mL) and to this was added In(OTf)$_3$ (51 mg, 92 µmol, 5 mol%). The solution was refluxed for 6 h and, after cooling, evaporated to dryness. The residue was purified by flash chromatography column eluted with CH$_2$Cl$_2$/CH$_3$OH [9:1] to give the title compound 185 as a thick colourless syrup (505 mg, 96%). The spectral data matched those reported.$^{401}$ CAS: 82207-46-3.

$^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 3.58 (dd, $J = 13.7$ Hz, $J = 2.9$ Hz, 1H, H-5’$_a$), 3.65 (dd, $J = 13.2$ Hz, $J = 6.4$ Hz, 1H, H-5’$_b$), 3.90-3.93 (m, 2H, H-3’ & H-4’), 4.14 (dd, $J = 10.9$ Hz, $J = 4.9$ Hz, H-2’), 5.30 (d, $J = 4.9$ Hz, 1H, OH-2’), 5.49 (d, $J = 5.4$ Hz, 1H, OH-3’), 5.75-5.76 (m, 1H, H-1’), 8.02 (d, $J = 7.3$ Hz, 1H, H-6), 11.92 (br s, 1H, NH). LRMS-ESI: $m/z$ = 310 [M+Na]$^+$
5'-Azido-5'-deoxy-2',3'-diacetyl-5-fluorouridine 186

5'-Azido-5'-deoxy-5-fluorouridine 185 (450 mg, 1.57 mmol, 1.0 equiv) was dissolved in pyridine (8 mL) and the solution was cooled with an external ice bath. Ac₂O (0.37 mL, 3.92 mmol, 2.5 equiv) was added dropwise and the mixture stirred while returning to room temperature. After 4 h, the starting material was no longer detectable by TLC and the mixture was taken up in EtOAc, washed with water, saturated NaHCO₃ and brine before being dried over MgSO₄, filtered and evaporated under reduced pressure. The resulting syrup 186 (535 mg, 92%) displayed satisfying purity for using immediately in the following step and the spectral data matched those reported. CAS: 1266253-85-3.

¹H NMR (500 MHz, CDCl₃): δ 2.11 (s, 3H, Ac), 2.15 (s, 3H, Ac), 3.74 (dd, J = 13.4 Hz, J = 3.0 Hz, 1H, H-5’ₐ), 3.84 (dd, J = 13.4 Hz, J = 2.7 Hz, 1H, H-5’ₖ), 4.24-4.26 (m, 1H, H-4’), 5.28-5.33 (m, 2H, H-2’ & H-3’), 6.11 (d, J = 5.7 Hz, 1H, H-1’), 7.66 (d, J = 6.0 Hz, 1H, H-6), 9.03 (br s, 1H, NH). LRMS-ESI: m/z = 394 [M+Na]⁺

6.1.4.3. Thymidine

3’-O-Acetyl-5’-tosylthymidine 188

Thymidine 152 (0.484 g, 2.00 mmol, 1.0 equiv.) was dissolved in pyridine (5.0 mL) and the solution was cooled to 0 °C. Tosyl chloride (0.42 g, 2.2 mmol, 1.1 equiv.) was added to the
cold solution with stirring. Stirring was continued for 3 h and Ac₂O (1.0 mL, 10.59 mmol, 10.6 equiv.) was added to the reaction mixture. The solution was stirred for an additional 16 h and the solution was diluted with CH₂Cl₂ (30 mL). The solution of the O-tosyl derivative, was washed with 1 M HCl and then with brine (30 mL). The product was purified by silica gel column chromatography eluting with CH₂Cl₂/CH₃OH [1:9]. Colourless syrup. The title compound 188 is known but the spectral data has not been reported to our knowledge. CAS: 75145-86-7. ¹H NMR (300 MHz, CDCl₃): δ 1.98 (s, 3H, CH₃-5), 2.20-2.29 (m, 1H, H-2’a), 2.41-2.48 (m, 1H, H-2’b), 2.51 (s, 3H, CH₃), 3.75-3.82 (m, 2H, H-5’), 4.17-4.21 (m, 1H, H-3’), 5.20-5.22 (m, Hz, 1H, H-4’), 6.35 (dd, J = 8.7 Hz, J = 5.2 Hz, 1H, H-1’), 7.40 (s, 1H, H-6), 8.15 (br s, 1H, NH). LRM-ESI: m/z = 461 [M+Na]⁺

3’-O-Acetyl-5’-azido-5’-deoxythymidine 189

![Chemical Structure](image)

This compound was prepared following procedure C using 3’-O-Acetyl-5’-tosylthymidine 188 (400 mg, 912 µmol, 1.0 equiv.), NaN₃ (297 mg, 1.82 mmol, 2.0 equiv.) and benzyltriethylammonium chloride (43 mg, 182 µmol, 0.2 equiv.) in dry 6 mL DMF. The suspension was stirred at 80 °C overnight and after work up, the title compound 189 was attained as a colourless syrup (262 mg, 93 %). The spectral data matched those reported.⁴⁰⁹ CAS: 27766-92-3. ¹H NMR (300 MHz, CDCl₃): δ 1.96 (s, 3H, CH₃-5), 2.11 (s, 3H, Ac), 2.21-2.27 (m, 1H, H-2’a), 2.39-2.44 (m, 1H, H-2’b), 3.71-3.79 (m, 2H, H-5’), 4.11 (dt, J = 5.4 Hz, J = 2.4 Hz, 1H, H-3’), 5.19 (dt, J = 7.3 Hz, J = 2.4 Hz, 1H, H-4’), 6.34 (dd, J = 8.8 Hz, J = 5.4 Hz, 1H, H-1’), 7.38 (s, 1H, H-6), 8.02 (br s, 1H, NH). LRM-ESI: m/z = 332 [M+Na]⁺
6.1.4.3. 2’-Deoxyuridine

\[3'\text{-O-Acetyl-5'}\text{-deoxy-5'}\text{-tosyluridine 190}\]

A solution of commercially available 2’-deoxyuridine 187 (600 mg, 2.63 mmol, 1.0 equiv.) in dry pyridine (5 mL) was cooled with an external ice bath and treated with tosyl chloride (551 mg, 2.89 mmol, 1.1 equiv.) and stirred at room temperature for 1 h. Ac₂O (1.25 mL) was added to the reaction mixture and stirred for another 3h. The solution was then taken up in CH₂Cl₂ and washed with 0.5 M hydrochloric acid, water and brine. The organic layer was dried on MgSO₄, filtered and evaporated under reduced pressure and the residue was purified using silica gel chromatography eluted with (CH₂Cl₂/EtOAc [2:1]) to give the title compound 190 as a pale yellow syrup (692 mg, 62%). \([\alpha]_D^{20} = 27.82 \, (c = 5 \, \text{mg.mL}^{-1}, \text{CH}_3\text{OH})\). \(^1\text{H NMR (300 MHz, CDCl}_3\): \(\delta 2.10 \, (s, 3H, \text{Ac}), 2.13-2.19 \, (m, 1H, H-2’\_a), 2.43-2.47 \, (m, 1H, H-2’\_b), 2.48 \, (s, 3H, \text{Ts-Ch}_3), 4.18-4.19 \, (m, 1H, H-3’), 4.33 \, (m, 2H, H5’), 5.19-5.21 \, (m, 1H, H-4’), 5.76 \, (d, J = 8.3 \, \text{Hz}, 1H, H-5), 6.33 \, (dd, J = 8.8 \, \text{Hz, J = 5.4} \, \text{Hz}, 1H, H-1’), 7.40 \, (d, J = 8.3 \, \text{Hz, 2H, H-3”/H-5”}), 7.53 \, (d, J = 7.8 \, \text{Hz, 1H, H-6}), 7.80 \, (d, J = 8.3 \, \text{Hz, 2H, H-2”/H-6”}), 8.14 \, (\text{br} s, 1H, \text{NH})\). \(^{13}\text{C NMR (75 MHz, CDCl}_3\): \(\delta 20.7 \, (\text{Ac-Ch}_3), 21.5 \, (\text{Ts-Ch}_3), 37.0 \, (C-2’), 68.9 \, (C-5’), 74.2 \, (C-3’), 81.9 \, (C-4’), 84.7 \, (C-1’), 103.0 \, (C-5), 127.7 \, (C-3’/C-5”), 130.0 \, (C-2”/C-6”), 131.9 \, (C-4’), 139.1 \, (C-6), 145.5 \, (C-1”), 150.4 \, (C-2), 163.3 \, (C-4), 170.4 \, (C=O)\). LRMS-ESI: \(m/z = 447 \, [\text{M+Na}]^+\), HRESI-MS: \(m/z\) calcd for C\(_{18}\)H\(_{20}\)N\(_2\)O\(_8\)SNa [M+Na]\(^+\): 447.0838; found 447.0839; \(\Delta = 0.22 \, \text{ppm}\)
This compound was prepared following procedure C using 3′-O-Acetyl-5′-deoxy-5′-tosyluridine 190 (400 mg, 942 µmol, 1.0 equiv.), NaN₃ (122 mg, 1.88 mmol, 2.0 equiv.) and benzyltriethylammonium chloride (43 mg, 188 µmol, 0.2 equiv.) in dry 6 mL DMF. The suspension was stirred at 80 °C overnight and after work up, the title compound 191 was attained as a colourless syrup (259 mg, 93 %). The spectral data matched those reported.  

CAS: 85144-91-8. ¹H NMR (300 MHz, CDCl₃): δ 2.02-2.26 (m, 1H, H-2′a), 2.09 (s, 3H, Ac), 2.39-2.47 (m, 1H, H-2′b), 3.73-3.75 (m, 2H, H-5′), 4.09-4.11 (m, 1H, H-4′), 5.16-5.18 (m, 1H, H-3′), 5.81 (d, J = 8.2 Hz, 1H, H-5), 6.31 (dd, J = 8.3 Hz, J = 5.7 Hz, 1H, H-1′), 7.58 (d, J = 8.2 Hz, 1H, H-6), 10.01 (br s, 1H, NH). ¹³C NMR (75 MHz, CDCl₃): δ 20.8 (CH₃), 37.2 (C-2′), 52.4 (C-5′), 74.6 (C-3′), 82.6 (C-4′), 84.6 (C-1′), 103.3 (C-5), 139.2 (C-6), 150.5 (C-2), 163.3 (C-4), 170.6 (C=O). LRMS-ESI: m/z = 318 [M+Na]⁺

6.1.4.5. 2′-Deoxy-2′-fluorouridine

3′-O-Acetyl-2′-deoxy-2′-fluoro-5′-tosyluridine 193

Commercially available 2′-deoxy-2′-fluorouridine 192 (600 mg, 2.44 mmol, 1.0 equiv.) was dissolved in pyridine (6 mL) and the solution was cooled to 0 °C. Tosyl chloride (511 mg, 2.69 mmol, 1.1 equiv.) was added to the cold solution with stirring. Stirring was continued for
3 h and Ac₂O (1.15 mL, 12.19 mmol, 5.0 equiv.) was added to the reaction mixture. The solution was stirred for additional 16 h and the solution was diluted with CH₂Cl₂. The solution of the O-tosyl derivative, was washed with 1 M HCl and brine before being dried over MgSO₄, filtered and evaporated under reduced pressure. The residue was purified by silica gel column chromatography eluted with CH₂Cl₂/EtOAc [2:1] to give the title compound 193 as a foam (507 mg, 47%). The spectral data matched those reported. CAS: 1668560-67-5.

**¹H NMR (500 MHz, CDCl₃):** δ 2.01 (s, 3H, Ac), 2.46 (s, 3H, Ts-CH₃), 4.04 (dt, J = 12.5 Hz, J = 4.8 Hz, 1H, H-4’), 4.28–4.34 (m, 2H, H-5’), 5.15-5.23 (m, 1H, H-3’), 5.30 (dd, J = 5.5 Hz, J = 4.9 Hz, 1H, H-2’), 5.57 (d, J = 21.8 Hz, H-5), 5.76 (d, J = 8.0 Hz, 1H, H-1’), 7.33 (d, J = 8.1 Hz, 1H, H-6), 7.38 (d, J = 8.2 Hz, 2H, ArH), 7.85 (d, J = 7.9 Hz, 2H, ArH), 9.92 (s, 1H, NH). **LRESI-MS:** m/z = 465 [M+Na]+

3’-O-Acetyl-5’-azido-2’,5’-dideoxy-2’-fluoro-5’-tosyluridine 194

This compound was prepared following procedure C using 3’-O-Acetyl-2’-deoxy-2’-fluoro-5’-tosyluridine 193 (450 mg, 1.02 mmol, 1.0 equiv.), NaN₃ (132 mg, 2.03 mmol, 2.0 equiv.) and benzyltriethylammonium chloride (46 mg, 203 µmol, 0.2 equiv.) in dry DMF (40 mL). The suspension was stirred at 80 °C overnight and after work up, the title compound 194 was attained as a white solid (283 mg, 89 %). m.p. 172-174 °C. [α]D²⁰ = 32.57 (c = 5 mg.mL⁻¹, CH₃OH). **¹H NMR (300 MHz, CDCl₃):** δ 2.17 (s, 3H, Ac), 3.60 (dd, J = 13.5 Hz, J = 4.4 Hz, 1H, H-5’a), 3.80 (dd, J = 13.5 Hz, J = 2.4 Hz, 1H, H-5’b), 4.29-4.32 (m, 1H, H-4’), 5.19-5.25 (m, 1H, H-3’), 5.32-5.44 (m, 1H, H-2’), 5.82 (d, J = 7.9 Hz, 1H, H-5), 5.86 (dd, J = 19.8 Hz, J = 2.0 Hz, 1H, H-1’), 7.42 (d, J = 7.9 Hz, 1H, H-6), 9.25 (br s, 1H, NH). **¹³C NMR (75 MHz, CDCl₃):** δ 20.4 (CH₃), 51.0 (C-5’), 70.2 (d, J = 15.3 Hz, C-3’), 79.4 (C-4’), 90.4
(d, $J = 126.6$ Hz, C-2'), 90.6 (C-4'), 91.5 (C-1'), 103.3 (C-5), 140.8 (C-6), 149.8 (C-2), 162.8 (C-4), 169.8 (C=O). **LRMS-ESI:** $m/z = 336$ [M+Na]+. **HRESI-MS:** $m/z$ calcld for C$_{11}$H$_{12}$FN$_{3}$O$_{5}$Na [M+Na]+: 336.0720; found 336.0734; $\Delta = 4.16$ ppm

6.1.3.5. 2'-O-Methyluridine

3'-Acetyl-5'-tert-butyldimethylsilyl-2'-methyluridine 198

A solution of 2'-methyluridine 195 (600 mg, 2.32 mmol, 1.0 equiv.) and imidazole (395 mg, 5.81 mmol, 2.5 equiv.) in dry DMF (5 mL) was treated with tert-butylidemethylsilyl chloride (385 mg, 2.55 mmol, 1.1 equiv.) and stirred at room temperature for 20 h. The reaction mixture was poured on crushed ice and the white precipitate 197 was collected by filtration, air dried and used in the following step without further purification. The solid was suspended in CH$_2$Cl$_2$ (12 mL) and added successively trimethylamine (0.5 mL, 3.48 mmol, 1.5 equiv.) and Ac$_2$O (0.26 mL, 2.79 mmol, 1.2 equiv.) and the resulting solution was stirred at room temperature. After 3 h, the starting material was no longer detectable by TLC and the solution was diluted with CH$_2$Cl$_2$ and washed with water, NaHCO$_3$ and brine and finally dried over MgSO$_4$, filtered and evaporated under reduced pressure. The residue was purified by silica gel chromatography eluted with CH$_2$Cl$_2$ to CH$_2$Cl$_2$/EtOAc [4:1] to give the title compound 198 as a colourless syrup (810 mg, 74 %). [$\alpha$]$_{D}^{20}$ = 25.3 (c = 5.0 mg.mL$^{-1}$, CH$_3$OH). **$^1$H NMR (300 MHz, CDCl$_3$):** $\delta$ 0.10 (s, 6H, 2 x SiCH$_3$), 0.92 (s, 9H, 3 x CH$_3$), 2.14 (s, 3H, Ac), 3.43 (s, 3H, OCH$_3$), 3.77-3.79 (m, 1H, H-2’), 3.97-4.00 (m, 2H, H-5’), 4.20-4.22 (m, 1H, H-4’), 5.12-5.14 (m, 1H, H-3’), 5.70 (d, $J = 7.9$ Hz, 1H, H-5), 6.06 (d, $J = 2.7$ Hz, 1H, C-1’), 7.92 (d,
$J = 7.9$ Hz, 1H, H-6), 9.60 (br s, H, NH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ -5.7 (SiCH$_3$), -5.6 (SiCH$_3$), 18.3 (SiC$_3$), 20.7 (Ac-CH$_3$), 25.8 (3 x CH$_3$), 58.9 (OCH$_3$), 62.0 (C-5’) 69.9 (C-4’) 82.5 (C-2’) 86.8 (C-1’) 102.5 (C-5) 139.6 (C-6) 150.3 (C-2) 163.2 (C-4) 170.2 (C=O).

**LRMS-ESI:** $m/z = 437$ [M+Na]$^+$, **HRESI-MS:** $m/z$ calcd for C$_{18}$H$_{30}$N$_2$O$_7$SiNa [M+Na]$^+:$ 437.5198; found 437.5199, $\Delta = 0.23$ ppm

### 3’-Acetyl-2’-methyluridine 199

![3’-Acetyl-2’-methyluridine](image)

To a solution of 3’-acetyl-5’-tert-butyldimethylsilyl-2’-methyluridine 198 (650 mg, 1.57 mmol, 1.0 equiv.) and AcOH (0.13 mL, 2.35 mmol, 1.5 equiv.) in dry THF (15 mL) was added a 1.0 M solution of TBAF in THF (2.00 mL, 2.04 mmol, 1.3 equiv.) dropwise at 0 °C. Stirring was continued for 6 h and the reaction mixture was evaporated to dryness. The residue was purified by silica gel chromatography column eluted with CH$_2$Cl$_2$/EtOAc [1:1] to give the title compound 199 as a white solid (358 mg, 76 %). The spectral data matched those reported.$^{472}$ **CAS:** 287101-01-3. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.16 (s, 3H, CH$_3$), 3.44 (s, 3H, CH$_3$), 3.79 (m, 1H, H-2’), 3.96 (m, 1H, H-3’), 4.20-4.23 (m, 2H, H-5’), 5.30 (m, 1H, H-4’), 5.77 (d, $J = 6.8$ Hz, 1H, H-5), 5.83 (d, $J = 4.4$ Hz, 1H, H-1’), 7.78 (d, $J = 7.8$ Hz, 1H, H-6), 9.64 (br s, 1H, NH). **LRMS-ESI:** $m/z = 323$ [M+Na]$^+$

### 3’-Acetyl-2’-methyl-5’-methylsulfonyluridine 200

![3’-Acetyl-2’-methyl-5’-methylsulfonyluridine](image)

A solution of 3’-acetyl-2’-methyluridine 199 (300 mg, 1.00 mmol, 1.0 equiv.) in dry CH$_2$Cl$_2$ (10 mL) was treated with Et$_3$N (0.1 mL, 1.50 mmol, 1.5 equiv.) and MsCl (0.09 mL,
1.20 mmol, 1.2 equiv.) at 0 °C. Stirring was continued for 16 h and the reaction mixture was washed with water, NaHCO₃ sat and brine before being dried over MgSO₄ and evaporated under reduced pressure. The residue was purified by silica gel chromatography and eluted with CH₂Cl₂/CH₃OH, [9:1] to give the title compound 200 as a colourless syrup (272 mg, 72 %). m.p. 156-157°C. The spectral data matched those reported.⁴⁷³ CAS: 1352173-41-1.

