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Synthesis of [60]fullerenyl amino acid derivatives for medicinal chemistry and material science applications

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Synthesis of [*60*]Fullerenyl Amino Acid Derivatives For Medicinal Chemistry and Material Science Applications

A thesis submitted in fulfilment of the requirements
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From

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By

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School of Chemistry

March 2015

Declaration

I, Sreenu Jennepalli, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Sreenu Jennepalli

March 2015

Publications

Sections of the work described in this thesis have been reported in the following publications:

1. Tatyana E. Shubina, Dmitry I. Sharapa, Christina Schubert, Dirk Zahn, Marcus Halik, Paul A. Keller, Stephen G. Pyne, **Sreenu Jennepalli**, Dirk Guldi, and Timothy Clark. Fullerene van der Waals Oligomers as Electron Traps. ***J. Am. Chem. Soc.*** **2014**, *136*, 10890–10893

2. Sreenu Jennepalli, Stephen G. Pyne and Paul A. Keller. [*60*]Fullerenyl Amino Acids and Peptides: A Review of their Synthesis and Applications. ***RSC Advances,*** **2014,** *4*, 46383-46398.

3. Sreenu Jennepalli, Katherine A. Hammer, Thomas V. Riley, Stephen G. Pyne and Paul A. Keller. Synthesis of Water Soluble Mono and Bis[*60*]fullerene Based Di-Cationic Peptoids. ***Eur. J. Org. Chem.*** **2015**, 195–201.

Abstract

Chapter 1 reviews the synthesis and applications of [*60*]fullerene amino acids and peptides, in material science and biological activities reported so far. We segregated the type of [*60*]fullerene amino acids based on their connectivity, such as, fullerenyl substituents directly substituted to the amino acid α-carbon, [*60*]fullerenyl *N*-capped peptides and amino acids, [*60*]fullerenyl *C*-capped peptides and amino acids and fullerenyl amino acids that could potentially be incorporated within peptide chains.

Chapter 2 demonstrated the synthesis of the novel [*60*]fullerene monopeptide **201** and dipeptide **204**. [*60*]Fullerenoproline acid **198** was coupled to lysine amine **200** to yield the monopeptide **201** in 62% yield, which was separately deprotected to its amine salt **202** using TFA and to its acid derivative **203** with $Sn(CH_3)_3OH$. An amide coupling reaction of the amine **202** and the acid **203** resulted in the dipeptide **204** (57%). Attempts to synthesise the tetrapeptide **207** using the same approach were not successful because of its poor solubility. Monopeptide **201** and the dipeptide **204** have been studied experimentally using spectroelectrochemical measurements by Prof. Timothy Clark and Prof. Dirk M. Guldi at the University of Erlangen-Nuremberg, Germany. The bis-fullerene-substituted peptide **204** provided experimental support for density-functional theory (DFT) calculations which indicate that van der Waals fullerene dimers can form between adjacent fullerenes in semiconductor layers resulting in interstitial electron traps.

An alternative synthesis of the tetrapeptide **207** was examined. The lysine tetrapeptide **216** (70% yield) and its tetramine **217** (100%) were successfully synthesised. Attempts to couple **217** with [*60*]fullerenoproline acid **198** to produce the tetrapeptide **207** were also unsuccessful, possibly due to steric hindrance problems because of the sterically

demanding fullerene groups. Accordingly, we thought that the cyclic peptide tetrapeptide **220** might help to solve these steric hindrance problems. Tetrapeptide **216** was successfully converted to the novel cyclic peptide **220** in three synthetic steps. However we did not pursue this route any further because of the poor chemical yields and the difficulties in purifying **220**.

Chapter 3 reported an extension of [*60*]fullerenyl oligomers with the incorporation of spacers between the fullerenyl groups. Two types of amino acid spacers (L-lysine and L-phenylalanine) were introduced in to the oligomers (hexapeptide **227** and hexapeptide **238**). The synthesis of tripeptides with L-lysine spacers **224** in 62%, and L-phenylalanine spacers **235** (in 60%) were achieved by coupling the acid **203** with the amine **213** and the amine **202** with the acid **234**, respectively. The previously used ester and *N*-Boc cleavage reaction conditions were adopted to synthesise the corresponding acids (**225** and **236**) and amines (**226** and **237**) of **224** and **235**. Their amide coupling reaction provided the hexapeptides of **227** (44%) and **238** (40%) respectively. Currently, these molecules are under studies to form supramolecular complexes between these [*60*]fullerene peptides and porphyrin and photoinduced charge separation in Prof. Fukuzumi's laboratory at Osaka University, Japan.

Chapter 4 reported the synthesis and antibacterial screening of mono substituted [*60*]fullerenyl peptides (**243**, **245**, **247**, **272-279**) and bis-[*60*]fullerenyl peptide (**285**). A range of tri, tetra and pentapeptides having an oxazole or isopentyl ester terminal group were synthesised. These peptides were then deprotected to form their HCl and TFA salts **247**, **276-279**. Antibacterial screening of these peptides (**243**, **245**, **247**, **272-279** and **285**) was performed at the University of Western Australia in Prof. Thomas Riley's laboratory; unfortunately, none of these compounds showed significant activity.

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Acknowledgements

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And finally, I would like to thank my extended family and friends for their constant help and encouragement during the completion of my PhD and life in general.

Abbreviations

Å	Angstrom
^{o}C	Degree/s Celsius
µL	Microlitre/s
µmol	Micromole/s
Ac	Acetate
Boc	*tertiary*-Butoxycarbonyl
c	Concentration
calcd	Calculated
CG	Conjugate Gradient
conc.	Concentrated
conf.	Configuration
d	Doublet
dd	Doublet of doublets
DFT	Density Functional Theory
DIPEA	Diisopropylethylamine
dm	Decimetre/s
DMAP	Dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNP	Double Numerical basis set with Polarization
dppf	Bis(diphenylphosphino)ferrocene
E	Energy
E	*Trans*
EI	Electron Impact
eq.	Equivalent/s
ESI	Electrospray Ionisation
ES+	Positive Ion Electrospray
ES−	Negative Ion Electrospray

eV	Electron volt/s
g	Gram/s
h	Hour/s
HPLC	High Performance Liquid Chromatography
HR	High Resolution
Hz	Hertz
IC_{50}	Median Inhibitory Concentration
IR	Infra-red
K	Degree/s Kelvin
kcal	Kilocalories
L	Litre/s
LA	Lewis Acid
LDA	Lithium Diisopropylamide
Lit.	Literature
m	Multiplet (NMR)
m	Medium (IR)
M	Molar
mbar	Millibar/s
mg	Milligram/s
min	Minute/s
mL	Millilitre/s
mmol	Millimole/s
mol	Mole/s
m.p.	Melting Point
MS	Mass Spectrometry
Ms	Methanesulfonyl
NBS	*N*-Bromosuccinimide
nm	Nanometre/s
NMR	Nuclear Magnetic Resonance
Nu	Nucleophile
o	*ortho*
PES	Potential Energy Surface
PG	Protecting Group

PMB	*para*-Methoxybenzyl
ppm	Parts Per Million
QST	*Quadratic Synchronous Transit*
R_F	Retention Factor
RMS	Root Mean Square
RT	Room Temperature
rot.	Rotation
s	Singlet (NMR)
s	Strong (IR)
SAR	Structure Activity Relationship
sat.	Saturated
SCF	Self-Consistent Field
t	Triplet
tert	*tertiary*
$t_{1/2}$	Half Life
Temp	Temperature
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
TS	Transition State
UV	Ultra-Violet
v	Volume
w	Weak (IR)
W	Watt/s
wt.	Weight
Z	*Cis*
ZPE	Zero Point Energy

[*60*]Fullerenyl Amino Acid Derivatives: Synthesis and Their Applications

A large portion of synthetic [*60*]fullerene chemistry is directed towards its functionalization providing biologically active derivatives and molecules with applications also in material science.[1-6] The impetus for such work was to exploit the physical properties of [*60*]fullerene (such as sensitization of singlet oxygen and electron acceptor characteristics) and combine this with the properties of biomolecules (water solubility and precise secondary and tertiary structure).[5,7-10] For example, [*60*]fullerene derivatives covalently linked to peptides and proteins has been the goal of a number of research groups concerned with the application of [*60*]fullerene-peptide conjugates to biological problems.[11-16] It was anticipated that the addition of biologically active compounds to [*60*]fullerene in a strict regioselective fashion would aid the activity and specificity of future therapies. In recent times, the field has matured significantly such that there are different types of fullerenyl amino acids that can be categorised according to properties of the amino acid itself, allowing for a broader perspective of how they could be incorporated into different research programs. In this chapter we report on the current progress in the synthesis of fullerenyl amino acids and related derivatives and attempt to categorise the molecules into functional types for different uses: these include directly attached fullerenyl amino acids, fullerenyl *N*- and *C*-capping amino acids, and those amino acids in which the [*60*]fullerene group is attached to the amino acid side chain. These first and last mentioned derivatives have the potential to be incorporated into non-terminal positions of peptides. The applications of these substrates by

integration into different biological and materials chemistry programs are then highlighted.