¹H NMR (300 MHz, CDCl₃): δ 2.14 (s, 3H, Ac), 3.10 (s, 3H, Ms-CH₃), 3.42 (s, 3H, OCH₃), 4.06-4.08 (m, 1H, H-2’), 4.32-4.43 (m, 1H, H-3’), 4.42 (dd, J = 11.2 Hz, J = 3.4 Hz, 1H, H-5’a), 4.54 (dd, J = 11.2 Hz, J = 3.4 Hz, 1H, H-5’b), 5.13-5.15 (m, 1H, H-4’), 5.77 (d, J = 8.3 Hz, 1H, H-5), 5.90-5.91 (m, 1H, H-1’), 7.50 (d, J = 8.3 Hz, 1H, H-6), 10.23 (br s, 1H, NH). LRMS-ESI: m/z = 401 [M+Na]+

3’-Acetyl-5’-azido-5’-deoxy-2’-methyluridine 201

This compound was prepared following procedure C using 3’-Acetyl-2’-methyl-5’-methylsulfonyluridine 200 (250 mg, 660 µmol, 1.0 equiv.), NaN₃ (86.0 mg, 1.32 mmol, 2.0 equiv.) and benzyltriethylammonium chloride (30 mg, 132 µmol, 0.2 equiv.) in dry 6 mL DMF. The suspension was stirred at 80 °C overnight and after work up, the title compound 201 was attained as a white foam (195 mg, 91 %). [α]D²⁰ = 37.86 (c = 5 mg.mL⁻¹, CH₃OH).

¹H NMR (500 MHz, CDCl₃): δ 2.17 (s, 3H, Ac), 3.44 (s, 3H, OCH₃), 3.66 (d, J = 13.2 Hz, 1H, H-5’a), 3.81 (d, J = 13.7 Hz, 1H, H-5’b), 4.03-4.04 (m, 1H, H-4’), 4.24 (m, 1H, H-3’), 5.07-5.09 (m, 1H, H-2’), 5.83 (d, J = 7.8 Hz, 1H, H-5), 5.98 (d, J = 3.4 Hz, 1H, H-1’), 7.56 (d, J = 7.8 Hz, 1H, H-6). ¹³C NMR (125 MHz, CDCl₃): δ 20.6 (Ac-CH₃), 51.6 (C-5’), 59.1 (OCH₃), 70.6 (C-3’), 80.0 (C-2’), 81.3 (C-4’), 88.0 (C-1’), 103.3 (C-5),
139.5 (C-6), 150.1 (C-2), 162.7 (C-4), 170.1 (C=O). **LRMS-ESI:** \( m/z = 348 \ [\text{M+Na}]^+ \), **HRESI-MS:** \( m/z \) calcd for \( \text{C}_{12}\text{H}_{15}\text{N}_{6}\text{O}_{6}\text{Na} \ [\text{M+Na}]^+ \): 348.0920; found 348.0917; \( \Delta = 0.86 \text{ ppm} \)

### 6.1.5. Experimental for chapter 4

#### 6.1.5.1. CuAAC reactions

**5’-(4-(α-Acetoxy-3-phenoxybenzyl)triazolo)-N⁴-Acetyl-5’-deoxy-2’,3’-O-isopropylidenycytidine 202**

![Chemical Structure](image)

This compound was prepared following procedure D, described p. 143, using **172** (250 mg, 714 \( \mu \text{mol}, 1.0 \text{ equiv.} \)), **111** (228 mg, 856 \( \mu \text{mol}, 1.2 \text{ equiv.} \)), \( \text{Cu(OAc)}_2 \) (32 mg, 178 \( \mu \text{mol}, 0.25 \text{ equiv.} \)) and sodium ascorbate (71 mg, 357 \( \mu \text{mol}, 0.5 \text{ equiv.} \)) in \( \text{CH}_3\text{CN/H}_2\text{O} \ [4:1] \). The suspension was stirred at r. t. for 24 h and after work up, the title compound **202** was attained as a dark yellow oil (282 mg, 64 %). \([α]_{D}^{20} = 19.68 \ (c = 5 \text{ mg.mL}^{-1}, \text{CH}_3\text{OH})\). **\( ^1\text{H NMR} \) (300 MHz, CDCl\textsubscript{3})**: \( δ \) 1.32 (s, 3H, CH\textsubscript{3}), 1.51 (s, 3H, CH\textsubscript{3}), 2.10 (s, 3H, Ac), 2.26 (s, 3H, Ac), 4.54 (m, 1H, H-4’), 4.75-4.77 (m, 2H, H-5’), 5.00-5.02 (m, 1H, H-3’), 5.14-5.17 (m, 1H, H-2’), 5.56 (m, 1H, H-1’), 6.87-6.90 (m, 1H, ArH), 6.96-6.99 (m, 3H, ArH), 7.07-7.17 (m, 3H, ArH), 7.25-7.34 (m, 4H, ArH), 7.44-7.47 (m, 1H, H-6), 7.54 + 7.60 (2 s, 1H, H-5’), 10.04 (br s, 1H, NH). **\( ^{13}\text{C NMR} \) (300 MHz, CDCl\textsubscript{3})**: \( δ \) 21.0 (CH\textsubscript{3}), 24.8 (Ac-CH\textsubscript{3}), 25.0 (CH\textsubscript{3}), 26.8 (Ac-CH\textsubscript{3}), 52.1 (C-5’), 69.4 + 69.5 (C-1”), 82.4 (C-3’), 84.4 (C-2’), 87.3 + 87.3 (C-4’), 97.1 (C-1’), 98.2 + 98.3 (C-5), 114.3 (C), 117.3 (ArCH), 118.1 + 118.2 (ArCH), 118.8 + 118.9 (ArCH), 121.6 + 121.7 (ArCH), 123.4 (ArCH), 123.4 + 123.5 (C-5”), 129.7 (ArH), 129.8
(ArCH), 140.1 + 140.2 (C-4”), 146.7 + 146.7 (C-6), 147.8 + 147.9 (C), 154.7 (C-4), 156.6 + 156.6 (ArC-O), 157.3 (ArC-O), 163.6 + 163.7 (C-2), 169.8 (C=O), 170.8 (C=O).

LRMS-ESI: m/z = 639 [M+Na]+. HRESI-MS: m/z calcd for C_{31}H_{33}N_{6}O_{8} [M+H]+: 617.2360; found 617.2375; Δ = 2.43 ppm

5’-(4-(α-Acetoxy-3-phenoxybenzyl)triazolo)-N^4-tert-butoxycarbonyl-5’-deoxy-2’,3’-O-isopropyldenylcytidine 203

This compound was prepared following procedure D using 174 (460 mg, 1.13 mmol, 1.0 equiv.), 111 (360 mg, 1.35 mmol, 1.2 equiv.), Cu(OAc)_2 (51 mg, 281 µmol, 0.25 equiv.) and sodium ascorbate (111 mg, 563 µmol, 0.5 equiv.) in CH_3CN/H_2O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 203 was attained as a dark amber oil (653 mg, 86 %). [α]_D^{20} = 23.17 (c = 5 mg.mL^{-1}, CH_3OH). 1H NMR (500 MHz, CDCl_3): δ 1.34 (s, 3H, CH_3), 1.52 (s, 12H, Boc/CH_3), 2.10 + 2.11 (2 s, 3H, Ac), 4.58 (m, 1H, H-4’), 4.76-4.77 (m, 2H, H-5”), 5.03-5.05 (m, 1H, H-3’), 5.22-5.23 (m, 1H, H-2’), 5.48-5.49 (m, 1H, H-5), 6.89-6.90 (m, 1H, ArH), 6.94 + 6.95 (s, 1H, CHOAc), 6.99-7.00 (m, 2H, ArH), 7.08-7.11 (m, 2H, ArH/H-5”), 7.14-7.17 (m, 1H, ArH), 7.26-7.36 (m, 4H, ArH), 7.47 + 7.52 (d, J = 7.3 Hz, 1H, H-6), 7.68 (br s, 1H, NH). 13C NMR (125 MHz, CDCl_3): δ 21.0 (CH_3), 25.1 (CH_3), 26.9 (Ac-CH_3), 27.9 (Boc-C(CH_3)_3), 52.3 (C-5’), 69.6 (CHOAc), 82.8 (C-4’), 83.2 (C-3’), 84.5 (C-2’), 87.6 (C-1’), 99.2 (C-5), 114.2 (C), 117.4 (ArCH), 118.2 (ArCH), 118.9 (2 x ArCH), 121.7 (ArCH), 123.4 (C-5”), 123.7 (ArCH), 129.7 (2 x ArCH), 129.8 (ArCH), 140.3 (C-4”), 147.5 (C-6), 150.9 (C-2), 156.7 (ArC-O), 157.3 (ArC-O), 163.6 (C-4), 169.7 (Ac-C=O). LRMS-ESI: m/z = 697 [M+Na]+. HRESI-MS: m/z calcd for C_{34}H_{38}N_{6}O_{9}Na [M+Na]+: 697.2598; found 697.2606. Δ = 1.15 ppm
5’-(4-(α-Acetoxy-3-phenoxybenzyl)triazolo)-5’-deoxy-N^4,2’,3’-triacetylcytidine 204

This compound was prepared following procedure D using 167 (120 mg, 304 µmol, 1.0 equiv.), 111 (97 mg, 365 µmol, 1.2 equiv.), Cu(OAc)_2 (14 mg, 76 µmol, 0.25 equiv.) and sodium ascorbate (30 mg, 152 µmol, 0.5 equiv.) in CH_3CN/H_2O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 204 was attained as an amber film (183 mg, 91 %). [α]_D^{20} = 17.53 (c = 5 mg.mL^{-1}, CH_3OH). \[^1\]H NMR (500 MHz, CDCl_3): δ 2.06 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.11 + 2.12 (2 s, 3H, Ac), 2.25 (s, 3H, Ac), 4.53 (m, 1H, H-4’), 4.72-4.82 (m, 2H, H-5’), 5.52-5.54 (m, 2H, H-2’/H-3’), 5.67 (m, 1H, H-1’), 6.89 + 6.91 (m, 1H, ArH), 6.95-6.99 (m, 3H, ArH/CHOAc), 7.08-7.12 (m, 2H, ArH/H-5’), 7.16-7.19 (m, 1H, ArH), 7.27-7.33 (m, 3H, ArH), 7.47-7.51 (m, 2H, ArH/H-6), 9.82 (br s, 1H, NH). \[^1\]C NMR (125 MHz, CDCl_3): δ 20.3 (2 x Ac-CH_3), 20.4 + 20.4 (Ac-CH_3), 21.0 + 21.1 (Ac-CH_3), 24.8 + 24.9 (CH_3), 51.4 + 51.4 (C-5’), 69.5 + 69.5 (CHOAc), 71.0 + 71.0 (C-3’), 73.4 (C-2’), 80.5 + 80.6 (C-4’), 93.7 + 93.7 (C-1’), 97.3 + 97.4 (C-5), 117.3 + 117.3 (ArCH), 118.2 + 118.3 (ArCH), 118.9 + 119.0 (2 x ArCH), 121.7 + 121.7 (ArCH), 123.4 (C-5’), 124.0 + 124.1 (ArCH), 129.7 + 129.8 (2 x ArCH), 129.9 (ArCH), 140.1 + 140.2 (C-4”), 146.4 + 146.5 (C-6), 147.0 + 147.1 (ArC), 154.5 + 154.5 (C-2), 156.6 (ArC-O), 157.4 + 159.4 (ArC-O), 163.3 + 163.4 (C-4), 169.5 + 169.6 (2 x Ac-C=O), 169.8 + 169.8 (Ac-C=O), 170.8 + 170.8 (Ac-C=O). LRMS-ESI: m/z = 683 [M+Na]^+. HRESI-MS: m/z calcd for C_{32}H_{32}N_{10}O_{10}Na [M+Na]^+: 683.2078; found 683.2094; Δ = 2.34 ppm
This compound was prepared following procedure D using 178 (350 mg, 1.13 mmol, 1.0 equiv.), 111 (362 mg, 1.36 mmol, 1.2 equiv.), Cu(OAc)$_2$ (51 mg, 283 µmol, 0.25 equiv.) and sodium ascorbate (112 mg, 566 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 205 was attained as a dark yellow oil (573 mg, 88 %). $[\alpha]_{D}^{20} = 91.88$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, CDCl$_3$): δ 1.32 (s, 3H, CH$_3$), 1.52 (s, 3H, CH$_3$), 2.10 (s, 3H, Ac), 4.40-4.50 (m, 1H, H-4’), 4.59-4.77 (m, 2H, H-5’), 4.89-4.99 (m, 1H, H-3’), 5.00-5.08 (m, 1H, H-2’), 5.53 (s, 1H, H-1’), 5.65-5.74 (m, 1H, H-5), 6.90 (dd, J = 8.1 Hz, J = 1.4 Hz, 1H, ArH), 6.95-7.02 (m, 3H, ArH), 7.03-7.21 (m, 4H, ArH), 7.24-7.36 (m, 4H, ArH), 7.44 + 7.47 (2 s, 1H, H-5”), 7.50 (d, J = 3.4 Hz, 1H, ArH), 10.23 (br s, 1H, NH). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 21.0 (CH$_3$), 25.1 (CH$_3$), 26.8 + 26.9 (Ac-CH$_3$), 51.7 (C-5’), 69.5 + 69.5 (CHOAc), 81.6 + 81.7 (C-3’), 84.1 (C-2’), 86.0 (C-4’), 95.9 + 95.9 (C-1’), 102.8 + 102.8 (C-5), 114.7 (C$_q$), 117.3 (ArCH), 118.2 + 118.2 (ArCH), 118.8 (ArCH), 118.9 (2 x ArCH), 121.7 (ArCH), 123.4 (ArCH), 123.6 + 123.7 (C-5”), 129.7 (2 x ArCH), 129.8 (ArCH), 140.1 + 140.2 (ArC), 143.2 + 143.2 (C-6), 146.7 + 146.8 (C-4”), 150.2 (C-2), 156.6 (ArC-O), 157.3 (ArC-O), 163.5 (C-4), 169.7 + 169.8 (Ac-C=O). LRMS-ESI: m/z = 598 [M+Na]$^+$. HRESI-MS: m/z calcd for C$_{27}$H$_{27}$N$_5$O$_7$Na [M+Na]$^+$: 598.1914; found 598.1931; Δ = 2.84 ppm
This compound was prepared following procedure D using 180 (250 mg, 708 µmol, 1.0 equiv.), 111 (226 mg, 849 µmol, 1.2 equiv.), Cu(OAc)₂ (32 mg, 177 µmol, 0.25 equiv.) and sodium ascorbate (70 mg, 354 µmol, 0.5 equiv.) in CH₃CN/H₂O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 206 was attained as a pale yellow oil (631 mg, 90 %). [α]D²⁰ = 11.64 (c = 5 mg.mL⁻¹, CH₃OH). \(^1\)H NMR (500 MHz, CDCl₃): δ 2.08 + 2.08 (2 s, 6H, 2 x Ac), 2.13 + 2.14 (s, 3H, Ac), 4.42-4.47 (m, 1H, H-4’), 4.67-4.76 (m, 2H, H-5’), 4.75-4.77 (m, 1H, H-2’), 5.26-5.30 (m, 1H, H-3’), 5.30-5.37 (m, 1H, H-1’), 5.62-5.64 (m, 1H, H-5), 5.69-5.72 (m, 1H, CHOAc), 6.90 (m, 1H, ArH), 6.95-7.02 (m, 3H, ArH), 7.03-7.21 (m, 3H, ArH), 7.24-7.36 (m, 2H, ArH), 7.44-7.47 (m, 1H, H-6), 7.49 + 7.50 (2 s, 1H, H-5”), 9.63 (br s, 1H, NH). \(^13\)C NMR (125 MHz, CDCl₃): δ 20.5 (Ac-CH₃), 20.6 (Ac-CH₃), 21.1/21.2 (Ac-CH₃), 51.0 (C-5’), 69.8 (CHOAc), 70.5 (C-3’), 72.8 (C-2’), 79.9 (C-4’), 91.2/91.3 (C-1’), 102.9/103.0 (C-5), 117.3 (ArCH), 118.2 (ArCH), 118.8 (CH, ArCH), 118.9 (2 x ArCH), 121.7 (ArCH), 123.4 (C-5”), 129.6 (2 x ArCH), 129.7 (ArCH), 139.0 (ArC), 141.2 (C-6), 146.6/146.7 (C-4”), 150.0 (C-2), 157.4 (ArC-O), 158.2 (ArC-O), 162.7 (C-4), 169.5 (Ac-C=O), 169.7 (Ac-C=O), 169.8 (Ac-C=O). LRMS-ESI: m/z = 642 [M+Na]⁺. HRESI-MS: m/z calcd for C₃₀H₂₉N₅O₁₀Na [M+Na]⁺: 642.1812; found 642.1837. Δ = 3.89 ppm
This compound was prepared following procedure D using 178 (310 mg, 1.00 mmol, 1.0 equiv.), 1-(3-methoxyphenyl)propargyl acetate 106 (246 mg, 1.20 mmol, 1.2 equiv.), Cu(OAc)$_2$ (45 mg, 250 µmol, 0.25 equiv.) and sodium ascorbate (99 mg, 501 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 207 was attained as a dark yellow syrup (479 mg, 93%). $[\alpha]_D^{25} = 10.46$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 1.33 (s, 3H, CH$_3$), 1.53 (s, 3H, CH$_3$), 2.13 (s, 3H, Ac), 3.79 (s, 3H, OCH$_3$) 4.42-4.47 (m, 1H, H-4'), 4.62-4.71 (m, 2H, H-5'), 4.93 (dd, $J = 16.9$ Hz, $J = 5.1$ Hz, 1H, H-3'), 5.00-5.02 (m, 1H, H-2'), 5.51 (m, 1H, H-5), 5.70 (t, $J = 7.1$ Hz, 1H, H-1' ), 6.85 (d, $J = 8.1$ Hz, 1H, H-6), 6.96 (d, $J = 2.7$ Hz, 1H, ArH), 7.00-7.05 (m, 3H, ArH), 7.25-7.29 (m, 1H, ArH), 7.41 + 7.43 (2 s, 1H, H-5''), 9.57 (br s, 1H, NH). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 20.9 (Ac-CH$_3$), 25.2 (CH$_3$), 27.0 (CH$_3$), 51.6 (C-5'), 55.2 (OCH$_3$), 69.8 (CHOAc), 81.5 (C-3'), 84.0 (C-2'), 85.7 (C-4'), 95.8 (C-1'), 102.9 (C-5), 113.1 (ArCH), 113.6 (ArCH), 114.9 (C$_q$), 119.4 (ArCH), 123.7 (C-5''), 129.7 (ArCH), 139.8 (ArC), 143.0 (C-6), 147.1 (C-4''), 150.0 (C-2), 159.7 (ArC-O), 163.2 (C-4), 169.8 (Ac-C=O). LRESI-MS: m/z = 536 [M+Na]$^+$. HRESI-MS: m/z calcld for C$_{24}$H$_{27}$N$_5$O$_8$Na [M+Na]$^+$: 536.1757; found 536.1783; $\Delta = 4.85$ ppm
This compound was prepared following procedure D using 178 (310 mg, 1.00 mmol, 1.0 equiv.), 1-(2,5-dimethoxyphenyl)propargyl acetate 107 (280 mg, 1.20 mmol, 1.2 equiv.), Cu(OAc)$_2$ (45 mg, 250 μmol, 0.25 equiv.) and sodium ascorbate (99 mg, 501 μmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 208 was attained as an amber syrup (480 mg, 88 %). [$\alpha$]$_D^{20}$ = 16.91 (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, CDCl$_3$): δ 1.32 (s, 3H, CH$_3$), 1.53 (s, 3H, CH$_3$), 2.12 (s, 3H, Ac), 3.74 (s, 3H, OCH$_3$), 3.77 (s, 3H, OCH$_3$), 4.41-4.45 (m, 1H, H-4’), 4.65-4.66 (m, 2H, H-5”), 4.91-5.00 (m, 2H, H-2’/H-3’), 5.55 (m, 1H, H-5), 5.67-5.79 (m, 1H, H-1’), 6.81 (s, 1H, CHOAc), 6.99-7.05 (m, 1H, H-6), 7.13 (m, 1H, ArH), 7.30 (d, J = 4.9 Hz, 1H, ArH), 7.43 (d, J = 4.4 Hz, 1H, ArH), 9.83 (br s, 1H, NH).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 21.1 (Ac-CH$_3$), 25.1 (CH$_3$), 27.0 (CH$_3$), 51.4 (C-5’), 55.6 (OCH$_3$), 56.3 (OCH$_3$), 64.4 (CHOAc), 81.3 (C-3’), 83.9 (C-2’), 85.4 (C-4’), 95.3 (C-1’), 102.9 (C-5), 112.1 (ArCH), 113.6 (C$_q$), 114.0 (ArCH), 114.9 (ArC), 123.9 (C-5”), 127.8 (ArCH), 142.9 (C-6), 146.6 (C-4”), 150.0 (ArC-O), 150.4 (C-2), 153.6 (ArC-O), 163.4 (C-4), 169.8 (Ac-C=O). LRESI-MS: $m/z$ = 566 [M+Na]$^+$. HRESI-MS: $m/z$ calcd for C$_{29}$H$_{29}$N$_5$O$_9$Na [M+Na]$^+$: 592.1863; found 592.1868; Δ = 0.84 ppm
This compound was prepared following procedure D using 178 (130 mg, 420 µmol, 1.0 equiv.), 1-(3-propoxyphenyl)propargyl acetate 108 (117 mg, 504 µmol, 1.2 equiv.), Cu(OAc)$_2$ (19 mg, 105 µmol, 0.25 equiv.) and sodium ascorbate (42 mg, 210 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 209 was attained as a pale yellow syrup (205 mg, 90%).