1.1 Synthesis of Fullerenyl Amino Acids

From a material science and medicinal chemistry perspective, fullerenyl amino acids are important targets potentially serving as central hubs in architecturally defined nanostructures or 3D-templates in drug design.[17-24] To date fullerenyl amino acids and peptide derivatives have been prepared by the initial attachment of a handle to the fullerene followed by coupling to a protected amino acid or peptide.[25-27]

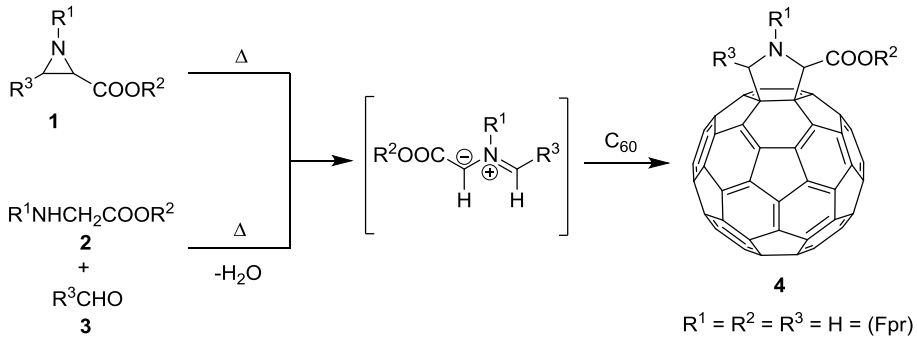
1.2 Synthesis of Derivatives with Fullerenyl Substituents Directly Substituted to the Amino Acid α-Carbon

There are surprisingly few examples of fullerenyl amino acids that are direct analogues of α-amino acids, *i.e.* the α-carbon is bonded directly to a C_{60} carbon atom. There are two examples of this type of molecule, the well-established fullerenoprolines, and there are reports of protected versions of amino acids whereby the fullerenyl substituent is directly coupled to the α-carbon.

1.2.1 Fullerenoproline derivatives

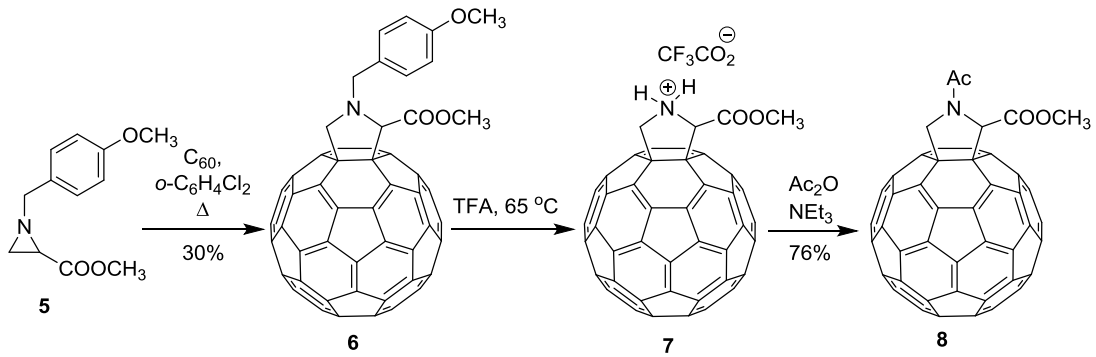
The first synthesis of α-substituted fullerenyl amino acids, the fullerenoprolines (Fpr) (**4**), was achieved by the addition of azomethine ylides to [*60*]fullerene.[28-30] The azomethine ylide intermediate can be generated in two different ways, either *via* a thermal ring-opening of aziridines **1** or *via* tautomerisation of iminium salts formed by the condensation of α-amino esters **2** with aldehydes **3** (Scheme 1.1). These reactions

allow for a significant number of Fpr derivatives to be generated by using different combinations of aldehydes and amino esters.



Scheme 1.1: The synthesis of fulleroprolines **4** can be achieved by thermal addition of aziridines or iminium salts to [*60*]fullerene.

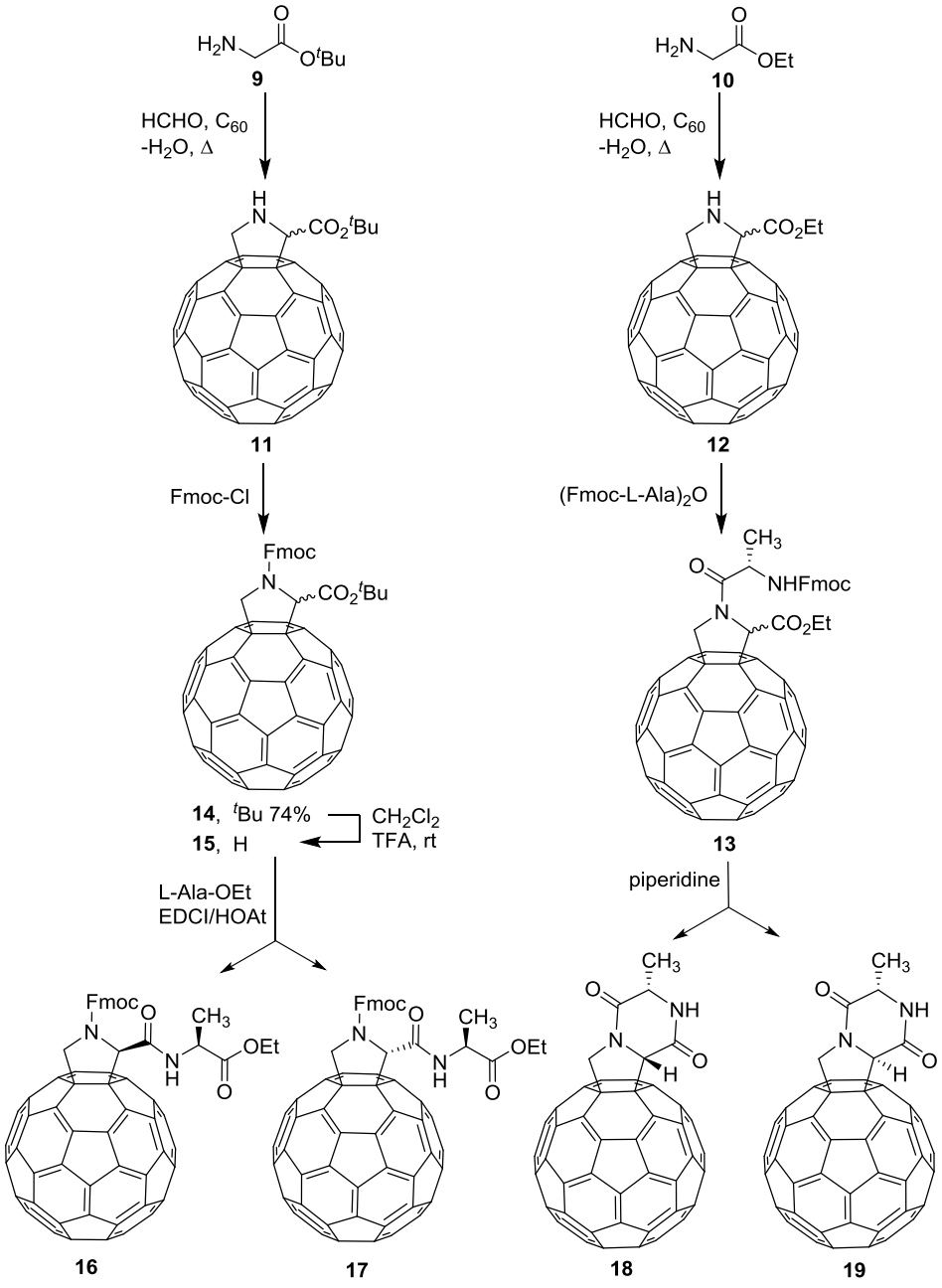
Fpr analogues can be prepared with the pyrrolidine nitrogen protected (Scheme 1.2) or unprotected (Scheme 1.3).[31] Addition of the aziridine **5** to [*60*]fullerene under thermal conditions formed the *N*-protected Fpr **6**. Subsequent treatment with TFA provided the secondary amine salt **7**, which could then be acylated with acetic anhydride to provide **8** (Scheme 1.2).[31]



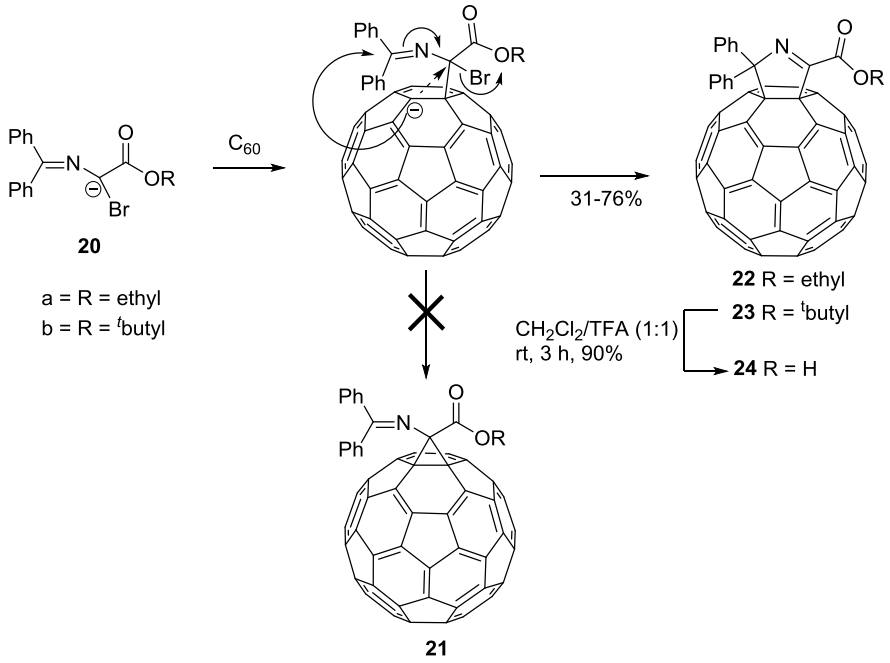
Scheme 1.2: Synthesis of the protected fulleroproline **8** was achieved through thermal addition of aziridine **5** to [*60*]fullerene.

To obtain more useful Fpr derivatives for peptide synthesis, the azomethine ylides, which are generated *in situ* from the reaction of glycinate esters **9** and **10** with paraformaldehyde, can be added to [*60*]fullerene to produce the free Fprs **11** and **12**, respectively. These were relatively unstable and had to be kept as dilute solutions in the dark.[31] However, the amine group was readily functionalized using standard acylation procedures with acid anhydrides and acid chlorides (Scheme 1.3), to deliver racemic *N*-protected Fpr ester of the type **13**. Alternatively the amine **11** was protected as the N-Fmoc derivative **14**, and then the *tert*-butyl ester converted to the acid **15** by treatment with TFA. Subsequent coupling with ethyl L-alaninate under EDCI/HOAt conditions afforded the diastereomers **16** and **17**. The diastereomeric ratio of **13** was determined by formation of the dioxopiperazines **18** and **19**, thus eliminating the additional complexity in characterization imposed by amide rotamers.[31]

A second type of fullerenyl amino acid was initially envisaged as a methanofullerene of the type **21** (Scheme 1.4). Strategies towards such molecules include the addition of diphenyliminoglycinates to [*60*]fullerene under Bingel cyclopropanation reaction conditions which was originally reported to furnish the corresponding methano[*60*]fullerene derivatives **21** (Scheme 1.4).[32,33] However subsequent extensive NMR studies revealed that the structure of the products was that of the dihydrofullerenoproline derivatives[33] **22**, and not the more strained three-membered (cyclopropane) ring products **21**. The dihydrofullerenoproline derivative **23** was hydrolysed to its carboxylic acid using TFA to yield **24** in 90% yield.

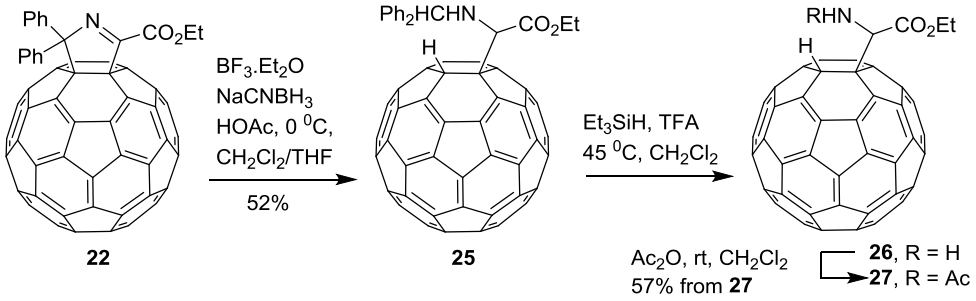


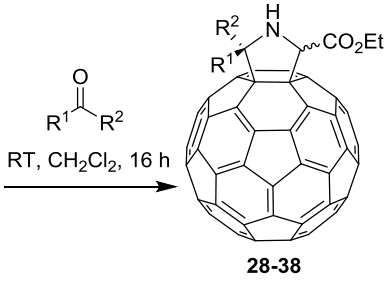
Scheme 1.3: Synthesis of Fpr peptide derivatives **16-19**.



Scheme 1.4: The addition of iminoglycinate to fullerene under Bingel cyclopropanation conditions provides the analogous [*60*]fullerenyldihydropyrrole **22** not the previously reported methano[*60*]fullerene **21**.

$BF_3.Et_2O$ and $NaCNBH_3$ mediated reductive ring-opening of dihydrofullerenoproline derivative[33] **22** yielded the ethyl *N*-benzhydrylfullerenyl[*60*]glycinate **25**, which was *N*-deprotected to give ethyl fullerenylglycinate **26**[34] a true fullerenyl α-amino ester which was fully characterised as its more stable *N*-acetyl derivative **27**. Therefore, the original structure **22** could be considered as a protected version of **26**. The free amine of **26** reacted with a variety of aldehydes and ketones in a Mannich-type process to produce 5-substituted and 5,5-disubstituted Fprs **28-38.** This process represents a versatile and general strategy to synthesise Fprs.

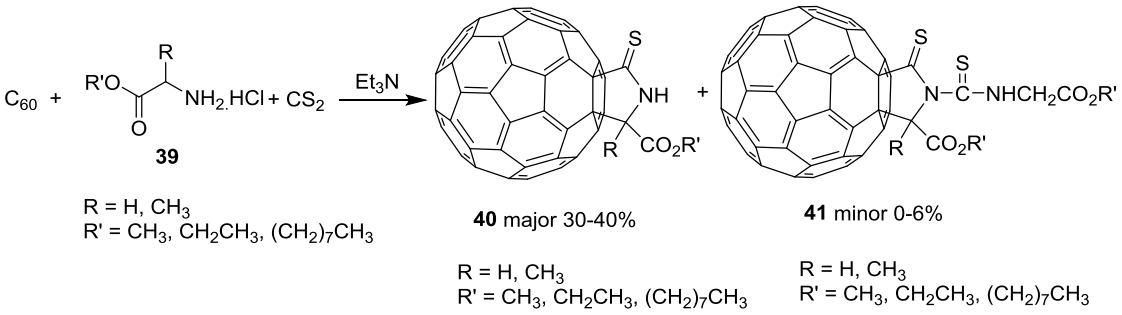




entry	R^1, R^2	product (yield from 25, %)	*cis:trans*
1	H, *i*-Pr	**28** (49)	3:1
2	H, *t*-Bu	**29** (47)	3:1
3	H, *c*-C_6H_{11}	**30** (48)	2:1
4	H, Ph	**31** (58)	2.5:1
5	H, ferrocenyl	**32** (37)	5:1
6	Me, Me	**33** (64)	-
7	Et, Et	**34** (61)	-
8	-$(CH_2)_4$-	**35** (54)	-
9	-$(CH_2)_5$-	**36** (51)	-
10	-$(CH_2)_6$-	**37** (54)	-
11	Me, Ph	**38** (14)	2.5:1

Scheme 1.5: Synthesis of 5-substituted and 5,5-disubstituted fulleroprolines **28-38**.

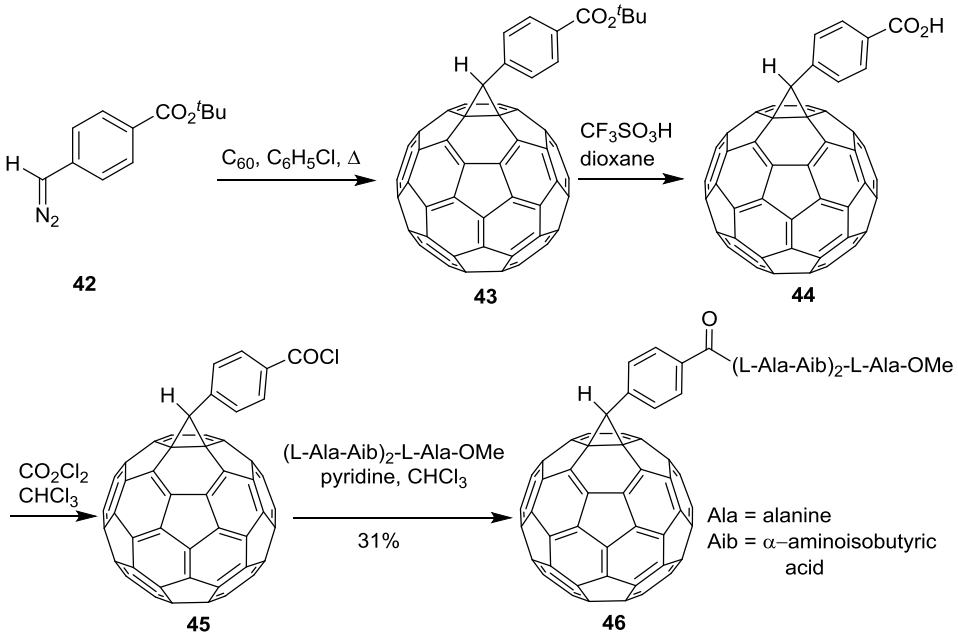
The reactions of amino acid ester hydrochlorides and CS_2 with [*60*]fullerene in the presence of Et_3N yields novel [*60*]fullerene derivatives **40** (major) and **41** (minor) containing biologically active amino acids,[35] thioamide, and thiourea units. The thiolactam groups in compounds **40** are sensitive to moisture and can easily be hydrolyzed to the corresponding lactams.



Scheme 1.6: Reactions of [*60*]fullerene with amino acid esters and CS_2.