$[\alpha]_D^{25} = 30.14$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, CDCl$_3$): δ 1.02 (t, $J = 7.8$ Hz, 3H, H-3’’’), 1.33 (s, 3H, CH$_3$), 1.53 (s, 3H, CH$_3$), 1.78 (q, $J = 6.8$ Hz, 2H, H-2’’’), 2.12 (s, 3H, Ac), 3.90 (t, $J = 6.4$ Hz, 2H, H-1’’’), 4.43-4.46 (m, 1H, H-4’), 4.62-4.71 (m, 2H, H-5’), 4.93 (dt, $J = 17.1$ Hz, $J = 4.9$ Hz, 1H, H-3’), 5.00-5.02 (m, 1H, H-2’), 5.52 (m, 1H, CHOAc), 5.69-5.71 (m, 1H, H-5), 6.84 (d, $J = 7.8$ Hz, 1H, H-1’), 6.95 (d, $J = 4.4$ Hz, 1H, ArH), 7.00-7.07 (m, 3H, ArH/H-6), 7.23-7.26 (m, 1H, ArH), 7.43 (d, $J = 7.8$ Hz, 1H, ArH), 9.83 (br s, 1H, NH). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 10.4 (C-3’’’), 21.1 (Ac-CH$_3$), 22.5 (C-2’’’), 25.2 (CH$_3$), 27.0 (CH$_3$), 51.6 + 51.7 (C-5’), 69.5 (C-1’’’), 69.8 + 69.9 (CHOAc), 81.5 + 81.6 (C-3’), 84.0 + 84.1 (C-2’), 85.8 + 85.7 (C-4’), 95.7 + 95.8 (C-1’), 102.9 (C-5), 109.9 (ArCH), 113.5 + 113.5 (C), 114.1 + 114.8 (ArCH), 119.0 + 119.1 (ArCH), 123.7 (C-5’’’), 129.6 (ArCH), 139.7 + 139.7 (ArC), 143.0 + 143.1 (C-6), 147.1 (C-4’’’), 150.1 (C-2), 159.2 (ArC-O), 163.4 (C-4), 169.8 (Ac-C=O). LRESI-MS: m/z = 564 [M+Na]$^+$. HRESI-MS: m/z calcld for C$_{26}$H$_{31}$N$_5$O$_8$Na [M+Na]$^+$: 564.2070; found 564.2088; Δ = 3.19 ppm
This compound was prepared following procedure D using 178 (350 mg, 1.13 mmol, 1.0 equiv.), 1-(3-cyclopentoxyphenyl)propargyl acetate 109 (351 mg, 1.36 mmol, 1.2 equiv.), Cu(OAc)$_2$ (51 mg, 283 µmol, 0.25 equiv.) and sodium ascorbate (112 mg, 566 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 210 was attained as a dark yellow oil (584 mg, 91 %). $[\alpha]_D^{25} = 18.58$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.33 (s, 3H, CH$_3$), 1.53 (s, 3H, CH$_3$), 1.77-1.88 (m, 8H, H-2''/H-3''/H-4''/H-5''), 2.12 + 2.12 (s, 3H, Ac), 4.45 (m, 1H, H-4'), 4.62-4.67 (m, 1H, H-5'a), 4.69-4.74 (m, 1H, H-1''/H-5'b), 4.92-4.97 (m, 1H, H-3'), 5.02-5.03 (m, 1H, H-2'), 5.55 (m, 1H, H-1'), 5.69-5.71 (m, 1H, H-5), 6.80 (d, $J = 7.8$ Hz, $J = 1.4$ Hz, 1H, ArH), 6.95-6.98 (m, 3H, CHOAc/ArH), 7.09 (d, $J = 7.8$ Hz, 1H, H-6), 7.23 (t, $J = 7.8$ Hz, 1H, ArH), 7.46 (d, $J = 6.3$ Hz, 1H, H-5''), 10.16 (br s, 1H, NH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 21.0 (Ac-CH$_3$), 23.9 (C-3''/C-4'''), 25.1 (CH$_3$), 26.9 (CH$_3$), 32.6 (C-2''/C-5'''), 51.6 (C-5'), 69.7 (CHOAc), 79.1 (C-1'''), 81.6 (C-3'), 84.0 (C-2'), 85.8 (C-4'), 95.6 (C-1'), 102.7 (C-5), 114.4 (ArCH), 114.6 (C), 114.9 (ArCH), 118.6 (ArCH), 123.6 (C-5''), 129.4 (ArCH), 139.6 (ArC), 143.1 (C-6), 147.0 (C-4''), 150.1 (C-2), 158.1 (ArC-O), 163.5 (C-4), 169.7 (Ac-C=O). LRMS-ESI: $m/z = 590$ [M+Na$^+$]. HRESI-MS: $m/z$ calcd for C$_{28}$H$_{33}$N$_5$O$_8$Na [M+H]$^+$: 590.2227; found 590.2249; $\Delta = 3.73$ ppm
This compound was prepared following procedure D using 178 (250 mg, 808 µmol, 1.0 equiv.), 1-(N-tert-butoxycarbonyl-3-indolyl)propargyl acetate 131 (319 mg, 970 µmol, 1.2 equiv.), Cu(OAc)₂ (38 mg, 202 µmol, 0.25 equiv.) and sodium ascorbate (80 mg, 404 µmol, 0.5 equiv.) in CH₃CN/H₂O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 211 was attained as a dark yellow oil (418 mg, 81%). 

$[\alpha]_{D}^{25} = 16.05$ (c = 5 mg.mL⁻¹, CH₃OH). ¹H NMR (300 MHz, CDCl₃): δ 1.32 (s, 3H, CH₃), 1.53 (s, 3H, CH₃), 1.65 (s, 9H, Boc), 3.82 (s, 3H, OCH₃), 4.43 (m, 1H, H-4’), 4.69 (m, 2H, H-5’), 4.91 (m, 1H, H-3’), 4.99-5.02 (m, 1H, H-2’), 5.46 (m, 1H, H-1’), 5.69 (m, 1H, OH), 6.99-7.13 (m, 2H, H-6/ArH), 7.27 (m, 1H, ArH), 7.47-7.73 (m, 3H, H-5’/ArH), 8.12 (m, 1H, ArH), 9.71 (br s, 1H, NH). ¹³C NMR (75 MHz, CDCl₃): δ 25.3 + 25.3 (CH₃), 27.1 + 27.1 (CH₃), 28.1 (Boc-C(CH₃)₃), 51.6 + 51.9 (C-5’), 53.8 + 53.8 (OCH₃), 81.2 + 81.5 (C-3’), 84.0 + 84.1 (C-2’), 84.3 (Boc-C(CH₃)₃), 85.5 + 85.9 (C-4’), 95.8 (C-1’), 96.2 (C-2’), 102.9 + 103.0 (C-5), 114.9 (C), 115.3 (ArCH), 121.1 + 121.2 (ArCH), 122.6 + 122.7 (C-5’), 124.5 + 124.6 (ArCH), 125.3 (ArCH), 125.5 (ArCH), 127.9 + 127.9 (ArC), 134.0 (ArC), 136.0 + 136.1 (ArC), 143.2 (C-6), 143.4 (C-4’), 149.6 + 149.6 (Boc-C=O), 150.0 + 150.1 (C-2), 163.2 + 163.3 (C-4), 171.2 (C=O). LRMS-ESI: m/z = 661 [M+Na]⁺. HRESI-MS: m/z calcd for C₃₀H₃₄N₆O₁₀Na [M+Na]⁺: 661.2234; found 661.2251; Δ = 2.57 ppm
This compound was prepared following procedure D using 180 (220 mg, 623 μmol, 1.0 equiv.), 1-(3-propoxyphenyl)propargyl acetate 108 (173 mg, 747 μmol, 1.2 equiv.), Cu(OAc)$_2$ (28 mg, 156 μmol, 0.25 equiv.) and sodium ascorbate (62 mg, 311 μmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 212 was attained as a dark amber syrup (328 mg, 90%). 

$[\alpha]_D^{20} = 12.56$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, CDCl$_3$): δ 1.02 + 1.02 (2 t, $J = 7.5$Hz, 3H, H-3’’’), 1.76-1.81 (m, 2H, H-2’’’’), 2.08 + 2.09 (2 s, 6H, 2 x Ac), 2.14 + 2.14 (2 s, 3H, Ac), 3.92 + 3.92 (2 t, $J = 6.4$ Hz, 2H, H-1’’’’), 4.41-4.46 (m, 1H, H-4’’), 4.66-475 (m, 2H, H-5’’), 5.27-5.32 (m, 1H, H-2’’), 5.35-5.38 + 5.40-5.43 (m, 1H, H-3’’), 5.62-5.63 (m, 1H, H-5), 5.69-5.72 (m, 1H, H-1’’), 6.84-6.86 (m, 1H, ArH), 6.94-7.03 (m, 4H, ArH, H-6/CHOAc), 7.25-7.28 (m, 1H, ArH), 7.50 + 7.53 (2 s, 1H, H-5’’’), 8.95 (br s, 1H, NH). 

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 10.5 (C-3’’’’), 20.4 (Ac-CH$_3$), 20.4 (Ac-CH$_3$), 21.1 (Ac-CH$_3$), 22.5 (C-2’’’’), 50.9 (C-5’’), 69.6 (C-1’’’’), 69.9 (CHOAc), 70.5 (C-3’’), 72.7 (C-2’’), 79.9 (C-4’’), 91.4 (C-1’), 103.4 (C-5), 113.6 (ArCH), 114.3 (ArC), 119.1 (ArCH), 124.1 (C-5’’’), 129.7 (ArCH), 139.7 (ArC), 141.3 (C-6), 147.5 (C-4’’’), 149.5 (C-2), 159.3 (ArC-O), 162.6 (C-4), 169.5 (Ac-C=O), 169.6 (Ac-C=O), 169.8 (Ac-C=O). LRESI-MS: m/z = 708 [M+Na]$^+$. HRESI-MS: m/z calcd for C$_{27}$H$_{31}$N$_5$O$_{10}$Na [M+Na]$^+$: 608.1969; found 608.2043; Δ = 12.17 ppm
This compound was prepared following procedure D using 180 (250 mg, 708 µmol, 1.0 equiv.), 1-(3-cyclohexoxyphenyl)propargyl acetate 109 (220 mg, 849 µmol, 1.2 equiv.), Cu(OAc)$_2$ (32 mg, 177 µmol, 0.25 equiv.) and sodium ascorbate (70 mg, 354 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 213 was attained as a dark yellow oil (385 mg, 89 %). $[\alpha]_D^{20} = 37.44$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, CDCl$_3$): δ 1.75-1.89 (m, 8H, H-2''/H-3''/H-4''/H-5''), 2.09 (m, 6H, 2 x Ac), 2.15 (s, 3H, Ac), 4.41-4.45 (m, 1H, H-4’), 4.65-4.72 (m, 2H, H-5’), 4.74-4.78 (m, 1H, H-2’), 5.25-5.29 (m, 1H, H-3’), 5.33-5.41 (m, 1H, H-1’), 5.61-5.63 (m, 1H, H-5), 5.69-5.72 (m, 1H, CHOAc), 6.82-6.84 (m, 1H, H-1’), 6.93 (d, $J = 8.0$ Hz, 1H, H-6), 6.95-7.00 (m, 3H, ArH), 7.24-7.27 (m, 1H, ArH), 7.46 + 7.50 (2 s, 1H, H-5’’), 8.56 (br s, 1H, NH). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 20.4 (Ac-CH$_3$), 20.4 (Ac-CH$_3$), 21.2 (Ac-CH$_3$), 24.0 (2 x CH$_2$), 32.8 (2 x CH$_2$), 50.9 (C-5’), 69.9 + 70.6 (CHOAc), 72.7 (C-3’), 79.3 (C-2’), 80.0 (C-4’), 91.3 (C-1’), 103.4 (C-5), 114.7 (ArCH), 115.2 (ArCH), 118.8 (ArCH), 124.1 (C-5’’), 129.7 (ArCH), 139.6 (ArCH), 141.3 (C-6), 147.6 (C-4’’), 149.6 (C-2), 158.3 (ArC-O), 161.8 (C-4), 169.6 (2 x Ac-C=O), 169.8 (Ac-C=O). LRMS-ESI: m/z = 634 [M+Na]$^+$. HRESI-MS: m/z calcd for C$_{29}$H$_{33}$N$_5$O$_{10}$Na [M+H]$^+$: 634.2125; found 634.2142; $\Delta = 2.68$ ppm
This compound was prepared following procedure D using 180 (250 mg, 708 µmol, 1.0 equiv.), 1-(3-cyclopent oxy-4-methoxyphenyl)propargyl acetate 110 (250 mg, 849 µmol, 1.2 equiv.), Cu(OAc)$_2$ (32 mg, 177 µmol, 0.25 equiv.) and sodium ascorbate (70 mg, 354 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 214 was attained as a dark yellow oil (381 mg, 84%).

$[α]_D^{20} = 15.37$ (c = 5 mg.mL$^{-1}$, CH$_3$OH).