1.3 Synthesis of [*60*]fullerenyl *N*-Capped Peptides and Amino Acids

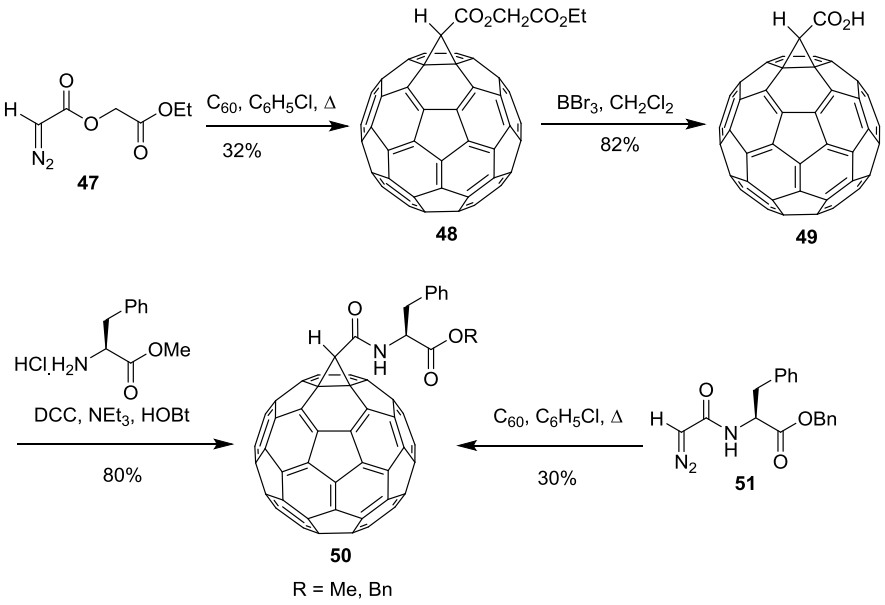
The thermal addition of the diazomethane derivative **42** to [*60*]fullerene and subsequent deprotection of the ester of the resulting methano[*60*]fullerene **43** provided the acid **44**.[36] This represented the first synthesis of a fullerenyl compound which had a synthetic handle to readily allow for peptide functionalization (Scheme 1.7). The acid **44** was converted to the reactive acid chloride **45** which underwent amide coupling with a pentapeptide under basic conditions to provide the first reported fullerenyl peptide **46**.[36]



Scheme 1.7: Synthesis of **46** through a diazo-addition of **42** to [*60*]fullerene.

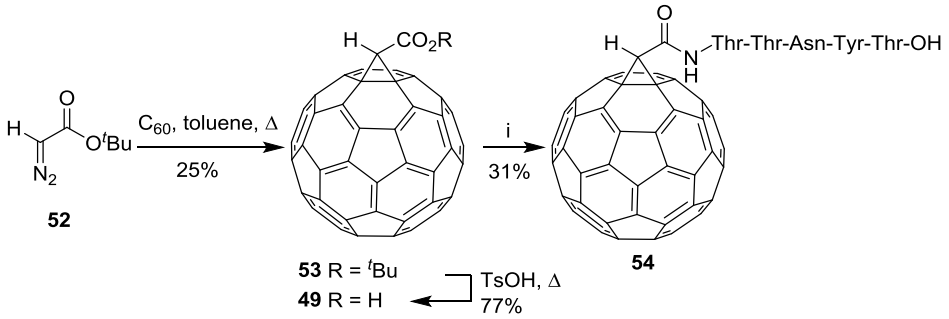
This work was extended to alkyl diazoacetates of the type **47**, which was added to [*60*]fullerene forming the methano[*60*]fullerene **48**, which was deprotected to the carboxylic acid **49** and then coupled to amino acids under standard DCC coupling reaction conditions to form fullerenyl amino acid esters of the type **50**.[37,38] Alternatively, a more efficient route was developed, that allowed the direct addition of

diazoamides to [*60*]fullerene. For example, a solution of [*60*]fullerene in toluene was treated with the diazoacetamide **51** at reflux for 48 h providing the fullerenyl amino acid **50** (R = Bn) in 30% yield (Scheme 1.8).[39]



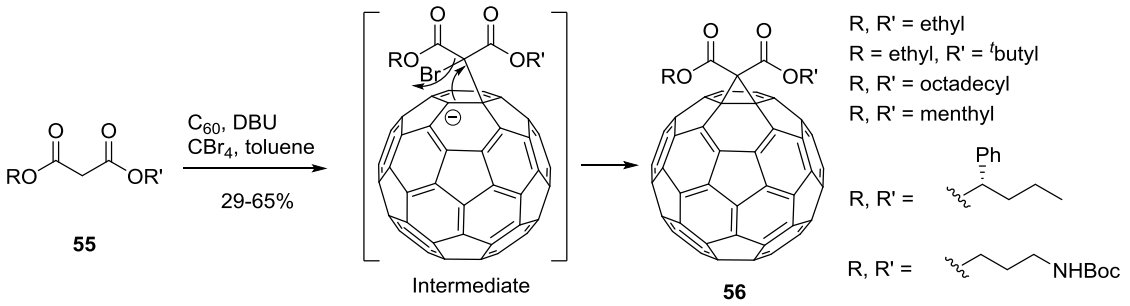
Scheme 1.8: Synthesis of **50** through a diazo-addition of **47** or **51** to [*60*]fullerene.

Thermal reactions of [*60*]fullerene with other diazo compounds such as diazomethanes,[40,41] diazoacetates,[42] diazoamides, diazomethylphosphonates,[43] and diazoketones, provides a broad variety of methano[*60*]fullerenes, having handles for further functionalization. The methano[*60*]fullerene carboxylic acid **49** can be accessed from either the *tert*-butyl ester **52** (Scheme 1.9) or the *O*-glycolic ester **47** (Scheme 1.8), followed by ester deprotection of the fullerenyl adducts **53** and **48**, respectively. The synthetic utility of the methano[*60*]fullerenyl carboxylic acid **49** was further demonstrated by its DCC/HOBt mediated coupling with peptides to produce the fullerenyl peptides **54**.[44,45]



Scheme 1.9: Synthesis of peptide **54** was achieved through addition of **52** to [*60*]fullerene. i) H-Thr-Thr-Asn-Tyr-Thr-OH, DCC, HOBt, C_6H_5Br/DMSO (6:1).

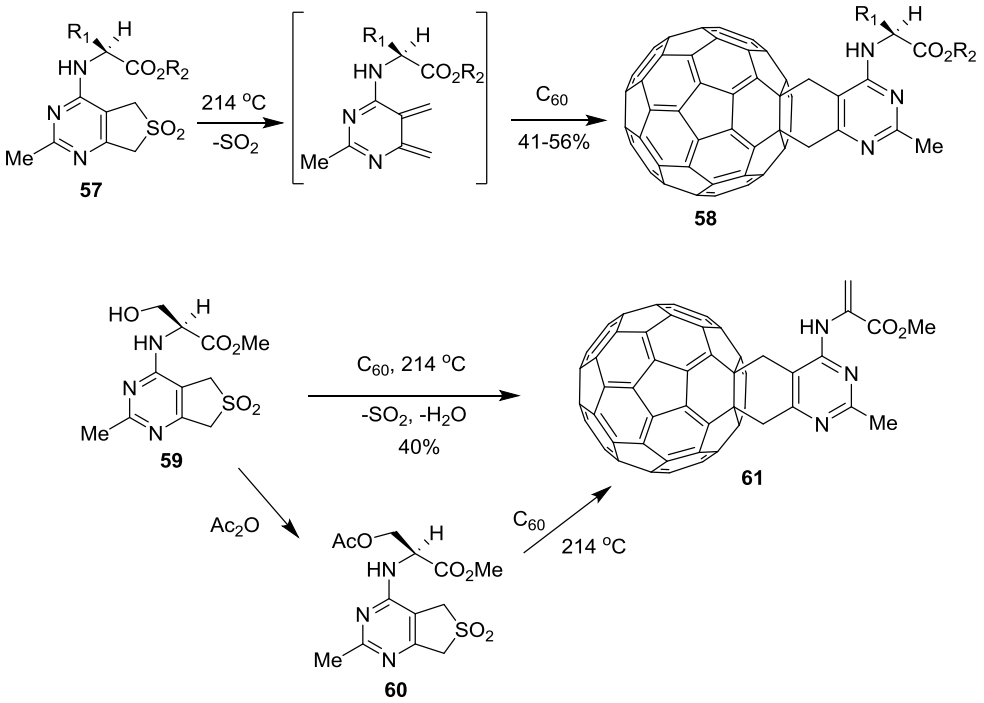
The Bingel reaction conditions have been modified to work, in moderate to excellent yields, with ketones, esters and iminoglycinates. This modification has allowed for the generation of the analogous α-haloanion *in situ* rather than the previous isolation of the halogenated intermediate. This efficient and reliable one-pot reaction has been used extensively with malonic esters **55** and derivatives, as illustrated in Scheme 1.10.[46-48]



Scheme 1.10: Malonates add to [*60*]fullerene under Bingel cyclopropanation conditions to provide methanofullerenes **56**.

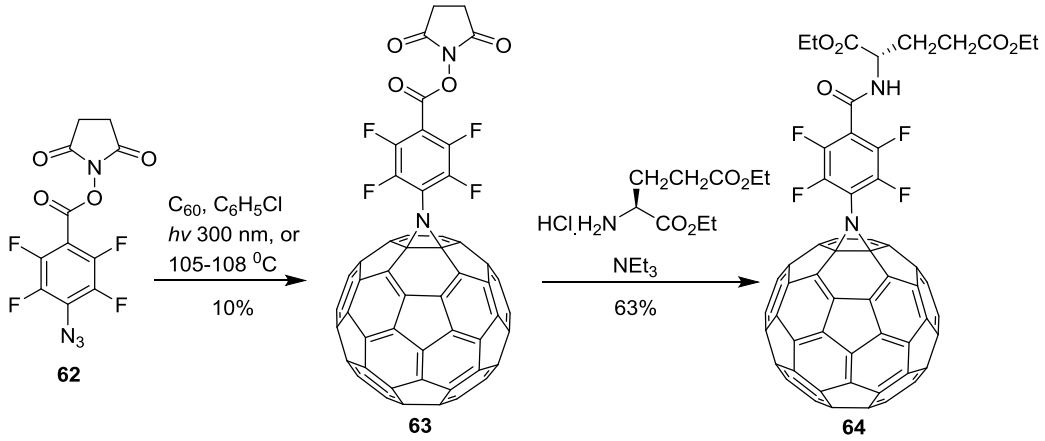
Thermolysis of sulfones **57** in the presence of [*60*]fullerene in 1,2,4-trichlorobenzene at reflux under a nitrogen atmosphere afforded the fullerene derivatives **58** (*via* the corresponding pyrimidine *o*-quinodimethanes) (Scheme 1.11). A

similar reaction with **59** did not give the expected adduct but the dehydration product **61** (Scheme 1.11).[49]