$^1$H NMR (500 MHz, CDCl$_3$): δ 1.56-1.59 (m, 2H, H-3'"/H-4'"), 1.77-1.90(m, 6H, H-2"'/H-3"'/H-4"'/H-5"'), 2.06 (m, 3H, Ac), 2.07 (m, 3H, Ac), 2.11 (s, 3H, Ac), 3.81 (s, 3H, OCH$_3$), 4.41-4.46 (m, 1H, H-4'), 4.66-4.72 (m, 2H, H-5'), 4.74-4.78 (m, 1H, H-2'), 5.27-5.31 (m, 1H, H-3'), 5.35-5.42 (m, 1H, H-1'), 5.63 (m, 1H, H-5), 5.68-5.74 (m, 1H, CHOAc), 6.81-6.83 (m, 1H, ArH), 6.89-6.92 (m, 1H, H-6), 6.95-7.03 (m, 2H, ArH), 7.04-7.06 (m, 1H, ArH), 7.50 + 7.52 (2 s, 1H, H-5"'), 9.67 (br s, 1H, NH).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 20.2 (Ac-CH$_3$), 20.3 (Ac-CH$_3$), 21.1 (Ac-CH$_3$), 23.9 (C-3"'/C-4"'), 32.6 (C-2"'/C-5"'), 50.9 (C-5'), 56.0 (OCH$_3$), 69.8 (CHOAc), 70.4 (C-3'), 72.7 (C-2'), 79.9 + 80.5 (C-4'), 91.3 (C-1'), 103.2 (C-5), 111.7 (ArCH), 114.3 (ArCH), 119.7 (ArCH), 123.9 (C-5”), 130.5 (ArC), 141.1 (C-6), 147.6 (C-4”), 149.6 (ArC-O), 150.1 (C-2), 157.1 (ArC-O), 162.9 (C-4), 169.6 (Ac-C=O), 169.9 (Ac-C=O), 170.0 (Ac-C=O). LRMS-ESI: m/z = 663 [M+Na]$^+$. HRESI-MS: m/z calced for C$_{36}$H$_{35}$N$_5$O$_{11}$Na [M+Na]$^+$: 666.2231; found 666.2246; Δ = 2.25 ppm
This compound was prepared following procedure \textbf{D} using \textit{180} (200 mg, 566 µmol, 1.0 equiv.), 1-(3-phenoxynaphthyl)propargyl acetate \textit{112} (178 mg, 679 µmol, 1.2 equiv.), Cu(OAc)$_2$ (26 mg, 141 µmol, 0.25 equiv.) and sodium ascorbate (56 mg, 283 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound \textbf{215} was attained as a dark yellow oil (310 mg, 89 %). \[\alpha\]$_{D}^{20}$ = 9.22 (c = 5 mg.mL$^{-1}$, CH$_3$OH). \textbf{1H NMR (300 MHz, CDCl$_3$): }δ 2.07 (Ac), 2.08 (Ac), 2.23 (Ac), 3.81 (s, 3H, OCH$_3$), 4.43-4.47 (m, 1H, H-4'), 4.66-4.76 (m, 2H, H-5'), 4.91-4.97 (m, 2H, H-3'), 5.01-5.05 (m, 1H, H-2'), 5.56-5.59 (m, 1H, H-1'), 5.71 + 5.72 (d, J = 8.1 Hz, 1H, H-5), 6.65-6.68 (m, 1H, CHOAc), 6.95-6.97 (m, 2H, ArH), 7.01-7.09 (m, 3H, ArH), 7.13-7.21 (m, 2H, ArH), 7.28-7.31 (m, 2H, ArH/H-6), 7.62 + 7.63 (m, 1H, H-5’’), 9.87 (br s, 1H, NH). \textbf{13C NMR (75 MHz, CDCl$_3$): }δ 20.7 (Ac-CH$_3$), 20.8 (Ac-CH$_3$), 21.0 (Ac-CH$_3$), 51.7 + 51.8 (C-5’’), 56.0 + 56.1 (OCH$_3$), 69.5 + 69.6 (CHOAc), 81.7 + 81.8 (C-3’’), 84.1 + 84.2 (C-2’’), 85.9 + 86.0 (C-4’’), 95.9 +96.0 (C-1’’), 102.9 +103.0 (C-5), 112.8 (ArCH, ), 114.9 +115.0 (ArCH), 116.9 +117.0 (2 x ArCH), 122.5 (ArCH), 123.5 + 123.6 (C-5’’’), 124.1 (ArCH), 129.5 (2 x ArCH), 131.2 + 131.3 (ArC), 141.5 + 141.6 (C-6), 147.1 + 147.2 (C-4’’’), 150.1 + 150.2 (C-2), 151.6 (ArC=O), 157.0 (ArC=O), 158.0 (ArC=O), 163.4 (C-4), 169.6 (Ac-C=O), 169.8 (Ac-C=O), 169.9 (Ac-C=O). \textbf{LRMS-ESI: }m/z = 672 [M+Na]$^+$, \textbf{HRESI-MS: }m/z calcd for C$_{31}$H$_{31}$N$_{11}$O$_{11}$Na [M+Na]$^+$: 672.1918; found 672.1913; Δ = 0.74 ppm
This compound was prepared following procedure D using 180 (160 mg, 453 µmol, 1.0 equiv.), 1-(3-phenoxymethyl)propargyl acetate 113 (154 mg, 543 µmol, 1.2 equiv.), Cu(OAc)$_2$ (20 mg, 113 µmol, 0.25 equiv.) and sodium ascorbate (45 mg, 226 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 216 was attained as a dark yellow oil (251 mg, 87%). $[\alpha]_{D}^{20} = 14.86$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (300 MHz, CDCl$_3$): δ 2.08 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.24 (s, 3H, Ac), 4.43-4.47 (m, 1H, H-4'), 4.66-4.76 (m, 2H, H-5'), 5.35-5.44 (m, 2H, H-3'/H-2'), 5.59 (m, 1H, H-1'), 5.71-5.75 (m, 1H, H-5), 6.90-6.92 (m, 1H, CHOAc), 6.94-6.96 (m, 2H, ArH), 7.04-7.10 (m, 2H, H-6/ArH), 7.14-7.24 (m, 3H, ArH), 7.29-7.32 (m, 2H, ArH), 7.59 + 7.61 (2 s, 1H, H-5''), 9.87 (br s, 1H, NH). $^{13}$C NMR (75 MHz, CDCl$_3$): δ 20.3 (Ac-CH$_3$), 20.3 (Ac-CH$_3$), 21.0 (Ac-CH$_3$), 51.1 (C-5'), 68.9 (CHOAc), 70.5 (C-3'), 72.8 (C-2'), 79.9 (C-4'), 91.9 (C-1'), 103.2 (C-5), 117.0 (2 x ArCH), 117.2 (ArCH), 120.9 (d, J = 6.0 Hz, ArCH), 123.2 (C-5''), 123.7 (d, J = 7.4 Hz, ArCH), 124.1 (d, J = 11.6 Hz, ArCH), 129.7 (2 x ArCH), 135.0 (d, J = 3.7 Hz, ArC-F), 141.8 (C-6), 143.4 (d, J = 11.6 Hz, ArCH), 146.7 (C-4''), 150.1 (C-2), 154.0 (d, J = 250.3 Hz, ArC-F), 157.0 (2 x ArC-O), 163.1 (C-4), 169.6 (Ac-C=O), 169.7 (Ac-C=O), 169.8 (Ac-C=O). LRMS-ESI: $m/z$ = 660 [M+Na]$^+$. HRESI-MS: $m/z$ calcd for C$_{30}$H$_{28}$FN$_3$O$_{10}$Na [M+Na]$^+$: 660.1718; found 660.1713; Δ = 0.76 ppm
This compound was prepared following procedure D using 180 (140 mg, 397 µmol, 1.0 equiv.), 1-(3-phenoxyphenyl)propargyl acetate 114 (135 mg, 475 µmol, 1.2 equiv.), Cu(OAc)$_2$ (18 mg, 99 µmol, 0.25 equiv.) and sodium ascorbate (39 mg, 198 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 217 was attained as a yellow oil (240 mg, 95%). [$\alpha$]$^D_{20}$ = 25.24 (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 2.08 + 2.08 (2 s, 3H, Ac), 2.09 (s, 3H, Ac), 2.13 + 2.13 (2 s, 3H, Ac), 4.44 - 4.49 (m, 1H, H-4’), 4.68-4.79 (m, 2H, H-5’), 5.34-5.38 (m, 1H, H-3’), 5.40-5.47 (m, 1H, H-2’), 5.65 (d, $J$ = 3.9 Hz, 1H, H-5), 5.70-5.73 (m, 1H, H-1’), 6.86-6.88 (m, 1H, ArH), 6.97-7.08 (m, 6H, H-6, CHOAc/ArH), 7.09-7.12 (m, 1H, ArH), 7.17-7.20 (m, 1H, ArH), 7.28-7.32 (m, 1H, ArH), 7.59 + 7.61 (2 s, 1H, H-5”), 9.83 (br s, 1H, NH). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 20.2 (Ac-CH$_3$), 20.3 (Ac-CH$_3$), 20.9 + 21.0 (Ac-CH$_3$), 51.0 + 51.0 (C-5’), 69.4 + 69.5 (CHOAc), 70.4 + 70.5 (C-3’), 72.7 + 7.2.7 (C-2’), 79.9 + 80.0 (C-4’), 91.4 + 91.5 (C-1’), 103.2 + 103.2 (C-5), 116.3 (d, $J$ = 23.5 Hz, 2 x ArCH), 116.8 + 116.8 (ArCH), 117.6 + 117.6 (ArCH), 120.6 + 120.7 (2 x ArCH), 121.6 + 121.6 (ArCH), 124.0 + 124.1 (C-5”), 129.9 (ArCH), 140.1 + 140.2 (C-6), 141.5 (ArC), 146.9 (C-4”), 150.1 + 150.1 (C-2), 152.3 + 152.3 (ArC-O), 157.6 + 157.8 (ArC-O), 158.9 (d, $J$ = 225.9 Hz, C-F), 163.0 (C-4), 169.6 (Ac-C=O), 169.6 (Ac-C=O), 169.8 (Ac-C=O). LRMS-ESI: $m/z$ = 660 [M+Na]$^+$. HRESI-MS: $m/z$ calcld for C$_{30}$H$_{28}$F$_{3}$N$_{5}$O$_{10}$Na [M+Na]$^+$: 660.1718; found 660.1733; $\Delta$ = 2.27 ppm
This compound was prepared following procedure D using 180 (300 mg, 849 μmol, 1.0 equiv.), 1-benzothienylpropargyl acetate 116 (235 mg, 1.02 mmol, 1.2 equiv.), Cu(OAc)₂ (38 mg, 212 μmol, 0.25 equiv.) and sodium ascorbate (84 mg, 424 μmol, 0.5 equiv.) in CH₃CN/H₂O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 218 was attained as a dark yellow oil (456 mg, 92%). [α]D²⁰ = 32.08 (c = 5 mg.mL⁻¹, CH₃OH). ¹H NMR (500 MHz, CDCl₃): δ 2.09 (s, 6H, 2x Ac), 2.16 (s, 3H, Ac), 4.45-4.49 (m, 1H, H-4'), 4.70-4.80 (m, 2H, H-5'), 5.31-5.33 (m, 1H, H-2'), 5.38-5.45 (m, 1H, H-3'), 5.60-5.63 (m, 1H, H-1'), 5.70 + 5.70 (2d, J = 8.0 Hz, 1H, H-5), 7.01 + 7.04 (2d, J = 8.0 Hz, 1H, H-6), 7.31-7.38 (m, 4H, CHOAc/ArCH), 7.72-7.74 (m, 2H, ArCH), 7.78 + 7.80 (2s, 1H, H-5''), 9.23-9.25 (m, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 20.3 (Ac-CH₃), 20.4 (Ac-CH₃), 21.0 + 21.1 (Ac-CH₃), 51.1 + 51.2 (C-5''), 66.2 + 66.3 (CHOAc), 70.5 + 70.6 (C-3''), 72.7 + 72.7 (C-2''), 80.0 + 80.1 (C-4''), 91.6 (C-1'), 103.3 + 103.4 (C-5), 122.3 (C-3''''), 123.8 + 123.8 (C-5'''), 124.0 (CHOAc), 124.3 + 124.3 (C-4''''), 124.6 (C-5''''), 124.9 (C-6''''), 138.9 (ArC), 139.8 + 139.9 (ArC), 141.2 + 141.2 (C-6), 141.5 + 141.5 (ArC), 146.3 + 146.3 (C-4''), 149.9 + 150.0 (C-2), 162.7 + 162.7 (C-4), 169.6 (Ac-C=O), 169.7 + 169.8 (Ac-C=O), 169.8 (Ac-C=O). LRMS-ESI: m/z = 606 [M+Na]+. HRESI-MS: m/z calcd for C₂₆H₂₅N₅O₉SNa [M+Na]+: 606.1271; found 606.1276; Δ = 0.82 ppm
5'-[(α-Acetoxy-(N-tert-butoxycarbonyl-3-yl)methyl)triazolo]-5'-deoxy-2',3'-O-isopropylidenyluridine 219

This compound was prepared following procedure D using 180 (350 mg, 991 µmol, 1.0 equiv.), 1-(N-tert-butoxycarbonyl-3-indolyl)propargyl acetate 130 (372 mg, 1.19 mmol, 1.2 equiv.), Cu(OAc)₂ (45 mg, 248 µmol, 0.25 equiv.) and sodium ascorbate (98 mg, 495 µmol, 0.5 equiv.) in CH₃CN/H₂O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 219 was attained as a pale yellow oil (594 mg, 90 %). [α]D²⁰ = 23.81 (c = 5 mg.mL⁻¹, CH₃OH). 

1H NMR (500 MHz, CDCl₃): δ 1.67 (s, 9H, Boc), 2.10 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.14 (s, 3H, Ac), 4.42-4.46 (m, 1H, H₄'), 4.64-4.70 (m, 1H, H₅'a), 4.76-4.79 (m, 1H, H₂'/H₃'), 5.30-5.44 (m, 2H, H₂'/H₃'), 5.57-5.58 (m, 1H, H₅), 5.69-5.73 (m, 1H, H₁'), 6.99-7.03 (m, 1H, CHOH), 7.21 (t, J = 7.3 Hz, 1H, ArH), 7.30-7.34 (m, 2H, ArH), 7.53-7.56 (m, 1H, ArH), 7.65-7.66 (m, 1H, H₆), 7.74 + 7.76 (2 s, 1H, H₈’), 8.12-8.13 (m, 1H, ArH), 8.92 (br s, 1H, NH).

13C NMR (125 MHz, CDCl₃): δ 20.3 (Ac-CH₃), 20.4 (Ac-CH₃), 21.1 (Ac-CH₃), 28.1 (Boc-C(CH₃)₃), 50.8 + 51.0 (C-5’), 63.8 + 63.9 (CHOAc), 70.2 + 70.5 (C-3’), 72.8 + 72.9 (C-2’), 79.9 + 80.0 (C-4’), 84.3 (Boc-C(CH₃)₃), 91.9 (C-1’), 103.3 + 103.3 (C-5), 115.5 (ArCH), 119.8 (ArCH), 122.8 (ArCH), 124.1 + 124.3 (C-5’’), 124.8 (ArCH), 125.2 (ArCH), 128.2 (ArC), 136.5 (ArC), 141.6 (C-6), 146.3 (C-4’’), 146.4 (C), 149.7 + 149.7 (C-2), 162.5 (C-4), 169.6 (Ac-C=O), 170.0 (Ac-C=O), 170.2 (Ac-C=O). LRMS-ESI: m/z = 645 [M+Na]+. HRESI-MS: m/z calcd for C₃₁H₃₄N₆O₁₁Na [M+Na]+: 689.2183; found 689.2198; Δ = 2.18 ppm
This compound was prepared following procedure D using 180 (250 mg, 707 µmol, 1.0 equiv.), 1-(N-tert-butoxycarbonyl-3-indolyl)propargyl acetate 131 (280 mg, 849 µmol, 1.2 equiv.), Cu(OAc)$_2$ (32 mg, 177 µmol, 0.25 equiv.) and sodium ascorbate (64 mg, 354 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 220 was attained as a dark yellow oil (391 mg, 81%). $[\alpha]_D^{20} = 33.40$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (300 MHz, CDCl$_3$): δ 1.67 (s, 9H, Boc), 2.10 (s, 3H, Ac), 2.12 (s, 3H, Ac), 3.85 (s, 3H, OCH$_3$), 4.43 (m, 1H, H-4’), 4.58-4.80 (m, 3H, OH, H-5’), 5.25-5.32 (m, 1H, H-2’), 5.39-5.40 (m, 1H, H-3’), 5.55-5.60 (m, 1H, H-1’), 5.69-5.75 (m, 1H, H-5), 6.92-7.01 (m, 1H, H-6), 7.13-7.19 (m, 1H, ArH), 7.29-7.33 (m, 2H, ArH), 7.47-7.52 (m, 1H, ArH), 7.65-7.77 (m, 2H, ArH/H-5”), 8.13-8.15 (m, 1H, ArH), 8.72 (br s, 1H, NH). $^{13}$C NMR (75 MHz, CDCl$_3$): δ 20.4 (Ac-CH$_3$), 20.4 (Ac-CH$_3$), 28.1 (Boc-C(CH$_3$)$_3$), 50.8 (C-5’), 53.9 (OCH$_3$), 70.4 (C-3’), 72.8 (C-2’), 79.9 (C-4’), 84.4 (Boc-C(CH$_3$)$_3$), 91.6 (C-1’) 103.4 (C-5), 115.3 (ArCH), 121.1 (ArCH), 122.7 (ArCH), 124.6 (C-5”), 136.0 (ArC), 143.9 (C-4”), 149.6 (Boc-C=O), 149.8 (C-2), 162.3 (C-4), 169.6 (Ac-C=O), 169.6 (Ac-C=O). LRMS-ESI: m/z = 705 [M+Na]$^+$. HRESI-MS: m/z calcd for C$_{31}$H$_{44}$N$_6$O$_{12}$Na [M+Na]$^+$: 705.2132; found 705.2140; Δ = 1.13 ppm
This compound was prepared following procedure D using 180 (300 mg, 849 µmol, 1.0 equiv.), 3-Acetoxy-3-ethynylindanone 133 (176 mg, 1.02 mmol, 1.2 equiv.), Cu(OAc)$_2$ (38 mg, 212 µmol, 0.25 equiv.) and sodium ascorbate (84 mg, 424 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 221 was attained as a dark yellow syrup (375 mg, 84%). $\alpha$$_D$ = 49.20 (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.00 + 2.02 (s, 3H, Ac), 2.04 + 2.04 (s, 3H, Ac), 4.42-4.48 (m, 1H, H-4’), 4.77-4.80 (m, 2H, H-5’), 5.37-5.46 (m, 2H, H-2’/H-3’), 5.67-5.70 (m, 1H, H-5), 5.86-5.89 (m, 1H, H-1’), 6.69 (m, 1H, OH), 6.84-6.86 (m, 1H, ArH), 6.93-6.97 (m, 1H, ArH), 7.20-7.25 (m, 2H, ArH), 7.59 + 7.68 (d, J = 8.3 Hz, 1H, H-6), 8.12 + 8.13 (s, 1H, H-5”), 10.42 + 10.43 (s, 1H, NH), 11.47 (br s, 1H, NH). $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ 20.1 (Ac-CH$_3$), 20.2 (Ac-CH$_3$), 50.6 + 50.7 (C-5’), 71.5 + 71.6 (C-3’), 72.9 + 73.0 (C-2’), 78.6 + 78.9 (C), 79.2 + 79.3 (C-4’), 88.0 + 88.6 (C-1’), 102.4 + 102.5 (C-5), 109.8 (ArCH), 121.8 + 121.8 (ArCH), 123.9 + 124.0 (C-5”), 125.2 + 125.2 (ArCH), 129.3 (ArCH), 132.0 (ArC), 141.6 + 141.8 (ArC), 141.8 (C-6), 148.1 (C-4”), 150.3 + 150.3 (C-2), 162.9 + 163.0 (C-4), 169.2 (C=O), 169.3 (C=O), 177.2 (C=O). LRMS-ESI: $m/z$ = 549 [M+Na]$^+$. HRESI-MS: $m/z$ calcd for C$_{21}$H$_{22}$N$_6$O$_9$Na [M+Na]$^+$: 549.1346; found 549.1351; $\Delta$ = 0.91 ppm
This compound was prepared following procedure D using 180 (250 mg, 707 µmol, 1.0 equiv.), 2-(phenyl)propargyl acetate 136 (206 mg, 849 µmol, 1.2 equiv.), Cu(OAc)$_2$ (32 mg, 177 µmol, 0.25 equiv.) and sodium ascorbate (64 mg, 354 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 222 was attained as a pale yellow oil (409 mg, 97%). 