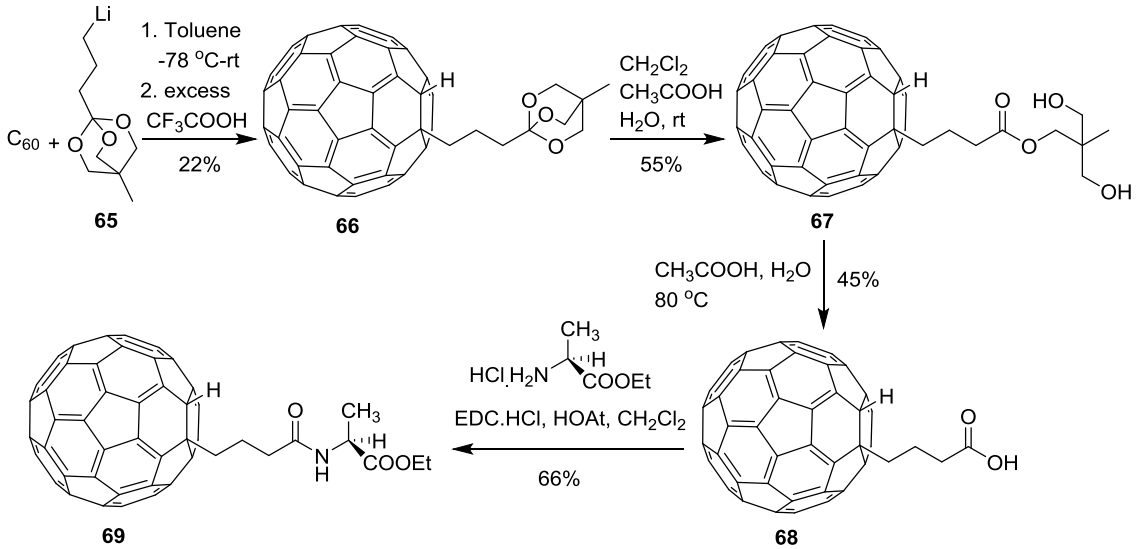
Scheme 1.11: Synthesis of [*60*]fullerene based tetrahydroquinazolines.

Organic azides act as 1,3-dipoles and can undergo [3+2]-cycloaddition reactions (either photochemically or thermally) with [*60*]fullerene to yield fulleroaziridines (Scheme 1.12).[50,51] Of these organic azides, photochemical or thermal reactions of [*60*]fullerene with perfluorophenylazide **62** produces the fulleroaziridine **63** with an activated *N*-hydroxysuccinamide ester. This active ester allows further functionalization, such as peptide coupling resulting in the formation of **64**.



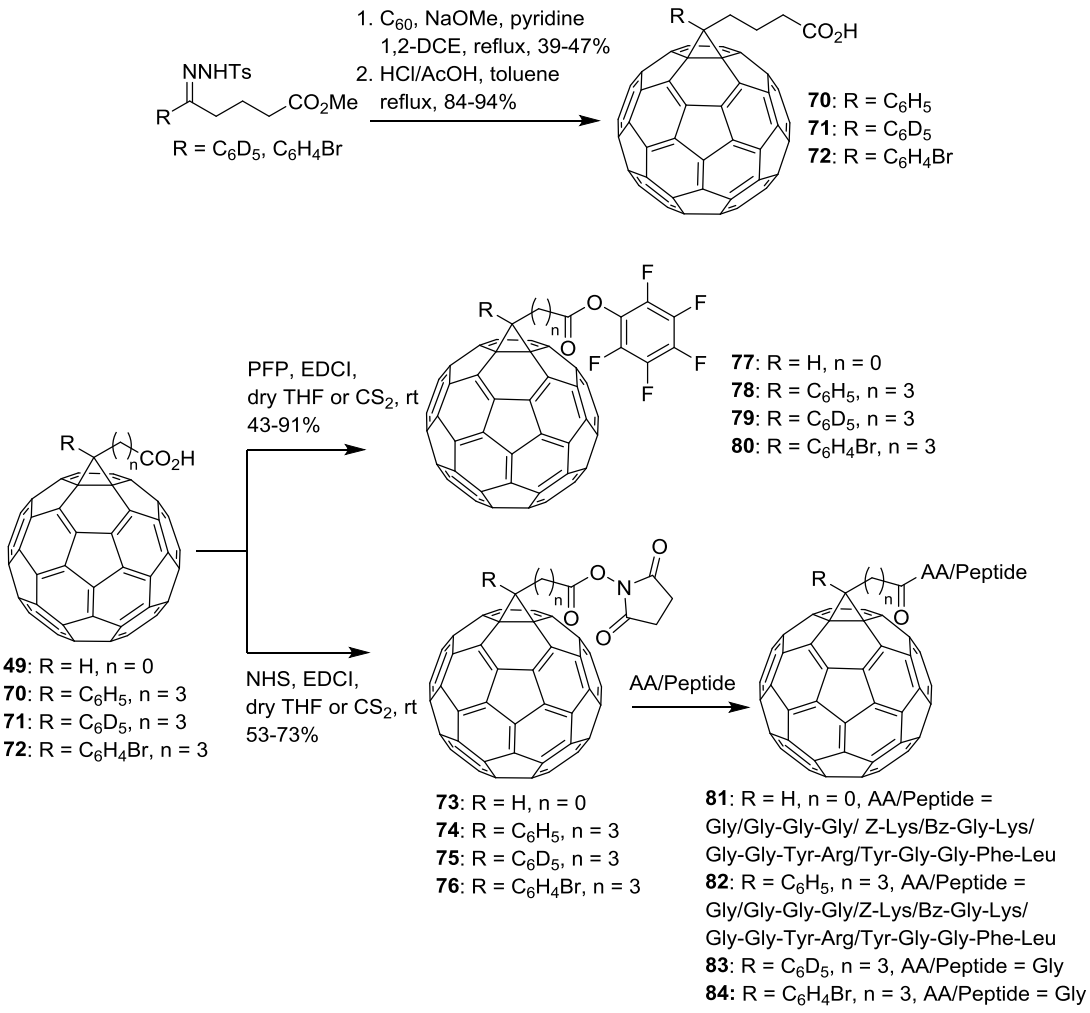
Scheme 1.12: Synthesis of fulleroaziridine

The reaction of [*60*]fullerene with organolithium and Grignard reagents have been exploited to incorporate a protected carboxylic acid functionality onto the C_{60} sphere.[52] The organolithium reagent made from the stable bicyclic orthoester **65**, prepared by following the general procedure described by Corey,[53,54] has been successfully added to [*60*]fullerene to give adduct **66**. This was converted to the acid **68** by two sequential hydrolysis steps and then coupled with L-alanine ethyl ester hydrochloride to give **69** (Scheme 1.13).



Scheme 1.13: Synthesis of 1-hydro-2-[3′-(L-Ala-OEt)propyl]-1,2-dihydro[*60*]fullerene **69**.

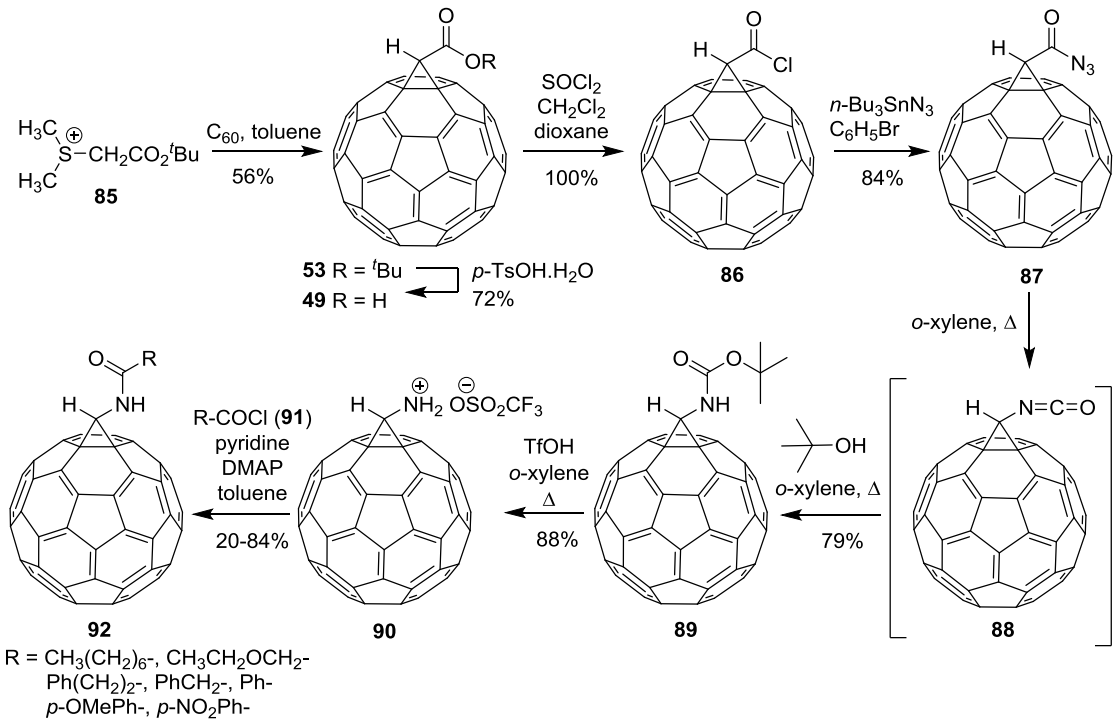
Methano[*60*]fullerene carboxylic acid derivatives **49** and **70-72** were converted to their active esters of *N*-hydroxysuccinimide (NHS) **73-76** and pentafluorophenol (PFP)[55] **77-80** and coupled with amino acids and peptides. These active esters have been reacted selectively with the amino group of the amino acid or peptide, or the hydroxy group but only under DMAP catalysis. Analysis by MALDI-TOF-MS revealed the potential utility of these active esters for the preparation of fullerene-modified amino acids or peptides for applications in biological studies (Scheme 1.14).