$[\alpha]^{20}_D = 29.53$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 2.07 (Ac), 2.10 (Ac), 2.23 (Ac), 4.43-4.50 (m, 1H, H-4'), 4.72-4.88 (m, 2H, H-5'), 5.09-5.11 + 5.22-5.26.42 (m, 1H, H-3’), 5.22-5.26 + 5.39-5.42 (m, 1H, H-2’), 5.44-5.45 + 5.66-5.67 (m, 1H, H-5), 5.72-5.73 + 5.80-5.81 (m, 1H, H-1’), 6.95-6.97 + 7.03-7.04 (m, 1H, H-6), 7.39-7.43 (m, 3H, ArH), 7.46-7.50 (m, 2H, ArH), 7.82-7.84 (m, 1H, H-5''), 9.34-9.39 (m, 1H, NH).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 20.3 (Ac-CH$_3$), 21.7 (Ac-CH$_3$), 50.6 + 50.9 (C-5’), 70.0 + 70.5 (C-3’), 72.0 + 72.4 (C-2’), 79.7 + 80.0 (C-4’), 89.3 + 90.4 (C-1’), 103.4 + 103.5 (C-5), 127.3 + 127.4 (C-2’’’/C-6’’’), 127.3-127.5 (m, CF$_3$), 128.2 + 128.3 (C-3’’’/C-5’’’), 129.3 + 129.5 (C-4’’’), 132.7 + 133.1 (ArC), 140.8 + 141.2 (C-6), 142.6 + 142.9 (C-4”), 150.2 + 150.2 (C-2), 162.7 + 162.8 (C-4), 167.7 + 168.0 (C=O), 169.4 + 169.6 (C=O), 169.6 + 169.7 (C=O). LRMS-ESI: $m/z$ = 618 [M+Na]$^+$. HRESI-MS: $m/z$ caled for C$_{25}$H$_{24}$N$_5$O$_9$Na [M+Na]$^+$: 618.1424; found 618.1437; $\Delta = 2.10$ ppm.
This compound was prepared following procedure D using 5’-Azido-3’-acetyl-5’-deoxythymidine 189 (200 mg, 647 µmol, 1.0 equiv.), 1-(3-phenoxyphe)nlypropargyl acetate 111 (207 mg, 776 µmol, 1.2 equiv.), Cu(OAc)₂ (29 mg, 162 µmol, 0.25 equiv.) and sodium ascorbate (64 mg, 323 µmol, 0.5 equiv.) in CH₃CN/H₂O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 223 was attained as a colourless syrup (350 mg, 94%). [α]D²⁰ = 48.27 (c = 5 mg.mL⁻¹, CH₃OH). ¹H NMR (500 MHz, CDCl₃): δ 1.89 (d, J = 0.9 Hz, 3H, CH₃), 2.10 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.13-2.17 (m, 1H, H-2’a), 2.32-2.36 (m, 1H, H-2’b), 4.27-4.30 (m, 1H, H-4’), 4.72-4.73 (m, 2H, H-5’), 5.21-5.29 (m, 1H, H-3’), 6.14-6.18 (m, 1H, H-1’), 6.87 (m, 1H, CHOAc), 6.92-6.94 (m, 1H, ArH), 6.96-7.01 (m, 3H, ArH), 7.10-7.12 (m, 2H, ArH), 7.17-7.18 (m, 1H, ArH), 7.30-7.35 (m, 3H, ArH), 7.51 (d, J = 9.8 Hz, 1H, ArH), 8.38. (br s, 1H, NH). ¹³C NMR (125 MHz, CDCl₃): δ 12.4 (CH₃), 20.8 (Ac-CH₃), 21.1 + 21.1 (Ac-CH₃), 36.1 (C-2’), 51.3 + 51.4 (C-5’), 69.6 (CHOAc), 74.2 + 74.4 (C-3’), 82.1 (C-4’), 85.4 (C-1’), 111.9 (C-5), 117.3 + 117.4 (ArCH), 118.4 (ArCH), 119.0 (2 x ArCH), 121.7 (ArCH), 123.6 (C-5’’), 124.0 + 124.1 (ArCH), 129.8 (2 x ArCH), 130.0 (ArCH), 135.4 + 135.5 (C-6), 140.0 + 140.1 (C-6), 147.2 (C-4’’), 150.0 (C-2), 156.7 (ArC-O), 157.6 (ArC-O), 163.0 (C-4), 169.7 (Ac-C=O), 170.7 (Ac-C=O). LRMS-ESI: m/z = 598 [M+Na]⁺. HRESI-MS: m/z calcd for C₂₉H₂₉N₅O₈Na [M+Na]⁺: 598.1914; found 598.1917; Δ = 0.50 ppm
This compound was prepared following procedure D using 5'-Azido-3'-acetyl-2',5'-dideoxyuridine 191 (245 mg, 830 μmol, 1.0 equiv.), 1-(3-phenoxyphenyl)propargyl acetate 111 (265 mg, 995 μmol, 1.2 equiv.), Cu(OAc)$_2$ (38 mg, 207 μmol, 0.25 equiv.) and sodium ascorbate (82 mg, 415 μmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 224 was attained as a pale yellow oil (394 mg, 84%). [α]$_D^{20}$ = 43.61 (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, CDCl$_3$): δ 2.02-2.06 (m, 1H, H-2’a), 2.09 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.30-2.36 (m, 1H, H-2’b), 4.28 (m, 1H, H-4’), 4.66-4.70 (m, 1H, H-5’a), 4.75-4.80 (m, 1H, H-5’b), 5.25-5.31 (m, 1H, H-3’), 5.67-5.70 (m, 1H, H-5), 6.16 (t, J = 6.7 Hz, 1H, H-1’), 6.91-7.00 (m, 4H, ArH), 7.04-7.06 (m, 1H, ArH), 7.08-7.12 (m, 2H, ArH), 7.16-7.19 (m, 1H, H-6), 7.29-7.33 (m, 3H, ArH), 7.52-7.56 (m, 1H, ArH), 9.88 (br s, 1H, NH). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 20.6 (Ac-CH$_3$), 21.0 (Ac-CH$_3$), 36.1 (C-2’), 51.1 (C-5’), 69.5 (CHOAc), 74.2 (C-3’), 82.1 (C-4’), 85.2 (C-1’), 103.2 (C-5), 117.2 (ArCH), 118.3 (ArCH), 119.0 (2 x ArCH), 121.6 (ArCH), 123.5 (C-5’), 125.8 (ArCH), 127.7 (ArCH), 128.0 (ArCH), 128.8 (ArCH), 129.4 (ArCH), 129.7 (2 x ArCH), 129.9 (ArCH), 134.5 (ArCH), 136.4 (ArCH), 140.0 (C-6), 147.0 (C-4’), 150.3 (C-2), 156.5 (ArC-O), 157.5 (ArC-O), 163.1 (C-4), 169.6 (Ac-C=O), 170.5 (Ac-C=O). LRMS-ESI: m/z = 584 [M+Na]$^+$, HRESI-MS: m/z calcd for C$_{28}$H$_{27}$N$_{5}$O$_{8}$Na [M+Na]$^+$: 584.1757; found 584.1749; Δ = 1.37 ppm
This compound was prepared following procedure D using 5’-Azido-2’,5’-dideoxy-2’-fluoro-2’,3’-diacetyluridine 194 (220 mg, 702 µmol, 1.0 equiv.), 1-(3-phenoxy-phenyl)propargyl acetate 111 (224 mg, 843 µmol, 1.2 equiv.), Cu(OAc)$_2$ (32 mg, 176 µmol, 0.25 equiv.) and sodium ascorbate (69 mg, 351 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 225 was attained as an off-white powder (382 mg, 94 %). [$\alpha$]$^D_{20} = 69.28$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). 1H NMR (400 MHz, CDCl$_3$): $\delta$ 2.09 (s, 3H, Ac), 2.11 (s, 3H, Ac), 4.46-4.52 (m, 1H, H$^{-4'}$), 4.62-4.67 (m, 1H, H$^{-5'}_a$), 4.71-4.77 (m, 1H, H$^{-5'}_b$), 5.22-5.33 (m, 1H, H$^{-3'}$), 5.37-5.40 (m, 1H, H$^{-2'}$), 5.69-5.72 (m, 1H, H$^{-3'}$), 5.89-5.92 (m, 1H, H-5), 6.89-6.92 (m, 1H, ArH), 6.97 (m, 1H, CHOAc), 6.98-6.99 (m, 1H, H$^{-1'}$), 7.00-7.01 (m, 1H, ArH), 7.02-7.06 (m, 1H, ArH), 7.08-7.10 (m, 1H, H-6), 7.12-7.14 (m, 1H, ArH), 7.17-7.20 (m, 1H, ArH), 7.28-7.34 (m, 3H, ArH/H$^{-5''}$), 7.55 (m, 1H, H-6), 9.68 (br s, 1H, NH). 13C NMR (400 MHz, CDCl$_3$): $\delta$ 20.2 (Ac-CH$_3$), 21.0 (Ac-CH$_3$), 50.8 (C$^{-5'}$), 69.6 (CHOAc), 71.0 (d, J = 15.5 Hz, C$^{-3'}$), 79.0 (C$^{-4'}$), 90.7 (d, J = 190.5 Hz, C$^{-2'}$), 93.8 (d, J = 37.7 Hz, C$^{-3'}$), 94.0 (C$^{-1'}$), 103.3 (C-5), 117.4 (ArCH), 118.3 (ArCH), 119.0 (2 x ArCH), 121.8 (ArCH), 123.5 (C$^{-5''}$), 124.0 (ArCH), 129.8 (2 x ArCH), 129.9 (ArCH), 140.2 (C-6), 142.4 (ArCH), 147.1 (C$^{-4''}$), 149.8 (C-2), 156.7 (ArC-O), 157.4 (ArC-O), 162.9 (C-4), 169.7 (Ac-C=O), 169.8 (Ac-C=O). LRMS-ESI: m/z = 602 [M+Na]$^+$. HRESI-MS: m/z calcd for C$_{28}$H$_{28}$FN$_3$O$_8$Na [M+Na]$^+$: 602.1663; found 602.1669. $\Delta = 0.99$ ppm
This compound was prepared following procedure D using 5’-Azido-3’-acetyl-5’-deoxyuridine 201 (160 mg, 492 µmol, 1.0 equiv.), 1-(3-phenoxyphenyl)propargyl acetate 111 (130 mg, 590 µmol, 1.2 equiv.), Cu(OAc)₂ (22 mg, 123 µmol, 0.25 equiv.) and sodium ascorbate (49 mg, 246 µmol, 0.5 equiv.) in CH₃CN/H₂O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 226 was attained as a dark yellow thick oil (241 mg, 83%). \([\alpha]D_{20}^2 = 54.72 \text{ (c = 5 mg mL}^{-1}, \text{ CH₃OH).} \]

**¹H NMR (300 MHz, CDCl₃):**

δ 2.13 (s, 3H, Ac), 2.14 (s, 3H, Ac), 3.36 + 3.37 (s, 3H, OCH₃), 3.93 - 3.95 + 3.96 - 3.98 (m, 1H, H-4’), 4.41-4.45 (m, 1H, H-3’), 4.69-4.70 (m, 2H, H-5’), 5.08-5.11 + 5.12-5.14 (m, 1H, H-2’), 5.67 + 5.69 (d, \(J = 8.8 \text{ Hz, 1H, H-5})

δ 7.08-7.13 (m, 2H, ArH), 7.18 (t, \(J = 7.3 \text{ Hz, 1H, ArH})

δ 7.30 - 7.35 (m, 2H, ArH), 7.49 + 7.53 (m, 1H, 5”), 9.21 (br s, 1H, NH). **¹³C NMR (75 MHz, CDCl₃):** δ 20.5 (Ac-CH₃), 21.1 (Ac-CH₃), 50.7 (C-5”), 59.1 (OCH₃), 69.6 (C-7’’’), 71.0 (C-2’), 79.5 (C-3”), 80.1 (C-4”), 90.3 (C-1”), 103.4 (C-5), 117.3 (ArCH), 118.4 (ArCH), 119.1 (2 x ArCH), 121.1 (ArCH), 123.6 (C-5’”), 124.3, 129.8 (2 x ArCH), 130.0 (ArCH), 140.0 + 140.0 (C-6), 140.3 + 140.5 (ArC), 147.2 + 147.2 (C-4’”), 149.9 + 149.9 (C-2), 156.6 + 156.6 (ArC-O), 157.5 + 157.6 (ArC-O), 162.6 + 162.7 (C-4), 169.8 (Ac-C=O), 170.0 (Ac-C=O). **LRMS-ESI:** \(m/z = 614 \text{ [M+Na]}^⁺. \)** **HRESI-MS:** \(m/z \text{ calcd for C}_{29}H_{29}N_{5}O_{9}Na [M+Na]^⁺: 614.1863; found 614.1858; Δ = 0.81 ppm**
This compound was prepared following procedure D using 5'-Azido-5'-deoxy-5-fluoro-2',3'-diacetyluridine 186 (220 mg, 592 µmol, 1.0 equiv.), 1-(3-phenoxyphenyl)propargyl acetate 111 (189 mg, 711 µmol, 1.2 equiv.), Cu(OAc)$_2$ (27 mg, 148 µmol, 0.25 equiv.) and sodium ascorbate (57 mg, 296 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 227 was attained as a dark yellow oil (363 mg, 96 %). $[\alpha]_D^{20} = 38.52$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, CDCl$_3$): δ 2.07 (Ac), 2.08 (Ac), 2.11 (Ac), 4.44-4.48 (m, 1H, H-4’), 4.67-4.78 (m, 2H, H-5’), 5.29-5.35 (m, 1H, H-3’), 5.37-5.41 (m, 1H, H-2’), 5.62-5.66 (m, 1H, H-5), 6.89-6.91 (m, 1H, H-1’), 6.97-7.00 (m, 3H, ArH), 7.08-7.14 (m, 2H, ArH), 7.17-7.20 (m, 1H, ArH), 7.22-7.23 (m, 1H, ArH), 7.27-7.32 (m, 3H, ArH/H-6), 7.58 + 7.60 (s, 1H, H-5’’), 9.89 (br s, 1H, NH). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 20.3 (Ac-CH$_3$), 20.3 (Ac-CH$_3$), 21.0 + 21.0 (Ac-CH$_3$), 50.9 (C-5’), 69.4 + 69.5 (CHOAc), 70.3 + 70.4 (C-3’), 72.6 + 72.7 (C-2’), 79.9 (C-4’), 90.9 + 91.2 (C-1’), 117.3 + 117.4 (ArCH), 118.2 + 118.3 (ArCH), 118.9 (2 x ArCH), 121.7 (ArCH), 123.5 (C-5’’), 124.1 + 124.2 (ArCH), 125.5 + 125.8 (2 d, $J = 34.5$ Hz, C-6), 129.7 (2 x ArCH), 129.9 + 129.9 (ArCH), 140.1 + 140.1 (ArC), 140.6 (d, $J = 239.4$ Hz, C-5), 147.1 + 147.2 (C-4’’), 148.8 + 148.8 (C-2), 156.6 (ArC-O), 156.6 + 156.7 (2 d, $J = 26.8$ Hz, C-4), 157.4 + 157.4 (ArC-O), 169.7 (Ac-C=O), 169.8 + 169.8 (Ac-C=O), 169.9 + 169.9 (Ac-C=O). LRMS-ESI: $m/z = 660$ [M+Na$^+$]. HRESI-MS: $m/z$ calcd for C$_{30}$H$_{28}$F$N_5$O$_{10}$Na [M+Na$^+$]: 660.1718; found 660.1733. $\Delta = 2.43$ ppm
This compound was prepared following procedure D using 5'-Azido-5'-deoxy-5-fluoro-2',3'-isopropylidenuridine 184 (250 mg, 763 µmol, 1.0 equiv.), 1-(3-phenoxy-phenyl)propargyl acetate 111 (244 mg, 917 µmol, 1.2 equiv.), Cu(OAc)$_2$ (35 mg, 191 µmol, 0.25 equiv.) and sodium ascorbate (76 mg, 382 µmol, 0.5 equiv.) in CH$_3$CN/H$_2$O [4:1]. The suspension was stirred at r. t. for 24 h and after work up, the title compound 228 was attained as a dark yellow oil (413 mg, 91%). \([\alpha]_D^{20} = 61.85\) (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.32 (s, 3H, CH$_3$), 1.53 + 1.53 (2 s, 3H,CH$_3$), 2.11 (s, 3H, Ac), 4.44-4.47 (m, 1H, H-4'), 4.62-4.72 (m, 2H, H-5'), 4.90-4.94 (m, 1H, H-3'), 5.00-5.04 (m, 1H, H-2'), 5.47-5.48 + 5.50-5.51 (m, 1H, H-1'), 6.88-6.91 (m, 1H, ArH), 6.97-7.00 (m, 3H, ArH/CHOAc), 7.27-7.33 (m, 3H, ArH/H-6), 7.49 + 7.50 (2 s, 1H, H-5''), 10.50 (br s, 1H, NH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 21.0 (Ac-CH$_3$), 25.1 (CH$_3$), 26.9 (CH$_3$), 51.9 (C-5''), 69.5 (CHOAc), 81.4 (C-3''), 83.9 (C-2''), 85.6 (C-4''), 95.5 (C-1''), 115.0 (C$_q$), 117.4 (ArCH), 118.3 (ArCH), 118.9 (2 x ArCH), 121.7 (ArCH), 123.5 (C-5''), 123.8 (ArCH), 127.1 (d, $J = 33.4$ Hz, C-6), 129.7 (2 x ArCH), 129.9 (ArCH), 140.1 (ArC), 141.9 (d, $J = 238.4$ Hz, C-5), 146.9 (C-4''), 148.8 (C-2), 156.6 (ArC-O), 157.1 (d, $J = 26.5$ Hz, C-4), 157.3 (ArC-O), 169.9 (Ac-C=O). LRMS-ESI: $m/z = 616$ [M+Na]$^+$. HRESI-MS: $m/z$ calcd for C$_{29}$H$_{38}$FN$_5$O$_8$Na [M+Na]$^+$: 616.1834; found 616.1833; $\Delta = 0.16$ ppm
6.1.5.2. Deprotection

5'-Deoxy-5'-(4-(α-hydroxy-3-phenoxymethyl)triazolo)uridine 229

This compound was prepared according to general procedure E, described p. 143, using 206 (130 mg, 0.21 mmol, 1.0 equiv.). After work up, the title compound 229 was attained as a white powder (96 mg, 93 %). m.p. 173-174 °C. [α]D20 = 27.63 (c = 5 mg.mL⁻¹, CH₃OH).

¹H NMR (300 MHz, DMSO-d₆): δ 3.97-4.00 (m, 1H, H-3'), 4.03-4.06 (m, 1H, H-2'), 4.14-4.16 (m, 1H, H-4'), 4.59-4.64 (m, 1H, H-5'a), 4.66-4.71 (m, 1H, H-5'b), 5.42-5.43 (s, 1H, OH-2'), 5.54-5.56 (m, 1H, OH-3'), 5.58-5.61 (m, 1H, H-5), 5.75-5.76 (m, 1H, H-1'), 5.81-5.82 (m, 1H, CHOH), 6.09 (m, 1H, OH), 6.86-6.88 (m, 1H, ArH), 6.99-7.01 (m, 2H, ArH), 7.09-7.11 (m, 1H, ArH), 7.13-7.16 (m, 2H, ArH), 7.31-7.34 (m, 1H, ArH), 7.36-7.39 (m, 2H, ArH), 7.43-7.44 + 7.48-7.50 (m, 1H, H-6), 7.85 + 7.85 (2 s, 1H, H-5''), 11.40 (br s, 1H, NH).

¹³C NMR (75 MHz, DMSO-d₆): δ 51.2 + 51.2 (C-5'), 67.5 (CHOH), 70.6 (C-3'), 72.1 + 72.2 (C-2'), 81.8 + 81.9 (C-4'), 88.5 + 88.6 (C-1'), 102.2 (C-5), 116.3 + 116.4 (ArCH), 117.2 + 117.2 (ArCH), 118.8 (2 x ArCH), 121.5 + 121.5 (ArCH), 123.0 + 123.1 (C-5''), 123.6 (ArCH), 129.8 (ArCH), 130.1 (2 x ArCH), 138.3 (ArC), 140.9 + 141.0 (C-6), 146.3 + 146.4 (C-4''), 150.7 + 150.7 (C-2), 151.2 + 151.3 (ArC-O), 156.6 + 156.7 (ArC-O), 163.1 + 163.1 (C-4). LRMS-ESI: m/z = [M+Na]+. HRESI-MS: m/z calcd for C_{27}H_{27}N_{5}O_{7}Na [M+Na]+: 598.1914; found 598.1931; Δ = 2.84 ppm
This compound was prepared according to general procedure E using 210 (280 mg, 0.458 mmol, 1.0 equiv.). After work up, the title compound 230 was attained as a colourless syrup (204 mg, 92 %). \(\left[\alpha\right]_{D}^{20} = 21.36\) (c = 5 mg.mL\(^{-1}\), CH\(_3\)OH). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 1.53-1.59 (m, 2H, H-3''/H-4''\(_a\)), 1.63-1.72 (m, 4H, H-2''/H-3''\(_b\)/H-4''\(_b\)/H-5''\(_a\)), 1.84-1.92 (m, 2H, H-2''/H-5''\(_b\)), 3.93-3.98 (m, 2H, H-2'/H-3'), 4.11-4.16 (m, 1H, H-4'), 4.57-4.70 (m, 2H, H-5'), 4.72-4.78 (m, 1H, H-1'''), 5.37-5.39 (m, 1H, OH-2'), 5.49-5.51 (m, 1H, OH-3'), 5.55-5.61 (m, 1H, H-5), 5.73-5.77 (m, 2H, H-1'/CHOH), 5.95-5.96 (m, 1H, OH), 6.74-6.77 (m, 1H, ArH), 6.90-6.92 (m, 2H, ArH), 7.17-7.21 (m, 1H, ArH), 7.42 + 7.47 (2 d, \(J = 8.1\) Hz, 1H, H-6), 7.82 + 7.82 (2 s, 1H, H-5''), 11.36 (br s, 1H, NH). \(^13\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 23.6 (C-3''/C-4'''), 32.3 (C-2''/C-5'''), 51.1 + 51.1 (C-5'), 67.8 (CHOH), 70.5 + 70.5 (C-3'), 72.1 + 72.1 (C-2'), 79.2 (C-1'''), 81.7 + 81.8 (C-4'), 88.4 + 88.5 (C-1'), 102.1 + 102.1 (C-5), 113.4 + 113.4 (ArCH), 113.7 + 133.8 (ArCH), 118.3 + 118.3 (ArCH), 122.8 + 122.9 (ArCH), 129.1 (ArCH), 140.8 + 140.9 (C-6), 145.6 + 145.6 (C-4''), 150.6 (C-2), 151.5 (ArC), 157.5 (ArC-O), 163.0 (C-4). LRESI-MS: \(m/z = 508\) [M+Na]. HRESI-MS: \(m/z\) calcd for C\(_{23}\)H\(_{27}\)N\(_3\)O\(_7\)Na [M+Na]: 508.1808; found 508.1804; \(\Delta = 0.79\) ppm
5’-Deoxy-5’-(4-(3-cyclopentoxy-α-methoxybenzyl)triazolo)uridine 231

5’-(4-(α-Acetoxy-3-cyclopentoxybenzyl)triazolo)-5’-deoxy-2’,3’-O-isopropylidenuridine 210