Scheme 1.14: Derivatization of carboxylic acids **49** and **70-72** with amino acids and peptides.

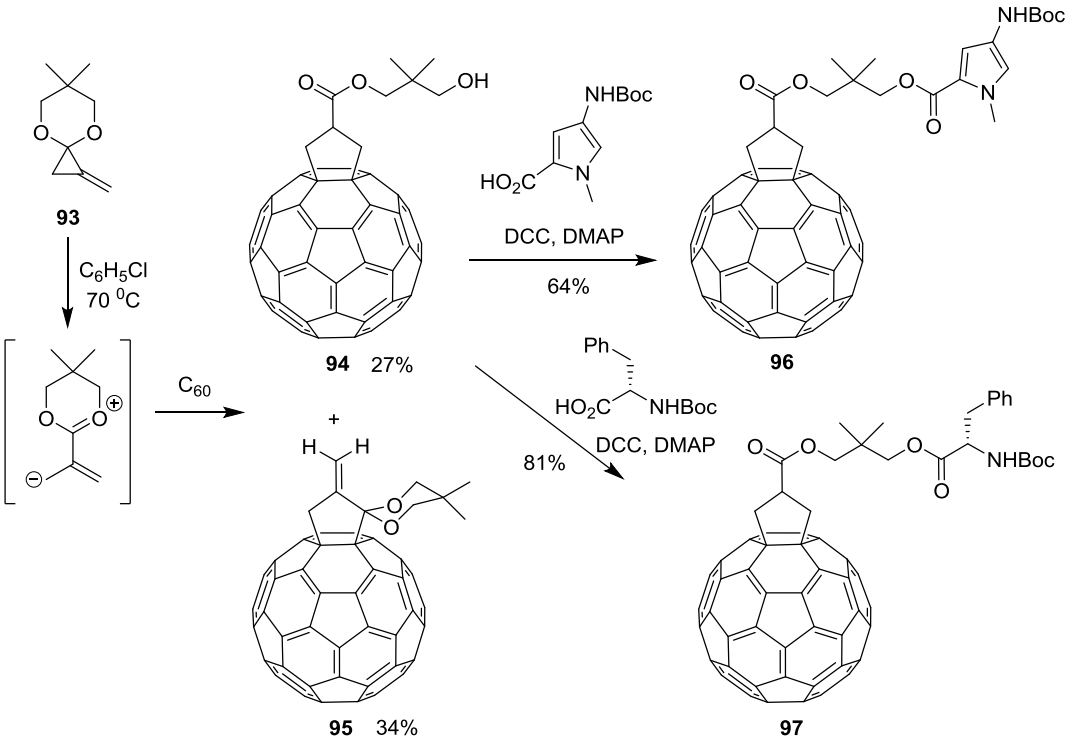
1.4 Synthesis of [*60*]Fullerenyl *C*-Capped Peptides and Amino Acids

Alternative methods to generate methano[*60*]fullerene derivatives *via* addition/elimination mechanisms have employed sulfonium and phosphonium ylides.[56,57] Deprotection of the ester moieties in these methano[*60*]fullerenes, as well as the malonate derivatives, has provided access to versatile handles, including the carboxylic acid **49** which was generated by the addition of the sulfonium ylide **85** to [*60*]fullerene, followed by cleavage of the *tert*-butyl ester **53** with *p*-TsOH (Scheme 16).[58] The acid **49** was then converted to its acid chloride **86**, which was subsequently treated with tributyltin azide to deliver the acyl azide **87** in good yield. Exposure of **87** to *o*-xylenes at reflux was expected to have afforded the isocyanate **88**, which was not isolated but trapped as the *tert*-butyl carbamate derivative **89**. Treatment of **89** with TfOH provided the amine salt **90**, which was coupled to various acyl chlorides **91** to generate the corresponding amide derivatives **92** (Scheme 1.15).[59]



Scheme 1.15: Synthesis of amides **92** was achieved *via* the Curtius rearrangement.

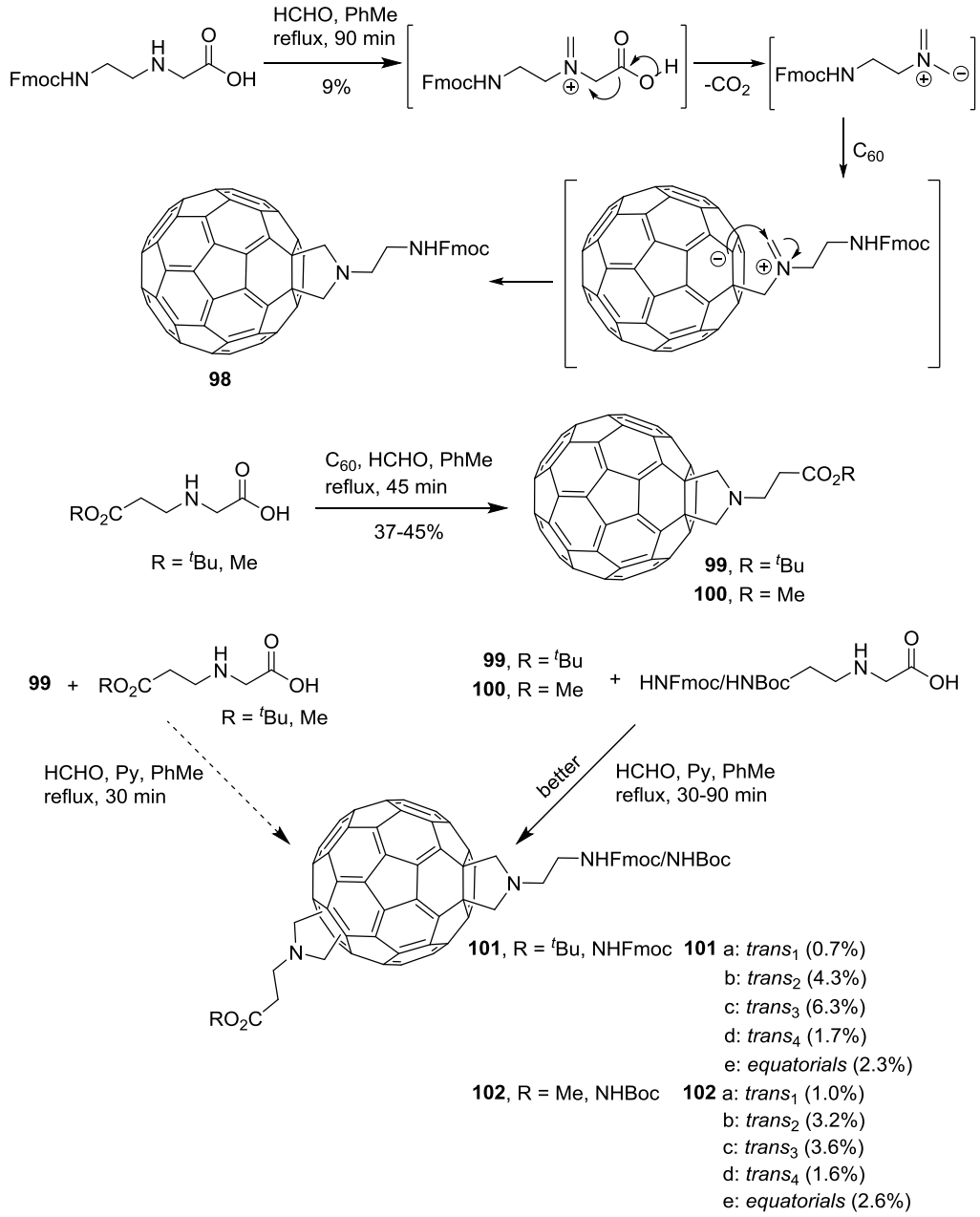
Thermal ring opening of methylenecyclopropanone ketal **93** in chlorobenzene in the presence of [*60*]fullerene followed by silica gel hydrolysis of the resulting ketene acetal gave a mixture of cycloadducts **94** and **95**.[60] The corresponding fullerenyl amino ester derivatives **96** and **97** were subsequently prepared from **94** by DCC coupling (Scheme 1.16).[61]



Scheme 1.16: Synthesis of fullerenyl amino ester derivatives *via* [3+2]-cycloaddition reactions.