(400 mg, 0.705 mmol, 1.0 equiv.) and In(OTf)$_3$ (20 mg, 0.035 mmol, 5 mol%) were dissolved in CH$_3$CN/H$_2$O [9:1] (20 mL) and the solution was stirred at r. t. overnight. At this stage, no reaction was occurring judging by TLC and the mixture was brought to reflux. After 6 h, the solvent was evaporated under reduced pressure and the remaining water was co-evaporated with methanol. The residue was purified by silica gel chromatography eluted with CH$_2$Cl$_2$/CH$_3$OH [9:1] to afford the title compound 231 was attained as a colourless syrup (169 mg, 48 %) along with 230 (99 mg, 29 %). $[\alpha]_D^{\text{20}} = 19.46$ (c = 5 mg.mL$^{-1}$, CH$_3$OH). $^1$H NMR (500 MHz, DMSO-$d_6$): δ 1.56 (m, 2H, H-3"'a/H-4"'a), 1.68 (m, 4H, H-2"'/H-3"'/H-4"'/H-5"'a), 1.88 (m, 2H, H-2"'/H-5"'b), 3.24 + 3.25 (2 s, 3H, OCH$_3$), 3.95 - 4.03 (m, 2H, H-2'/H-3'), 4.13 (m, 1H, H-4'), 4.58-4.64 (m, 1H, H-5'a), 4.67-4.68 (m, 1H, H-5'b), 5.35-5.36 (d, $J = 5.1$ Hz, 1H, OH-2'), 5.41 (m, 1H, H-1"'a), 5.48-5.49 (d, $J = 5.5$ Hz, 1H, OH-3"'), 5.55-5.57 (m, 1H, H-5), 5.72-5.73 (m, 1H, H-1"'), 6.80-6.81 (m, 1H, ArH), 6.90-6.92 (m, 2H, ArH), 7.21-7.25 (m, 1H, ArH), 7.40 + 7.46 (2 d, $J = 8.1$ Hz, 1H, H-6), 7.90 + 7.91 (2 s, 1H, H-5''), 11.32 (br s, 1H, NH). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 23.5 (C-3"'/C-4"'), 32.2 + 32.2 (C-2"'/C-5'''), 56.2 + 56.2 (C-5"'), 70.5 + 70.5 (C-3"'), 72.0 + 72.1 (C-2"'), 77.4 + 77.4 (CH), 78.5 + 78.5 (C-1"''), 81.6 + 81.7 (C-4"'), 88.5 + 88.6 (C-1"'), 102.0 (C-5), 113.8 + 113.8 (ArCH), 114.4 + 114.5 (ArCH), 118.8 (ArCH), 123.5 (C-5'''), 129.4 (ArCH), 140.8 + 140.8 (C-6), 141.9 (ArC), 148.2 + 148.2 (C-4"'), 150.6 (C-2), 157.6 (ArC-O), 162.9 (C-4). LRESI-MS: $m/z = 522$ [M+Na]$^+$. HRESI-MS: $m/z$ calcd for C$_{24}$H$_{29}$N$_5$O$_7$Na [M+Na]$^+$: 522.1965; found 522.1953; $\Delta = 2.30$ ppm
This compound was prepared according to general procedure E using 212 (250 mg, 0.427 mmol, 1.0 equiv.). After work up, the title compound 232 was attained as a colourless syrup (184 mg, 94 %). \([\alpha]_D^{20} = 17.86\) (c = 5 mg.mL\(^{-1}\), CH\(_3\)OH). \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 0.96 + 0.96 (2 t, \(J = 7.3\) Hz, 3H, H-3”), 1.68-1.73 (m, 2H, H-2”), 3.86-3.90 (m, 2H, H-1”), 4.08-4.15 (m, 1H, H-4’), 4.57-4.63 (m, 1H, H-5’\(_a\), 4.65-4.69 (m, 1H, H-5’\(_b\), 5.37-5.38 (m, 1H, OH-2’), 5.49-5.51 (m, 1H, OH-3’), 5.56 + 5.60 (2 d, \(J = 8.1\) Hz, 1H, H-5), 5.73-5.74 (m, 1H, H-1’), 5.76-5.77 (m, 1H, CH\(_2\)OH), 5.96-5.97 (m, 1H, OH), 6.78-6.80 (m, 1H, ArH), 6.93-6.94 (m, 1H, ArH), 6.96 (m, 1H, ArH), 7.18-7.22 (m, 1H, ArH), 7.42 + 7.47 (2 d, \(J = 8.1\) Hz, 1H, H-6), 7.83 + 7.83 (2 s, 1H, H-5”), 11.36 (br s, 1H, NH). \(^1^3\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 10.3 + 10.5 (C-3”), 22.0 (C-2”), 51.0 + 51.2 (C-5”), 67.7 + 67.8 (CH\(_2\)OH), 70.4 + 70.6 (C-3’), 71.9 + 72.0 (C-1”), 72.1 + 72.2 (C-2’), 81.7 + 81.8 (C-4’), 88.3 + 88.5 (C-1’), 102.1 + 102.1 (C-5), 112.3 + 112.4 (ArCH), 112.9 + 113.0 (ArCH), 118.4 + 118.5 (ArCH), 123.0 + 123.0 (C-5”), 129.0 (ArC), 129.2 (ArCH), 140.7 + 141.0 (C-6), 145.6 + 145.6 (C-4”), 150.6 (C-2), 158.5 (ArC-O), 162.9 + 162.9 (C-4). LRESI-MS: \(m/z = 459\) [M+Na]\. HRESI-MS: \(m/z\) calc'd for C\(_{21}\)H\(_{25}\)N\(_5\)O\(_7\)Na [M+Na]\. Δ = 3.11 ppm
This compound was prepared according to general procedure E using 214 (230 mg, 0.358 mmol, 1.0 equiv.). After work up, the title compound 233 was attained as a white powder (166 mg, 90 %). m.p. 236-238 °C. \([\alpha]_D^{20} = 15.07 (c = 5 \text{ mg mL}^{-1}, \text{ CH}_3\text{OH})\). \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 1.55 (m, 2H, H-3''/H-4''), 1.69 (m, 4H, H-2'''/H-3'''/H-4'''/H-5''''), 1.81-1.82 (m, 2H, H-2'''/H-5'''), 3.72 (s, 3H, OCH\(_3\)), 4.43 (m, 1H, H-3'), 4.73-4.75 (m, 3H, H-5'/H-1'''), 5.35-5.36 (m, 1H, H-2'), 5.40-5.47 (m, 1H, H-4'), 5.63-5.66 (m, 1H, H-5), 5.84-5.85 (m, 1H, H-1'), 6.87-6.91 (m, 2H, H-2'''/H-5'''), 6.93-6.94 (m, 1H, H-6'''), 7.60-7.65 (m, 1H, H-6'''), 7.90 + 7.92 (2 s, 1H, H-5''), 11.49 (br s, 1H, NH). \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 23.5 (C-3''/C-4'''), 32.1 (C-2'''/C-5''''), 50.5 (C-5'), 55.5 (OCH\(_3\)), 70.3 (C-3'), 71.6 (C-2'), 77.2 (C-1'''), 79.4 (C-4'), 88.4 (C-1'), 102.3 (C-5), 112.0 + 112.0 (C-2'''), 113.5 (C-6'''), 119.4 (C-5''''), 123.3 + 123.4 (C-5''), 132.6 (C-1'''), 141.8 (C-6), 146.9 (C-4''), 148.7 (C-3'''), 149.2 (C-4'''), 150.2 (C-2), 162.9 (C-4). LRESI-MS: \(m/z = 538 \text{ [M+Na]}^+\). HRESI-MS: \(m/z\) calcd for C\(_{29}\)H\(_{27}\)FN\(_5\)O\(_8\)Na \[M+Na]\(^+\): 538.1914; found 538.1921. \(\Delta = 1.30 \text{ ppm}\)
This compound was prepared according to general procedure E using 215 (240 mg, 0.370 mmol, 1.0 equiv.). After work up, the title compound 234 was attained as a white powder (164 mg, 85%).

**m.p.** 293-295 °C. \([\alpha]_D^{25} = 1.02\) (c = 5 mg.mL\(^{-1}\), CH\(_3\)OH).

**\(^1\)H NMR (500 MHz, DMSO-d\(_6\))**: \(\delta\) 2.88 (s, 3H, OCH\(_3\)), 3.08-3.21 (m, 2H, H-2’/H-3’), 3.26-3.34 (m, 1H, H-4’), 3.73-3.87 (m, 2H, H-5’), 4.48-4.56 (m, 1H, OH-3’), 4.65 (m, 1H, OH-2’), 4.74 (t, \(J = 8.2\) Hz, 1H, H-1’), 4.88-4.93 (m, 2H, H-5, CHOH), 5.10 (m, 1H, OH), 5.98 (d, \(J = 7.8\) Hz, 2H, ArH), 6.18 (t, \(J = 7.2\) Hz, 1H, ArH), 6.31-6.22 (m, 2H, ArH), 6.35 (d, \(J = 8.2\) Hz, 1H, ArH), 6.45 (t, \(J = 7.5\) Hz, 2H, ArH), 6.59 (dd, \(J = 24.8, 8.0\) Hz, 1H, H-6), 10.51 (s, 1H, NH).

**\(^13\)C NMR (125 MHz, DMSO-d\(_6\))**: \(\delta\) 51.0 + 51.1 (C-5’), 55.7 + 55.7 (OCH\(_3\)), 67.1 + 67.1 (CHOH), 70.5 (C-3’), 72.1 + 72.1 (C-2’), 81.6 + 81.7 (C-4’), 88.5 + 88.7 (C-1’), 102.0 (C-5), 113.0 (ArCH), 116.0 (ArCH), 119.6 (ArCH), 122.0 (ArCH), 122.8 (ArCH), 123.2 (ArCH), 123.3 (C-5”), 1296 (ArCH), 1371 + 137.1 (C), 140.8 + 140.9 (C-6), 143.2 (C-4”), 150.3 + 150.6 (C-2), 151.4 + 151.4 (ArC-O), 157.7 (ArC-O), 162.9 (C-4). **LRESI-MS:** \(m/\varepsilon = 628\) [M+Na\(^+\)]. **HRESI-MS:** \(m/\varepsilon\) calcd for C\(_{30}\)H\(_{31}\)N\(_5\)O\(_9\)Na [M+Na\(^+\)]: 628.2019; found 628.2047; \(\Delta = 4.46\) ppm
This compound was prepared according to general procedure E using 216 (180 mg, 0.282 mmol, 1.0 equiv.). After work up, the title compound 235 was attained as a white powder (128 mg, 89 %). m.p. 221-223°C. [α]D20 = 12.64 (c = 5 mg.mL⁻¹, CH3OH). 

**1H NMR** (500 MHz, DMSO-d6): δ 3.24-3.10 (m, 2H, H-2’, H-3’), 3.34-3.26 (m, 1H, H-4’), 3.89-3.74 (m, 2H, H-5’), 4.56 (d, J = 4.7 Hz, 1H, OH-3’), 4.69-4.66 (m, 1H, OH-2’), 4.78-4.74 (m, 1H, H-5), 4.91 (d, J = 5.2 Hz, 1H, H-1’), 4.98 (d, J = 3.3 Hz, 1H, CHOH), 5.29-5.24 (m, 1H, OH), 6.14 (d, J = 7.5 Hz, 2H, ArH), 6.32-6.26 (m, 1H, ArH), 6.42-6.35 (m, 2H, ArH), 6.57-6.47 (m, 3H, ArH), 6.63 (dd, J = 29.0 Hz, J = 8.0 Hz, 1H, H-6), 10.52 (s, 1H, NH). 

**13C NMR** (125 MHz, DMSO-d6): δ 52.2 + 52.0 (C-5’), 67.7 + 67.8 (CHOH), 71.4 + 71.5 (C-3’), 72.9 + 72.9 (C-2’), 82.5 + 82.6 (C-4’), 89.4 + 89.6 (C-1’), 103.0 (C-5), 117.8 (2 x ArCH), 117.9 (ArCH), 120.7 (d, J = 6.1 Hz, ArCH), 123.8 + 124.0 (C-5”), 124.2 (d, J = 11.6 Hz, ArCH), 130.8 (2 x ArCH), 131.1 (ArC), 141.6 + 141.7 (C-6), 143.3 (d, J = 11.7 Hz, ArCH), 151.6 + 151.9 (C-2), 153.5 (d, J = 249.7 Hz, C-F), 157.7 (2 x ArC-O), 163.9 (C-4).

**LRESI-MS**: m/z = 534 [M+Na]⁺. **HRESI-MS**: m/z calcd for C24H22FN3O7Na [M+Na]⁺: 534.1401; found 534.1427; Δ = 4.87 ppm
This compound was prepared according to general procedure E using 217 (180 mg, 0.282 mmol, 1.0 equiv.). After work up, the title compound 236 was attained as a white powder (131 mg, 91%). m.p. °C. [α]D20 = 41.19 (c = 5 mg.mL−1, CH3OH). 1H NMR (400 MHz, DMSO-d6): δ 3.94-3.98 (m, 1H, H-3’), 4.00-4.07 (m, 1H, H-2’), 4.11-4.15 (m, 1H, H-4’), 4.57-4.71 (m, 2H, H-5’), 5.39 (br s, 1H, OH-3’), 5.51 (br s, 1H, OH-2’), 5.56 & 5.59 (2 d, J = 8.3 Hz, 1H, H-5), 5.73-5.75 (m, 1H, H-1’), 5.80 (m, 1H, CHOH), 6.04 (br s, 1H, OH), 6.82-6.90 (m, 1H, ArH), 7.03-7.07 (m, 3H, ArH), 7.12-7.14 (m, 1H, ArH), 7.19-7.25 (m, 2H, ArH), 7.20-7.33 (m, 1H, ArH), 7.41-7.48 (m, 1H, H-6), 7.83 + 7.84 (2 s, 1H, H-5”), 10.96 (br s, 1H, NH). 13C NMR (125 MHz, DMSO-d6): δ 51.1 (C-5’), 67.4 (CHOH), 70.5 (C-3’), 72.0 + 72.1 (C-2’), 81.7 + 81.8 (C-4’), 88.4 + 88.5 (C-1’), 102.0 + 102.1 (C-5), 115.7 + 115.8 (ArCH), 116.5 (ArCH), 116.6 (d, J = 24.2 Hz, 2 x ArCH), 120.7 + 120.8 (2 d, J = 8.1 Hz, 2 x ArCH), 121.3 (ArCH), 122.9 + 123.0 (C-5”), 129.7 (ArCH), 140.1 + 140.2 (ArC), 141.5 (C-6), 150.6 + 150.6 (C-2), 151.1 + 151.2 (C-4”), 152.4 + 152.4 (ArC-O), 157.0 (ArC-O), 158.2 (d, J = 240.6 Hz, ArC-F), 162.9 (C-4).

LRMS-ESI: m/z = 534 [M+Na]+. HRESI-MS: m/z calcd for C24H22FN5O7Na [M+Na]+: 534.1401; found 534.1422. Δ = 3.93 ppm
This compound was prepared according to general procedure E using 218 (135 mg, 0.23 mmol, 1.0 equiv.). After work up, the title compound 237 was attained as a off white powder (99 mg, 94%). m.p. 202-204°C. [α]_D^{25} = 29.08 (c = 1 mg.mL^{-1}, CH₃OH). ^1H NMR (500 MHz, DMSO-d₆): δ 3.97 (m, 1H, H-3’), 4.10 (m, 1H, H-2’), 4.13-4.17 (m, 1H, H-4’), 4.61-4.67 (m, 1H, H-5’ₐ), 4.69-4.71 + 4.72-4.73 (m, 1H, H-5’ₐ), 5.41 (br s, 1H, OH-3’), 5.53 (br s, 1H, OH-2’), 5.58 + 5.62 (2 d, J = 8.1 Hz, 1H, H-5), 5.74 + 5.75 (m, 1H, H-1’), 6.16 (m, 1H, CHOH), 6.55 (m, 1H, OH), 7.26-7.35 (m, 3H, H-3’/H-5’/H-6’’), 7.48-7.50 + 7.53-7.55 (m, 1H, H-6), 7.75-7.77 (m, 1H, H-4’’’), 7.88-7.90 (m, 1H, H-7’’’), 7.99 + 8.01 (2 s, 1H, H-5’’), 11.37 (br s, 1H, NH). ^13C NMR (125 MHz, DMSO-d₆): δ 51.2 + 51.2 (C-5’), 64.6 + 64.7 (CHOH), 70.5 (C-3’), 72.0 + 72.0 (C-2’), 81.7 + 81.8 (C-4’), 88.4 + 88.5 (C-1’), 102.1 + 102.2 (C-5), 120.4 + 120.5 (C-3’’), 122.4 (C-7’’’), 123.2 + 123.2 (C-5’’), 123.5 (C-4’’’), 124.1 (C-5’’’), 124.2 (C-6’’’), 138.9 + 138.9 (ArC), 139.2 + 139.2 (ArC), 141.0 + 141.0 (C-6), 149.2 + 149.2 (C-4’’), 150.2 + 150.3 (C-2), 150.7 + 150.7 (C-2’’’), 163.0 (C-4). LRMS-ESI: m/z = 480 [M+Na]^+. HRESI-MS: m/z calcd for C_{20}H_{19}N_{5}O_{6}SNa [M+Na]^+: 480.0954; found 480.0972; Δ = 3.75 ppm
This compound was prepared according to general procedure E using 219 (300 mg, 0.450 mmol, 1.0 equiv.). After work up, the title compound 238 was attained as a white powder (202 mg, 83 %). m.p. 215-216 °C. [α]D20 = 38.30 (c = 5 mg.mL⁻¹, CH3OH). ¹H NMR (500 MHz, DMSO-d₆): δ 1.62 (s, 9H, Boc), 3.95-3.96 (m, 1H, H-3’), 4.00-4.03 (m, 1H, H-2’), 4.09-4.15 (m, 1H, H-4’), 4.58-4.64 (m, 1H, H-5’a), 4.66-4.71 (m, 1H, H-5’b), 5.38 (br s, 1H, OH-2’), 5.50 (br s, 1H, OH-3’), 5.55-5.59 (m, 1H, H-5), 5.72-5.78 (m, 1H, H-1’), 6.01-6.03 (m, 1H, OH), 6.07 (m 1H, CHOH), 7.12-7.17 (m, 1H, ArH), 7.27-7.30 (m, 2H, H-5'/ArH), 7.44-7.46 (m, 1H, H-6), 7.49-7.52 (m, 1H, ArH), 7.57-7.58 (m, 1H, ArH), 8.03-8.05 (m, 1H, ArH), 11.34 (br s, 1H, NH). ¹³C NMR (125 MHz, DMSO-d₆): δ 27.7 (Boc-C(CH₃)₃), 51.2 (C-5’), 61.8 (CHOH), 70.5 (C-3’), 72.0 (C-2’), 81.8 (C-4’), 83.7 (Boc-C(CH₃)₃), 88.4 (C-1’), 102.1 (C-5), 114.7 (ArCH), 120.5 (ArCH), 122.5 (ArCH), 123.3 (ArC), 123.7 (ArCH), 124.3 (C-5”), 128.3 (ArCH), 135.1 (ArC), 140.9 (C-6), 149.1 (C-4”), 150.3 (Boc-C=O), 150.6 (C-2), 162.9 (C-4). LRMS-ESI: m/z = 563 [M+Na]⁺. HRESI-MS: m/z calcd for C₂₅H₂₈N₆O₈Na [M+Na]⁺: 563.1866; found 563.1891; Δ = 4.40 ppm
This compound was prepared according to general procedure E using 220 (250 mg, 0.366 mmol). After work up, the title compound 239 was attained as an off white powder (173 mg, 79%). m.p. > 280 °C, decomposed. [α]_D^{20} = 36.28 (c = 5 mg.mL⁻¹, CH₃OH).