Protected bis-fulleropyrrolidine amino acids derivatives **101** and **102** have been synthesised and characterised from two successive [3+2]-cycloaddition reactions of azomethine ylides to [*60*]fullerene, prepared *in situ* from the reactions of *N*-glycine derivatives and formaldehyde (Prato reaction). These reactions resulted in two different fullereopyrrolidine couples having unsymmetrical, orthogonally protected amino and acid functional groups.[62] These peptides could be used as models for fullerene-based

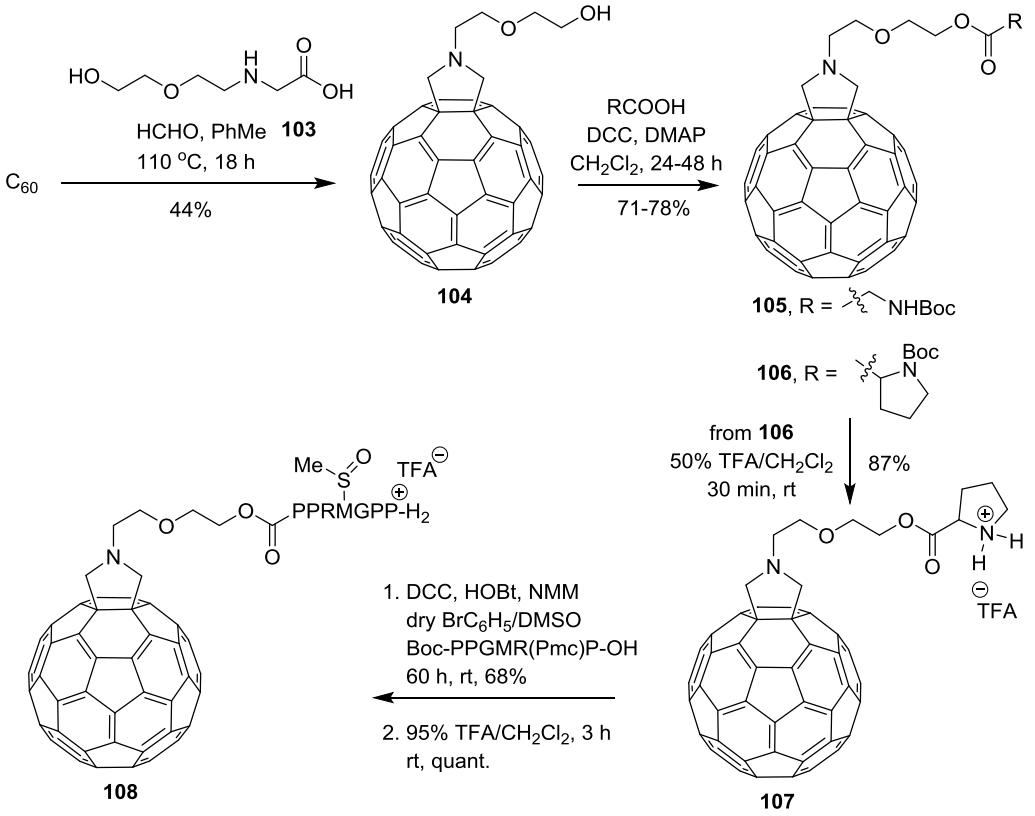
peptidomimetics with the carbon sphere inserted into the peptide backbone (Scheme 1.17).



Scheme 1.17: Synthesis of mono-fulleropyrrolidines and unsymmetrical bis-fulleropyrrolidines.

The 1,3-dipolar cycloaddition reaction of the *N*-substituted glycine derivative **103** to [*60*]fullerene produced the fulleropyrrolidine **104** having a hydroxyl group at the terminus of the *N*-substituent (Scheme 1.18). Subsequent esterification of this alcohol

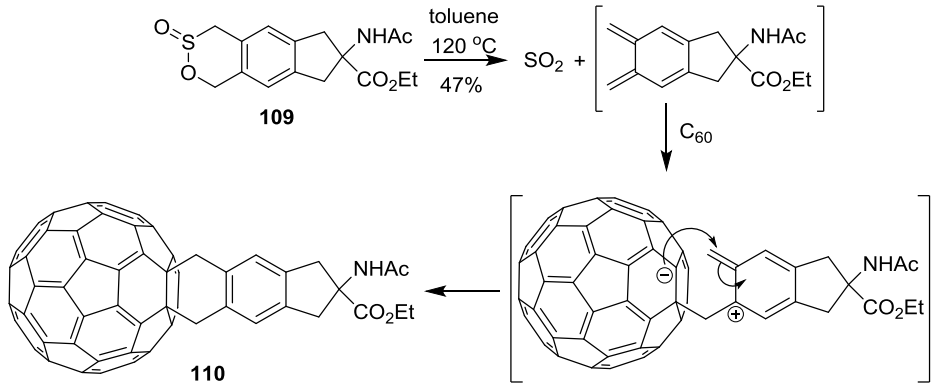
with the *C*-terminus of the protected amino acids gave **105** and **106**. Deprotection of **106** to its TFA salt **107** and then coupling with a hexapeptide followed by final deprotection with TFA resulted in the fullereno peptide **108**, having an oxidised methionine residue.[63]



Scheme 1.18: Synthesis of [*60*]fulleropeptides.

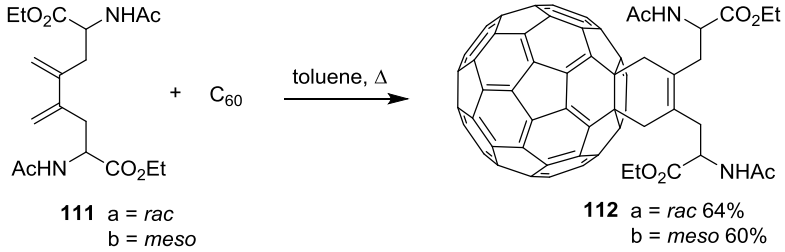
1.5 Synthesis of Fullerenyl Amino Acids That Could Potentially be Incorporated Within Peptide Chains

The synthesis of highly functionalized benzo-annulated indane-based α-amino acid (AAA) derivatives was reported via a [4+2]-cycloaddition strategy using the sultine derivative **109**, containing an AAA ester moiety, as a reactive diene component. By adopting this strategy, a new α,α-dialkylatedindane-based [*60*]fullerene containing a rigid AAA unit **110** was realized (Scheme 1.19).[64]



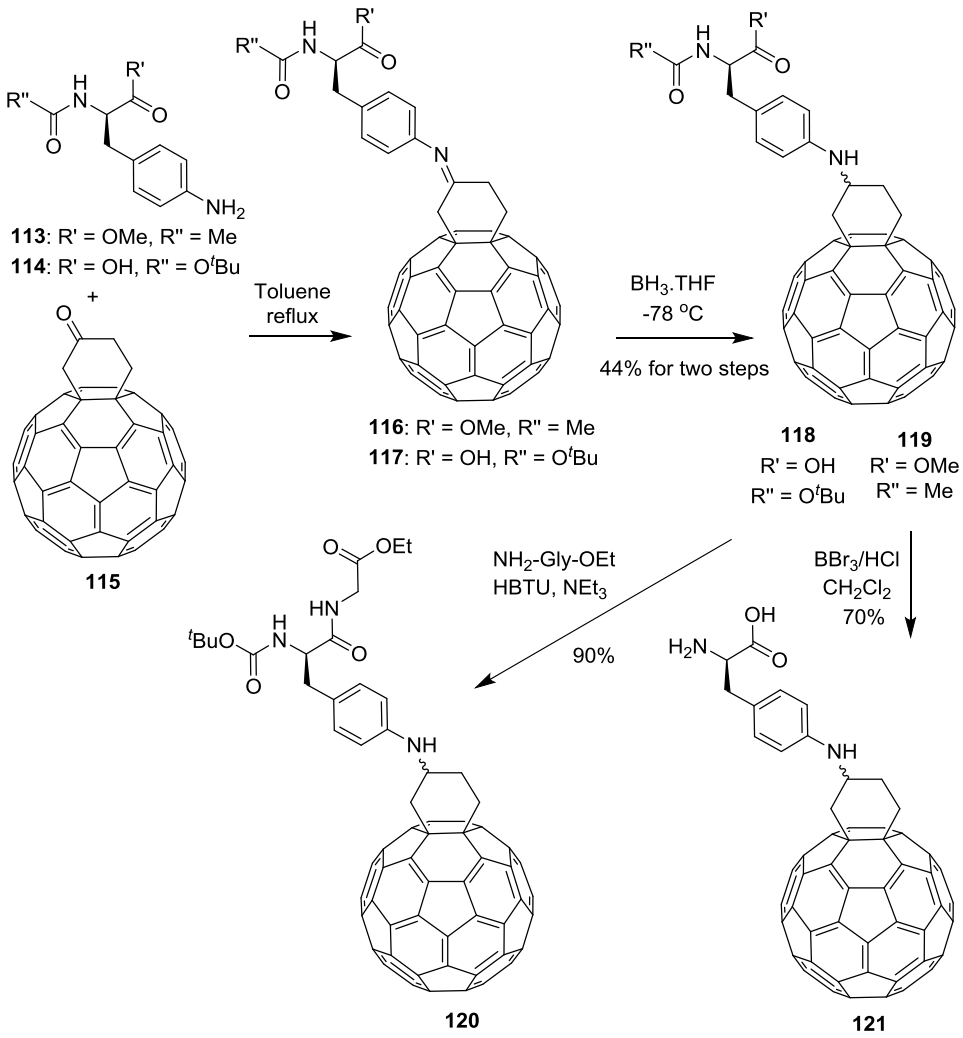
Scheme 1.19: Synthesis of α,α-dialkylatedindane-based [*60*]fullerene containing AAAs.