^1^H NMR (500 MHz, DMSO-d₆): δ 1.62 (s, 9H, Boc), 3.67 + 3.67 (2s, 3H, OCH₃), 3.96-4.01 (m, 2H, H-2'/H-3''), 4.14-4.18 (m, 1H, H-4''), 4.63-4.67 (m, 1H, H-5'ₐ), 4.71-4.74 (m, 1H, H-5'ₗ), 5.38-5.39 (m, 1H, OH-2') 5.50-5.51 (m, 1H, OH-3''), 5.56-5.58 (m, 1H, H-5), 5.57-5.76 (m, 1H, H-1'), 6.85 (s, 1H, OH-2''), 7.10-7.15 (m, 1H, ArH), 7.26-7.31 (m, 1H, ArH), 7.39-7.47 (m, 2H, ArH), 7.55 (d, J = 9.1 Hz, 1H, H-6), 7.97-8.01 (m, 1H, H-5''), 8.04-8.06 (m, 1H, ArH). ^1^3^C NMR (125 MHz, DMSO-d₆): δ 27.6 (Boc-C(CH₃)₃), 51.2 (C-5''), 52.6 (OCH₃), 70.5 + 70.5 (C-3'), 72.0 + 72.1 (COH), 72.5 + 72.6 (C-2'), 81.7 + 81.8 (C-4'), 84.0 + 84.0 (Boc-C(CH₃)₃), 88.3 + 88.3 (C-1'), 102.1 (C-5), 114.5 (ArCH), 122.0 + 122.1 (ArCH), 122.4 (ArC), 124.1 (ArCH) 124.3 (C-5''), 128.0 (ArCH) 133.2 (ArC) 135.1 (ArCH), 137.7 + 137.8 (ArC), 140.8 (C-6), 148.6 (C-4''), 148.9 (Boc-C=O), 150.6 + 150.7 (C-2), 162.9 (C-4), 172.0 (C=O). LRMS-ESI: m/z = 621 [M+Na]^+ . HRESI-MS: m/z calcd for C_{27}H_{30}N_{6}O_{10}Na [M+Na]^+: 621.1921; found 621.1930; Δ = 1.45 ppm.
This compound was prepared according to general procedure E using 221 (250 mg, 0.475 mmol, 1.0 equiv.). After work up, the title compound 240 was attained as a white powder (185 mg, 88 %). m.p. 241-243°C. [α]D^20 = 62.54 (c = 5 mg.mL^{-1}, CH₃OH). ¹H NMR (400 MHz, DMSO-d₆): δ 3.91-4.03 (m, 2H, H-2'/H-3’), 4.14-4.18 (m, 1H, H-4’), 4.62-4.74 (m, 2H, H-5’), 5.41 (br s, 1H OH-2’), 5.51 (br s, 1H, OH-3’), 5.62 + 5.63 (2 d, J = 8.1 Hz, H-5), 5.75-5.77 (m, 1H, H-1’), 6.70 (br s, 1H, OH-3’’’), 6.85-6.87 (m, 1H, ArH), 6.94-6.98 (m, 1H, ArH), 7.21-7.26 (m, 2H, ArH), 7.40 + 7.49 (2 d, J = 8.3 Hz, 1H, H-6), 8.09 + 8.10 (2 s, 1H, H-5’’’), 10.43 (br s, 1H, NH-1’’’), 11.33 (br s, 1H, NH-3). ¹³C NMR (125 MHz, DMSO-d₆): δ 51.1 + 51.2 (C-5’), 70.4 + 70.5 (C-3’), 72.0 + 72.1 (C-2’), 73.0 + 73.0 (C), 81.7 + 81.8 (C-4’), 88.2 + 88.4 (C-1’), 102.2 + 102.2 (C-5), 109.8 (ArCH), 121.8 (ArCH), 124.0 + 124.2 (C-5’’’), 125.2 + 125.3 (ArC), 129.4 (ArCH), 132.1 (ArCH), 140.8 + 140.9 (ArC), 141.8 + 141.9 (C-6), 148.0 + 148.0 (C-4’’’), 150.6 + 150.7 (C-2), 163.0 (C-4), 177.2 (C=O). LRMS-ESI: m/z = 465 [M+Na]^+. HRESI-MS: m/z calcd for C₁₉H₁₉N₆O₇Na [M+Na]^+: 465.1135; found 465.1135; Δ = 0 ppm
This compound was prepared according to general procedure E using 222 (300 mg, 0.504 mmol, 1.0 equiv.). After work up, the title compound 241 was attained as a white powder (203 mg, 86%).

**m.p.** 122-123 °C. \([\alpha]_D^{25} = 18.67\) (c = 5 mg.mL\(^{-1}\), CH\(_3\)OH).

**\(^1\text{H NMR}\) (500 MHz, DMSO-\(d_6\)):**
\[\delta = 3.96-4.00\) (m, 1H, H-3’), 4.03-4.09 (m, 1H, H-2’), 4.14-4.21 (m, 1H, H-4’), 4.66-4.79 (m, 2H, H-5’), 5.36-5.38 (m, 1H, OH-2’), 5.45-5.61 (m, 3H, H-5/OH/OH-3’), 5.74-5.76 (m, 1H, H-1’), 7.37-7.42 (m, 2H, ArH), 7.44-7.47 (m, 1H, ArH), 7.47 + 7.55 (2 d, J = 8.3 Hz, 1H, H-6), 7.59-7.62 (m, 2H, ArH), 8.12 + 8.13 (2 s, 1H, H-5’’), 11.29 (br s, 1H, NH).

**\(^{13}\text{C NMR}\) (125 MHz, DMSO-\(d_6\)):**
\[\delta = 51.3 + 51.3\) (C-5’’), 70.5 + 70.6 (C-3’’), 72.0 + 72.0 (C-2’’), 81.6 + 81.7 (C-4’’), 88.6 + 88.7 (C-1’’), 102.0 + 102.1 (C-5), 125.0 + 125.0 (C-5’’), 125.1 (ArCH), 127.4 (d, J = 4.4 Hz, 2 x ArCH), 127.8 (2 x ArCH), 128.0 (d, J = 286.8 Hz, CF\(_3\)), 128.3 (d, J = 28.6 Hz, C), 137.8 + 137.9 (ArC), 140.9 + 141.0 (C-6), 146.6 (C-4’’), 150.7 (C-2), 162.9 (C-4).

**LRMS-ESI:** \(m/z = 492\) [M+Na]\(^+\). **HRESI-MS:** \(m/z\) calcd for C\(_{19}\)H\(_{18}\)FN\(_5\)O\(_6\)Na [M+Na]\(^+\): 492.1107; found 492.1123. \(\Delta = 3.25\) ppm
This compound was prepared according to general procedure E using 204 (90 mg, 136 µmol, 1.0 equiv.). After work up, the title compound 242 was attained as a white powder (58 mg, 87%).

**m.p.** 231-233 °C. \([\alpha]^{20}_D = 23.19\) (c = 5 mg.mL\(^{-1}\), CH\(_3\)OH). \(^1\text{H NMR}\) (300 MHz, DMSO-\(d_6\)): \(\delta\) 3.93 (m, 1H, H-3’), 3.98 (m, 1H, H-2’), 4.11-4.12 (m, 1H, H-4’), 4.57-4.63 (m, 1H, H-5’a), 4.65-4.71 (m, 1H, H-5’b), 5.34 (br s, 1H, OH-2”), 5.44 (br s, 1H, OH-3”), 5.72 (m, 1H, CHOH), 5.74-5.76 (m, 1H, H-5), 5.80-5.81 (m, 1H, H-1’), 6.05-6.07 (m, 1H, OH), 6.85-6.87 + 6.88-6.89 (m, 1H, ArH), 6.98-7.01 (m, 2H, ArH), 7.08-7.15 (m, 3H, ArH), 7.21 (br s, 1H, NH), 7.26 (br s, 1H, NH), 7.30-7.33 (m, 1H, ArH), 7.34-7.43 (m, 3H, ArH/H-6), 7.83 + 7.83 (2 s, 1H, H-5”). \(^{13}\text{C NMR}\) (75 MHz, DMSO-\(d_6\)): \(\delta\) 51.3 + 51.4 (C-5’), 67.5 + 67.5 (CHOH), 70.7 + 70.8 (C-3’), 72.8 + 72.8 (C-2’), 81.2 + 81.3 (C-4’), 90.1 + 90.3 (C-1’), 94.5 + 94.5 (C-5), 116.3 + 116.4 (ArCH), 117.1 (ArCH), 118.7 (2 x ArCH), 121.4 + 121.5 (ArCH), 122.9 (ArCH), 123.5 (C-5”), 129.7 (ArCH), 130.0 (2 x ArCH), 141.6 + 141.7 (C-4’), 146.3 + 146.3 (C-6), 151.2 + 151.2 (C-2), 155.2 + 155.3 (ArC-O), 156.6 (ArC-O), 165.6 (C-4). \(\text{LRMS-ESI:}\) \(m/z = 515\) [M+Na]*. \(\text{HRESI-MS:}\) \(m/z\) calcd for C\(_{24}\)H\(_{24}\)N\(_6\)O\(_6\)Na [M+Na]*: 555.1655; found 555.1669; \(\Delta = 2.52\) ppm
5'-Deoxy-5'-(4-(α-hydroxy-3-phenoxybenzyl)triazolo)thymidine 243

This compound was prepared according to general procedure E using 223 (280 mg, 0.486 mmol, 1.0 equiv.). After work up, the title compound 243 was attained as a white powder (232 mg, 97%). m.p. 218-220 °C. [α]D20 = 46.96 (c = 5 mg.mL⁻¹, CH3OH). ¹H NMR (500 MHz, DMSO-d₆): δ 1.77 (s, 3H, CH₃), 2.07-2.13 (m, 2H, H-2’), 4.07 (m, 1H, H-4’), 4.26 (m, 1H, H-3’), 4.54-4.60 (m, 1H, H-5’a), 4.65-4.68 (m, 1H, H-5’b), 5.48 (m, 1H, OH-3’), 5.80 (m, 1H, CHO), 6.03 (m, 1H, OH), 6.16 (m, 1H, CH-1’), 6.86 (d, J = 7.8 Hz, 1H, ArH), 6.98 (m, 2H, ArH), 7.06 (m, 1H, ArH), 7.11-7.24 (m, 2H, C-6, ArH), 7.30-7.32 (m, 2H, ArH), 7.36-7.39 (m, 2H, ArH), 7.85 (d, J = 8.8 Hz, 1H, ArH), 11.27 (br s, 1H, NH). ¹³C NMR (125 MHz, DMSO-d₆): δ 11.9 (CH₃), 37.9 (C-2’), 51.1 + 51.2 (C-5’), 67.4 + 67.5 (CHOH), 70.8 + 70.8 (C-3’), 83.9 + 83.9 (C-4’), 84.0 (C-1’), 109.8 (C-5), 116.3 + 116.3 (ArCH), 117.0 + 117.1 (ArCH), 188.5 + 118.6 (2 x ArCH), 121.4 (ArCH), 122.7 + 122.7 (C-5”), 123.4 + 123.4 (ArCH), 129.6 (ArCH), 129.9 (2 x ArCH), 135.8 + 135.9 (C-6), 146.3 + 146.3 (C-4”), 150.3 (C-2), 151.1 + 151.2 (ArC-O), 156.5 (ArC-O), 156.5 + 156.5 (C), 163.6 + 163.6 (C-4).

LRMS-ESI: m/z = 514 [M+Na]+. HRESI-MS: m/z calcd for C₂₅H₂₅N₅O₆Na [M+Na]+: 514.1703; found 514.1722; Δ = 3.69 ppm
This compound was prepared according to general procedure E using 224 (250 mg, 0.445 mmol, 1.0 equiv.). After work up, the title compound 244 was attained as a white powder (195 mg, 92%). m.p. 232-233°C. \([\alpha]_D^{20} = 51.16\) (c = 5 mg.mL\(^{-1}\), CH\(_3\)OH). \(^1\)H NMR (500 MHz, DMSO-d\(_6\)): \(\delta\) 2.08 (m, 2H, H-2’), 4.04-4.08 (m, 1H, H-4’), 4.23-4.24 (m, 1H, H-3’), 4.53-4.58 (m, 1H, H-5’a), 4.63-4.67 (m, 1H, H-5’b), 5.49-5.50 (m, 1H, H-3’), 5.57-5.59 (m, 1H, H-5), 5.79-5.80 (m, 1H, CHOH), 6.03-6.04 (m, 1H, OH), 6.13-6.15 (m, 1H, H-1’), 6.86-6.88 (m, 1H, ArH), 6.99-7.00 (m, 2H, ArH), 7.07 (m, 1H, ArH), 7.12-7.15 (m, 2H, ArH), 7.31-7.32 (m, 1H, ArH), 7.36-7.39 (m, 2H, ArH), 7.47-7.53 (m, 1H, H-6), 7.84 (m, 1H, H-5”), 11.33 (br s, 1H, NH). \(^{13}\)C NMR (125 MHz, DMSO-d\(_6\)): \(\delta\) 38.1 (C-2’), 51.2 + 51.2 (C-5’), 67.4 (CHOH), 70.7 + 70.8 (C-3’), 84.1 + 84.2 (C-4’), 84.3 + 84.4 (C-1’), 102.0 (C-5), 116.3 + 116.3 (ArCH), 117.1 + 117.1 (ArCH), 118.6 (ArC), 118.7 (2 x ArCH), 121.4 + 121.4 (ArCH), 122.8 (C-5”), 123.4 + 123.4 (ArCH), 129.6 (ArCH), 130.0 (2 x ArCH), 140.6 + 140.7 (C-6), 146.3 (C-4”), 150.4 (C-2), 151.1 + 151.2 (ArC-O), 156.5 + 156.5 (ArC-O), 163.0 (C-4). LRMS-ESI: \(m/z = 500\) [M+Na]+. HRESI-MS: \(m/z\) calcd for C\(_{24}\)H\(_{23}\)N\(_5\)O\(_6\)Na [M+Na]+: 500.1546; found 500.1583 \(\Delta = 7.40\) ppm
2',5'-Dideoxy-2'-fluoro-5'-(4-(α-hydroxy-3-phenoxybenzyl)triazolo)uridine 245

This compound was prepared according to general procedure E using 225 (250 mg, 0.431 mmol, 1.0 equiv.). After work up, the title compound 245 was attained as a white powder (205 mg, 96%).

m.p. 164-166 °C. \([\alpha]_{D}^{20} = 73.04 \) (c = 5 mg.mL\(^{-1}\), CH\(_3\)OH). \(^1\)H NMR (500 MHz, DMSO-\(d_6\)):
\[\delta 4.15-4.17 \text{ (m, 2H, H-5')}, 4.61-4.66 \text{ (m, 1H, H-4')}, 4.73-4.76 \text{ (m, 1H, H-3')}, 5.08-5.21 \text{ (m, 1H, H-2')}, 5.53-5.57 \text{ (m, 1H, H-5)}, 5.80-5.81 \text{ (m, 1H, CHOH)}, 5.86 \text{ (m, 1H, OH-3')}, 5.90 \text{ (br s, 1H, OH)}, 6.04 \text{ (m, 1H, H-1')}, 6.86 \text{ (m, 1H, ArH)}, 6.99-7.01 \text{ (m, 2H, ArH)}, 7.09 \text{ (m, 1H, H-6)}, 7.11-7.16 \text{ (m, 2H, ArH)}, 7.31-7.39 \text{ (m, 4H, ArH)}, 7.87-7.89 \text{ (m, 1H, H-5")}, 11.39 \text{ (br s, 1H, NH)}. \]  
\(^1\)C NMR (125 MHz, DMSO-\(d_6\)):
\[\delta 50.6 \text{ (C-5')}, 67.4 \text{ (CHOH)}, 69.6 \text{ (d, J = 16.3 Hz, C-3')}, 80.1 \text{ (C-4')}, 90.7 \text{ (d, J = 190.5 Hz, C-2')}, 93.8 \text{ (d, J = 37.7 Hz, C-3')}, 93.4 + 93.4 \text{ (C-1')}, 101.9 + 102.0 \text{ (C-5)}, 116.3 + 116.4 \text{ (ArCH)}, 117.1 \text{ (ArCH)}, 118.6 \text{ (2 x ArCH)}, 121.5 \text{ (ArCH)}, 122.9 + 123.0 \text{ (ArCH)}, 123.4 \text{ (C-5")}, 129.6 \text{ (ArCH)}, 130.0 \text{ (2 x ArCH)}, 141.2 + 141.3 \text{ (C-6)}, 146.3 \text{ (C-4")}, 150.1 \text{ (C-2)}, 151.2 \text{ (ArC-O)}, 156.5 + 156.5 \text{ (ArC-O)}, 163.1 \text{ (C-4)}. \]  
LRMS-ESI: \(m/z = 496 \text{ [M+H]}^+\). HRESI-MS: \(m/z\) calcd for C\(_{24}\)H\(_{22}\)FN\(_5\)O\(_6\)Na [M+Na]\(^+\):
518.1452; found 518.1459, \(\Delta = 1.35 \) ppm.
This compound was prepared according to general procedure E using \(226\) (180 mg, 0.304 mmol, 1.0 equiv.). After work up, the title compound \(246\) was attained as a white powder (147 mg, 95%).

**m.p.** 205-207 °C. \([\alpha]_D^{20} = 56.23\) (c = 5 mg mL\(^{-1}\), CH\(_3\)OH). **\(^1H\) NMR** (500 MHz, DMSO-\(d_6\)): \(\delta\) 3.35 (s, 3H, OCH\(_3\)). 3.77-3.79 + 3.81-3.83 (m, 1H, H-2’), 4.08-4.16 (m, 3H, H, H-3’), 4.60-4.65 (m, 1H, H-5’a), 4.68-4.69 + 4.71-4.72 (m, 1H, H-5’b), 5.53-5.55 (m, 1H, H-4’), 5.57 + 5.59 (2 d, \(J = 8.2\) Hz, 1H, H-5), 5.80 (m, 2H, H-1’/CHOH), 6.07-6.08 (m, 1H, OH), 6.85-6.87 (m, 1H, ArH), 6.98-7.00 (m, 2H, ArH), 7.08 (m, 1H, ArH), 7.11-7.15 (m, 2H, ArH), 7.30-7.33 (m, 1H, ArH), 7.36-7.39 (m, 2H, ArH), 7.43 + 7.48 (2 d, \(J = 8.2\) Hz, 1H, H-6), 7.87 + 7.88 (2 s, 1H, H-5”), 11.39 (br s, 1H, NH). **\(^{13C\) NMR** (125 MHz, DMSO-\(d_6\)): \(\delta\) 50.9 + 50.9 (C-5’), 57.6 + 57.7 (OCH\(_3\)), 67.4 (CHOH), 69.3 + 69.3 (C-1’), 81.1 + 81.1 (C-2’), 81.9 + 81.9 (C-3’), 86.9 + 87.0 (C-4’), 102.1 + 102.2 (C-5), 116.3 + 116.3 (ArCH), 117 (ArCH), 118.6 (2 x ArCH), 121.4 (ArCH), 122.9 (ArCH), 123.4 (C-5”), 128.9 (ArC), 129.6 (ArCH), 130.0 (2 x ArCH), 140.5 + 140.6 (C-6), 146.3 (C-4”), 150.3 (C-2), 151.2 (ArC-O), 156.5 (ArC-O), 162.9 (C-4). **LRMS-ESI:** \(m/z\) = 530 [M+Na]+. **HRESI-MS:** \(m/z\) calcd for C\(_{25}\)H\(_{25}\)N\(_5\)O\(_7\)Na [M+Na]+: 530.1652; found 530.1674; \(\Delta = 4.15\) ppm
5′-Deoxy-5-fluoro-5′-(4-(α-hydroxy-3-phenoxybenzyl)triazolo)uridine 247

This compound was prepared according to general procedure E using 227 (260 mg, 0.408 mmol, 1.0 equiv.). After work up, the title compound 247 was attained as a white powder (204 mg, 98 %).

**m.p.** 193-195 °C. [α]D25 = 25.47 (c = 5 mg.mL⁻¹, CH₃OH).

**¹H NMR** (500 MHz, DMSO-d₆):

δ 3.96 (m, 1H, H-3′), 4.08-4.11 (m, 1H, H-2′), 4.12-4.15 (m, 1H, H-4′), 4.62-4.71 (m, 2H, H-5′), 5.39 (br s, 1H, OH-2′), 5.53 (br s, 1H, OH-3′), 5.75 + 5.76 (m, 1H, H-1′), 5.80 (m, 1H, CH(OH)), 6.03-6.06 (m, 1H, OH), 6.86-6.88 (m, 1H, ArH), 6.99-7.01 (m, 2H, ArH), 7.07 + 7.08 (m, 1H, ArH), 7.12-7.15 (m, 2H, ArH), 7.30-7.33 + 7.31-7.34 (m, 1H, ArH), 7.37-7.40 (m, 2H, ArH), 7.86 + 7.86 (s, 1, H-5′′), 7.94-7.95 + 7.98-8.00 (m, 1H, H-6), 11.80 (br s, 1H, NH).

**¹³C NMR** (125 MHz, DMSO-d₆): δ 51.2 + 51.2 (C-5′), 67.4 + 67.5 (CHOH), 70.5 (C-3′), 72.0 + 72.0 (C-2′), 81.9 + 82.0 (C-4′), 88.6 + 88.6 (C-1′), 116.3 + 116.4 (ArCH), 117.1 (ArCH), 118.7 + 118.7 (2 x ArCH), 121.4 + 121.4 (ArCH), 122.7 + 122.8 (C-5′′), 123.4 + 123.5 (ArCH), 125.2 + 125.5 (2 d, J = 34.5 Hz, C-6), 129.6 + 129.7 (ArCH), 130.0 (2 x ArCH), 140.2 + 140.8 (d, J = 231.9 Hz, C-5), 146.3 + 146.3 (C-4′′), 149.4 (C-2), 151.2 + 151.3 (ArC-O), 156.5 + 156.6 (ArC-O), 157.0 + 157.0 (2 d, J = 26.2 Hz, C-4).

**LRMS-ESI:** m/z = 512 [M+Na]+. **HRESI-MS:** m/z calcd for C_{24}H_{22}FN_{5}O_{7}Na [M+Na]+: 534.1401; found 534.1412; Δ = 2.06 ppm
This compound was prepared according to general procedure E using 202 (160 mg, 259 µmol, 1.0 equiv.). After work up, the title compound 248 was attained as a white powder (134 mg, 97 %). m.p. 179-181 °C. [α]_{D}^{20} = 13.42 (c = 5 mg.mL^{-1}, CH_{3}OH). \(^{1}\)H NMR (300 MHz, DMSO-d_{6}): \(\delta\) 1.25 (s, 3H, CH_{3}), 1.45 (s, 3H, CH_{3}), 4.30-4.38 (m, 1H, H-4’), 4.53-4.70 (m, 2H, H-5’), 4.79-4.85 (m, 1H, H-3’), 5.03 (dd, \(J = 14.2\) Hz, \(J = 6.8\) Hz, 1H, H-2’), 5.36 (m, 1H, OH), 5.75 (dd, \(J = 11.2\) Hz, \(J = 7.3\) Hz, 1H, H-1’), 5.93 (m, 1H, H-5), 6.80-6.81 (m, 1H, CHOH), 6.90-7.03 (m, 4H, ArH), 7.11 (m, 2H, ArH), 7.18-7.26 (m, 3H, ArH), 7.43 + 7.44 (s, 1H, H-5”), 7.69 (br s, 1H, NH). \(^{13}\)C NMR (75 MHz, DMSO-d_{6}): \(\delta\) 25.0 (CH_{3}), 26.9 (CH_{3}), 51.5 (C-5’), 67.4 + 67.4 (CHOH), 82.1 (C-3’), 84.2 (C-4’), 85.9 + 86.0 (C-2’), 94.3 (C-5), 95.2 + 95.3 (C-1’), 112.9 (C_{q}), 116.4 (ArCH), 117.1 (ArCH), 118.7 (2 x ArCH), 121.5 (ArCH), 122.5 (ArCH), 123.4 (C-5”), 129.6 (ArCH), 130.0 (2 x ArCH), 144.7 (C-4”), 146.3 (C-6), 151.2 + 151.2 (C-2), 154.8 (ArC-O), 156.5 (ArC-O), 166.2 (C-4). LRMS-ESI: \(m/z = 555\) [M+Na]^+. HRESI-MS: \(m/z\) calcd for C_{27}H_{28}N_{6}O_{6}Na [M+Na]^+: 555.1968; found 555.1965; \(\Delta\) = 0.54 ppm
This compound was prepared according to general procedure E using 205 (110 mg, 0.19 mmol, 1.0 equiv.). After work up, the title compound 249 was attained as a white powder (96 mg, 94 %). m.p. 143-144 °C. [α]25 = 7.68 (c = 1 mg.mL\(^{-1}\), CH\(_3\)OH). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 1.28 (s, 3H, CH\(_3\)), 1.46 (s, 3H, CH\(_3\)), 4.33-4.39 (m, 1H, H-4’), 4.57-4.63 (m, 1H, H-5’a), 4.67-4.72 (m, 1H, H-5’b), 4.85-4.88 (m, 1H, H-3’), 5.08-5.11 (m, 1H, H-2’), 5.60-5.63 (m, 1H, H-5), 5.77-5.80 (m, 2H, H-1’/CHOH), 6.01-6.03 (m, 1H, OH), 6.85 + 6.87 (d, \(J = 2.4\) Hz, 1H, ArH), 6.99 + 7.00 (m, 2H, ArH), 7.07 (m, 1H, ArH), 7.13-7.15 (m, 2H, ArH), 7.30-7.33 (m, 1H, ArH), 7.37-7.40 (m, 2H, ArH), 7.62-7.65 (m, 1H, H-6), 7.90 + 7.90 (2 s, 1H, H-5”), 11.46 (br s, 1H, NH). \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 25.0 + 25.0 (CH\(_3\)), 26.8 (CH\(_3\)), 51.1 (C-5’), 67.4 + 67.4 (CHOH), 81.4 + 81.4 (C-3’), 83.5 (C-2’), 85.0 + 85.2 (C-4’), 93.0 + 93.1 (C-1’), 101.9 + 101.9 (C-5), 113.4 (C), 116.3 + 116.4 (ArCH), 117.0 (ArCH), 118.6 + 118.6 (2 x ArCH), 119.0 (ArCH), 121.4 (ArCH), 122.5 + 122.5 (ArCH), 123.4 (C-5”), 129.5 (ArCH), 129.9 (2 x ArCH), 130.2 (ArCH), 143.3 (C-6), 146.3 (C-4”), 150.3 (C-2), 151.2 (ArC-O), 156.5 (ArC-O), 163.4 (C-4). LRMS-ESI: \(m/z = 556\) [M+Na]\(^+\). HRESI-MS: \(m/z\) calcd for C\(_{27}\)H\(_{27}\)N\(_5\)O\(_7\)Na [M+Na]\(^+\): 534.1989; found 534.2000; \(\Delta = 2.06\) ppm
6.2. Biological procedures

6.2.1. ST inhibition assay

19 triazoles derivatives were evaluated by the Gerardy-Schahn group at the university of Hannover against ST8Sia-II using a fluorimetric assay as follows. Sodium cacodylate (100 mM, pH 6.7), Glycerol (50 %), MgCl₂ (1 M), MnCl₂ (100 mM), CMP-Neu5Ac (10 mM), ST8Sia-II (250 ng), and the acceptor DMB-DP3 250 (25 µM, used as a labelled acceptor, fig. 6.1) along with the inhibitor sample (final concentration of 100 µM) were incubated at 25°C for the indicated times. The reactions were terminated by 10-fold dilution in Tris–HCl (100 mM, pH 8.0) / ethylenediamine-tetraacetic acid (EDTA, 20 mM) followed by 10 min incubation at 50°C. Finally, the samples were centrifuged and analysed on a DNAPAC PA 100 analytical anion exchange column (Ex. 373 nm/Em. 448 nm). The elution system used was a mixture of H₂O and 5.0 M ammonium acetate buffer (pH 7.4). HPLC was carried out using a Waters 2695 Separations Module connected to an RF-10A spectrofluorometric detector at a flow rate of 1 mL/min.

![Figure 6.1](image_url). Structure of the fluorescent reagent DMB-DP3 250.
6.2.2. Cell lines and cell culture conditions

The cancer cell lines MiaPaCa-2, SKOV3 and MCF-7 were cultured in DMEM medium containing 2 mM L-glutamine and 10 % foetal bovine serum.

RAW264.7 cells were regularly cultured in vitro in culture medium consisting of RPMI-1640 medium, along with 2 mM L-glutamine, 5.6 % (2 g/L) NaHCO₃ and 5 % fetal calf serum.

**Determination of cell number:** Cell counts were performed by mixing 20 µL of media containing cells with 20 µL of Trypan blue and dispensing 20 µL of this mixture under a cover-slip on the haemocytometer. Count the number of cells using the following equation:

\[
\text{No. of Cells} = \frac{\text{(# of cells in 4 grids)}}{4} \times 2 \times 10000
\]

6.2.3. MTS Cell proliferation assay

The MTS assay described here utilises Promega’s MTS CellTiter 96® AQueous One Solution Cell Proliferation assay and is performed according to the manufacturer’s instructions.⁴⁷⁴-⁴⁷⁵

**Day 1:** The chosen cells were suspended in DMEM medium containing 10% fetal bovine serum and seeded in 96-well tissue culture plates at cells per well. The plates were incubated at 37 °C for 24 h. Three rows were kept on the plate for controls and blank which include cells alone, cells treated with 10% DMSO and samples in media.

**Sample preparation:** The samples are prepared from a stock solution in DMSO at a concentration of 1 mM. The stock solution is diluted with media in a separate plate before being added to the cells in order to minimise the DMSO concentration to a maximum of 1 %.

**Day 2:** The samples were transferred from the dilution plate to the cells and incubated at 37 °C for 24 to 48 h.
Day 3: The MTS reagent 86 is added to the plate (10 µL in each well) and incubated for 3 h at 37 °C. The MTS reagent is light sensitive so this step has to be performed with the lights off in the cytotoxic cabinet and it is preferable to cover the plate with foil during the final incubation. The absorbance of the final mixture is read at 490 nm.

The cell viability when exposed to the samples is deducted from the absorbance read by comparing with those of the cells in media alone (which correspond to the 100 % viability) and those treated with 10 % DMSO (which correspond to the 0 % viability). The blank (media containing the sample only) was substracted before proceeding in order to eliminate the false positive due to the absorbance of the compound added itself.

6.2.4. Griess reagent assay

The assay was performed by following reported procedures. The assay requires three sets of plates: one for the sample preparation, one for seeding the cells and a reading plate.

Day 1: RAW264.7 cells were suspended in RPMI-1640 medium containing 10 % fetal bovine serum and 1 % Glutamax and seeded in 96-well tissue culture plates at 5000 cells per well. The plates were incubated at 37 °C for 24 h. Three raws were kept on the plate for controls and blank which include cells alone, cells treated with 10 % DMSO and samples in media.

Sample preparation: The samples are prepared from a stock solution in DMSO at a concentration of 1 mM. The stock solution is diluted with media in a separate plate before being added to the cells in order to minimise the DMSO concentration to a maximum of 1 %.

Day 2: The samples were transfered from the dilution plate to the cells, added 25 µM of a 1 mg.mL⁻¹ solution of LPS and incubated at 37 °C for 24 h.
**Calibration curve:** A solution is prepared with 69 mg of NaNO\(_2\) in 1 mL MilliQ H\(_2\)O. 1 µL of this solution was diluted in 10 mL MilliQ H\(_2\)O from which 11 dilutions were prepared as described in table 6.1. 10 µL of these solutions were transferred to the reading plate in duplicate.

**Table 6.1.** Concentration of the NaNO\(_2\) solutions used for the calibration curve in the Griess reagent assay.

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<th>Standard (µM)</th>
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<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
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<td>NaNO(_2) solution</td>
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</table>

**Griess Reagent:** Two solutions prepared from 1.0 g sulphanilamide 88 in 100 mL 5 % HCl and 0.1 g N-(1-Naphthyl)ethylenediamine dihydrochloride 89 in 100 mL MilliQ H\(_2\)O were stored separately at 4 °C in dark glass bottles.

**Day 3:** 50 µL of the cell supernant and standard were transferred to the reading plate. Each well was added 20 µL of 1 % sulphanilamide and 25 µL of 0.1 % of N-(1-naphthyl)-ethylenediamine dihydrochloride and incubated for 10 min at room temperature. The absorbance of each well was read at 540 nm using a microplate reader.

Calculate nitrite concentration of samples from the absorbances using interpolation from the standard curve (0-100 µM of sodium nitrite in a 10 µM stepwise increment).

### 6.2.5. Antibacterial activity evaluation

This assay was performed by WADI (Worldwide Antibiotic Discovery Initiative). 23 compounds were tested, including 18 α-hydroxyphosphonate derivatives and 5 triazolonucleosides, on 5 Gram negative bacteria strains (*Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC700603), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 43300)).
results were compared to those of control compounds (Colistin, Polymyxin B, Vancomycin and Daptomycin) tested at 10 mg/mL in water.

**Single point bacterial inhibition assay:** Bacteria were cultured in Muller Hinton broth (MHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh MHB broth and incubated at 37 °C for 1.5-3 h. The compounds were plated at a single concentration of 64 μg/mL in non-binding surface 96-well plates (Corning; Cat. No 3641, NBS). For bacterial inhibitor controls, Colistin, Polymyxin B, Vancomycin and Daptomycin were serially diluted two-fold across the wells, with compound concentrations ranging from 0.03 to 4 μg/mL for Colistin and Polymyxin B, and from 0.125 to 16 μg/mL for Vancomycin and Daptomycin. The resultant mid-log phase cultures were diluted to the final concentration of 5 x 10⁵ CFU/mL, then 50 μL was added to each well of the plates, giving a final compound concentration of 32 μg/mL and a concentration range for the controls of 0.015 to 2 μg/mL for Colistin and Polymyxin B, 0.06 to 8 μg/mL for Vancomycin and Daptomycin. All the plates were covered and incubated at 37 °C for 24 h.

Inhibition of bacterial growth was determined visually, classifying wells into either with or without any bacterial growth.

**MIC (Minimum Inhibitory Concentration) assay:** Bacteria were cultured in Muller Hinton broth (MHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh MHB broth and incubated at 37 °C for 1.5-3 h. The compounds were serially diluted two-fold across the wells of non-binding surface 96-well plates (Corning; Cat. No 3641, NBS), with compound concentrations ranging from 0.03 μg/mL to 64 μg/mL, plated in duplicate. The resultant mid-log phase cultures were diluted to the final concentration of 5 x 10⁵ CFU/mL, then 50 μL was added to each well of the compound-containing 96-well plates, giving a final compound concentration range of 0.015 μg/mL to 32 μg/mL. All the plates were covered and incubated at 37 °C for 24 h.
The inhibition of bacterial growth was determined visually after 24 h, where the MIC is recorded as the lowest compound concentration with no visible growth.

### 6.3. Computational procedures

#### 6.3.1. Protein and Ligand

For docking purposes, the crystallographic coordinates of the porcine ST3Gal-I, the human ST6Gal-I and the human ST3Sia-III complexed with CMP were attained from the RCSB Protein Data Bank (PDB code 2WNB, 4JS2, 5BO6, 5BO7 and 5CXY respectively). The ligand was then removed to leave the receptor complex using Discovery Studio 4, which was used for the subsequent docking studies. The three-dimensional structures of the inhibitors were prepared utilizing ChemDraw 14.0 and Discovery Studio 4. The PDBQT files for the STs and the inhibitors were generated using Autodock Tools 1.5.6 and used as the input for AutoDock Vina 1.1.2. When docking, the grid scale was set as 30 Å x 30 Å x 30 Å based on grid module, cantered at the active site. The dimension was chosen to ensure it was big enough to cover all of the flexible residues. The docking procedure was validated by re-docking to replicate the crystallographically determined ST-CMP complex. Gasteiger charges were set for both substrates and protein. The protein was kept rigid, all torsional bonds of the ligands were kept free and water molecules have been removed from the PDB file.

#### 6.3.2. Docking

In the context of this study, docking into the crystal structures was performed by using AutodockVina. It is the lastest member of the AutoDock suite introduced in 2010 which has been developed at The Scripps Research Institute. The enzymes and ligands structures were prepared using AutoDock Tools as well as the docking parameters, such as the size of
A series of comparisons were made for models generated for different exhaustiveness values. The exhaustiveness is a function that controls how “exhaustive” a search the program performs to find a global minimum. Default settings had an exhaustiveness value of 12.

### 6.3.3. Protein alignment

The protein sequence alignments were performed using the online platform T-coffee. The acronym ‘T-coffee’ stands for ‘Tree-based Consistency Objective Function For alignment Evaluation’ and allows multiple alignments. The protein sequences were collected from the Universal Protein Ressource (Uniprot) and used in the FASTA format.

Structures were superimposed using Discovery Studio 4 (Copyright 2005-2013, Accelrys Software Inc.).
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References


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References


References


References


References


APPENDICES
Appendix A

Figure A.1. $^1$H NMR of compound 228 in CDCl$_3$

Figure A.2. APT NMR of compound 228 in CDCl$_3$
Figure A.3. COSY NMR of compound 228 in CDCl$_3$

Figure A.4. HMBC NMR of compound 228 in CDCl$_3$

Figure A.5. HSQC NMR of compound 228 in CDCl$_3$
### Table B.1. Percentage inhibition of the triazole derivatives at 100 µM against STBSia-II.

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Appendix B

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This appendix contains the front pages of selected published journal articles:

Advancement of Sialyltransferase Inhibitors: Therapeutic Challenges and Opportunities

Rimi Szabo¹ and Danielle Skropeta¹,²

¹School of Chemistry, University of Wollongong, Wollongong, NSW, 2522, Australia
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Abstract: Hypersialylation of tumor cell surface proteins along with a marked upregulation of sialyltransferase (ST) activity is a well-established hallmark of cancer. Due to the critical role of STs in tumor growth and progression, ST inhibition has emerged as a potential new antimetastatic strategy for a range of cancers including pancreatic and ovarian. Human STs are divided into subtypes based on their linkage and acceptor molecule, with each subtype controlling the synthesis of specific sialylated structures with unique biological roles. This has important implications for inhibitor development, as STs also play significant roles in immune response, inflammation, viral infection, and neurological disorders. Thus, the current goal in order to advance to the clinic is the development of subtype selective, cell-permeable and synthetically accessible, small-molecule ST inhibitors. Herein is a comprehensive review of the latest developments in ST inhibitors from design, Nature, and high-throughput screening, addressing both the challenges and opportunities in targeting cell surface sialylation. The review features an overview of the biological evaluation methods, computational and imaging tools, inhibitor molecular diversity, and selectivity toward ST subtypes, along with the emerging role of ST inhibitors as diagnostic tools for disease imaging.


Keywords: sialyltransferase inhibitors; structure-based design; anticancer agents; antimetastasis; diagnostic tools

I. INTRODUCTION

Sialic acid is the body’s most important sugar next to glucose.¹ Sialylation, the addition of sialic acid to cell surface molecules via sialyltransferase (ST) enzymes, is integral to cell function, governing numerous important biological processes including cell-cell recognition, adhesion, protein targeting, and fertilization.² However, aberrant sialylation is a hallmark of cancer, with hypersialylation of up to 30% seen in many cancers, along with marked upregulation of ST activity. This is directly correlated with increased metastatic potential and poor patient prognosis.³,⁴ Sialylation mediates tumor metastasis via several routes including stimulating tumor invasion and migration through integrin-mediated processes,⁵,⁶ promoting tumor survival by blocking galectin binding,⁷ and inhibiting Fas-mediated apoptosis.⁸,⁹ Due to the critical role of STs in

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Appendix B