A Diels-Alder approach has been developed to prepare dicarba analogues of cystine. Treatment of diene **111** with [*60*]fullerene in toluene at reflux furnished the stable cycloadduct **112** in good yield (Scheme 1.20).[65]



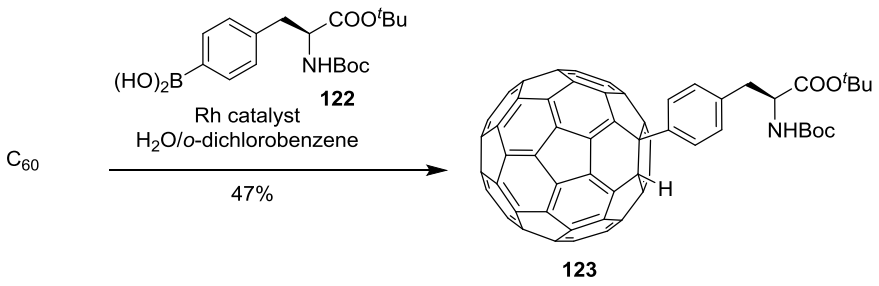
Scheme 1.20: [*60*]Fullerene-based dicarba analogue of cysteine **112**.

Ketone **115** has been used to synthesise [*60*]fullerene substituted *D*-phenylalanine derivatives (Scheme 1.21).[14] The *para*-amino substituted phenylalanine derivatives **113** and **114** readily undergo condensation reactions with **115** forming the analogous imines **116** and **117**, which were converted to their corresponding amines **118** and **119** by borane reduction. The *N*-Boc-glycine derivative **118** was coupled to ethyl glycinate under HBTU mediated coupling conditions to provide the peptide **120**. Treatment of the methyl glycinate derivative **119** with BBr_3 led to the isolation of the free glycine analogue **121**.



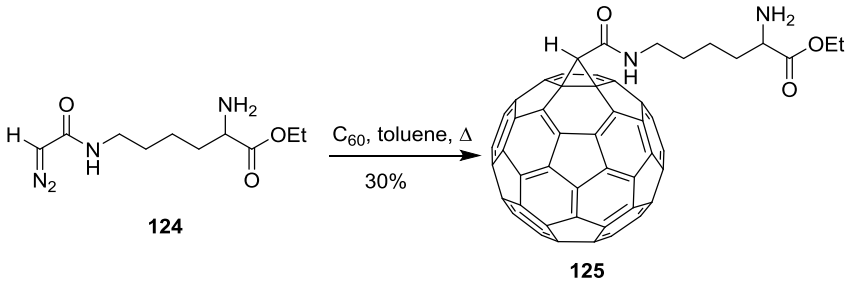
Scheme 1.21: Synthesis of [*60*]fullerenyl amino acids **120** and **121**.

A rhodium catalyzed reaction of [*60*]fullerene with the boronic acid derivative of phenylalanine **122** is shown in Scheme 1.22. This reaction gave the fullerene-tagged amino acid **123** in 47% yield.[66]



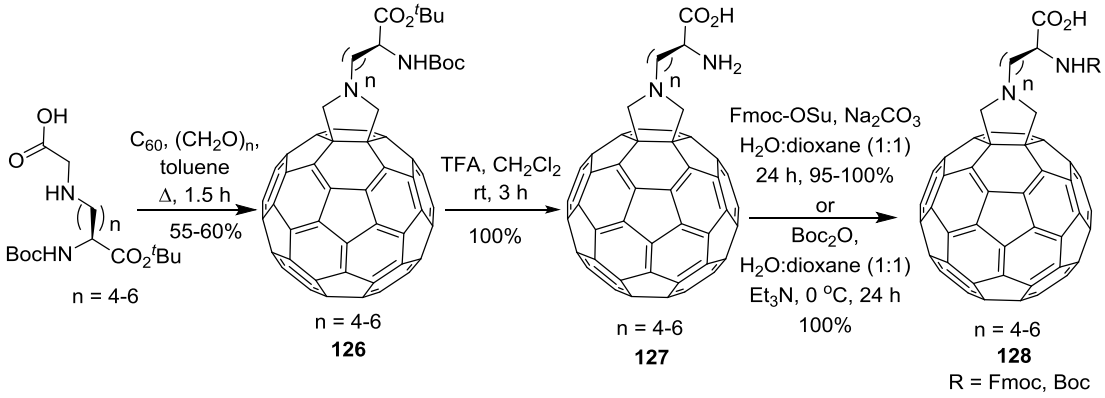
Scheme 1.22: Synthesis of 1-substituted 1,2-dihydro[*60*]fullerene derivatives.

A direct route was developed to produce fullerenyl peptides **125** by prolonged thermolysis of diazoamides (e.g. **124**) in the presence of [*60*]fullerene (Scheme 1.23).[39]



Scheme 1.23: Thermal addition of diazoamide to [*60*]fullerene provides access to fullerenyl amino esters.

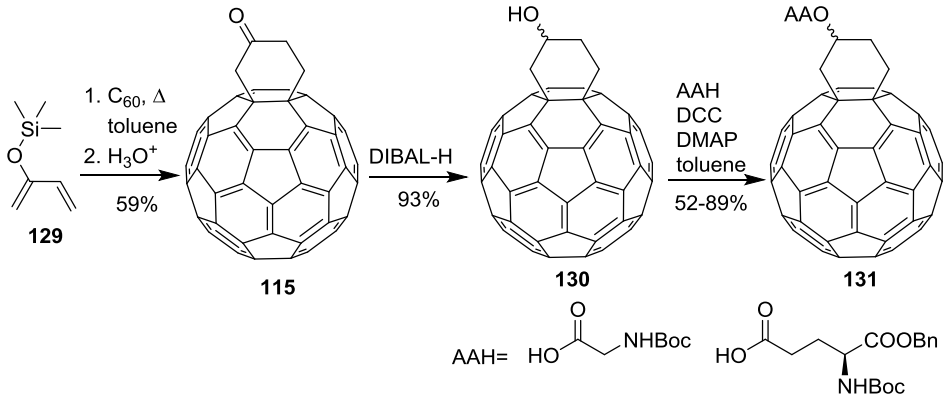
[*60*]Fullerene functionalized amino acids with 4–6 methylene spacers from the α-carbon to the nitrogen atom of fulleropyrrolidine **126**, and their corresponding *N*-protected versions **128**, have been synthesized from the reactions of *N*-glycine derivatives, formaldehyde and [*60*]fullerene under Prato's reaction conditions as shown in Scheme 1.24.[67]



Scheme 1.24. Synthesis of [*60*]fullerene functionalized amino acids with 4-6 methylene spacers.

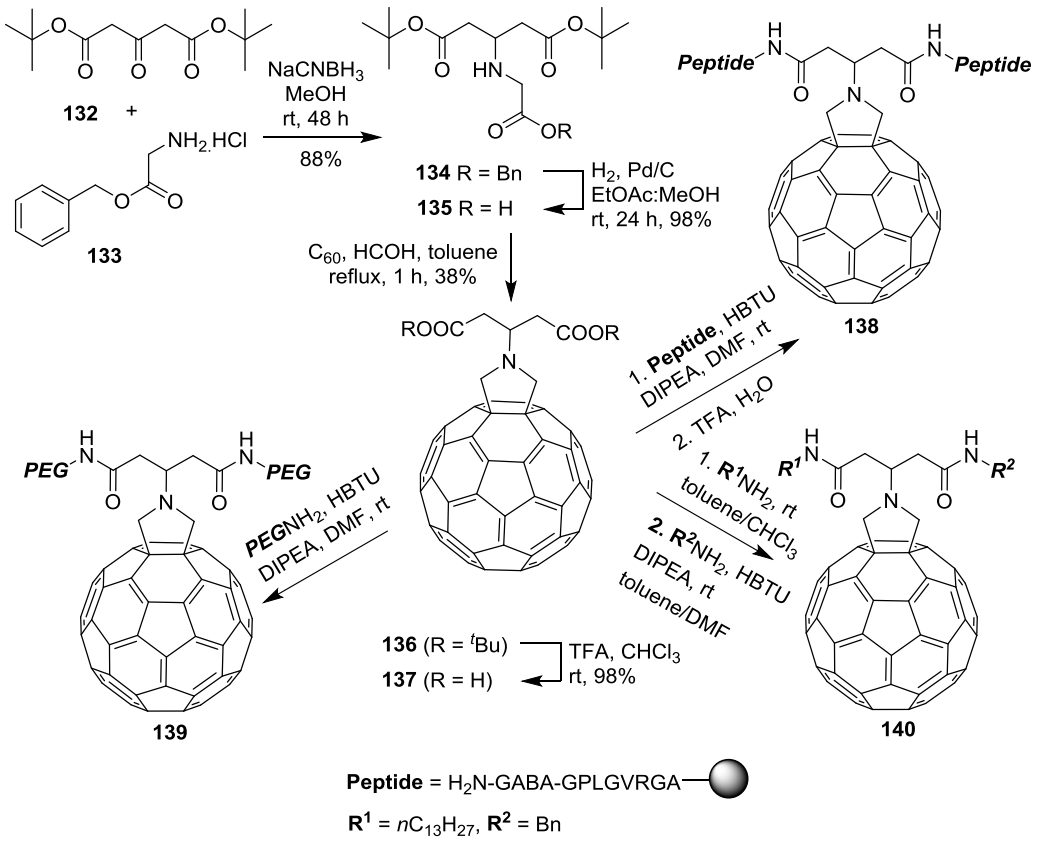
The [4+2]-cycloaddition of the 2-trimethylsilyloxybutadiene **129** to [*60*]fullerene provided the ketone **115**, after hydrolysis. Subsequent reduction with DIBAL-H afforded the racemic alcohol **130**, a versatile synthon for the generation of fullerenyl amino acid derivatives (Scheme 1.25). For example, the DCC mediated

esterification of **130** with alanine and glutamate derivatives provided the protected fullerenyl amino acids **131**.[13]



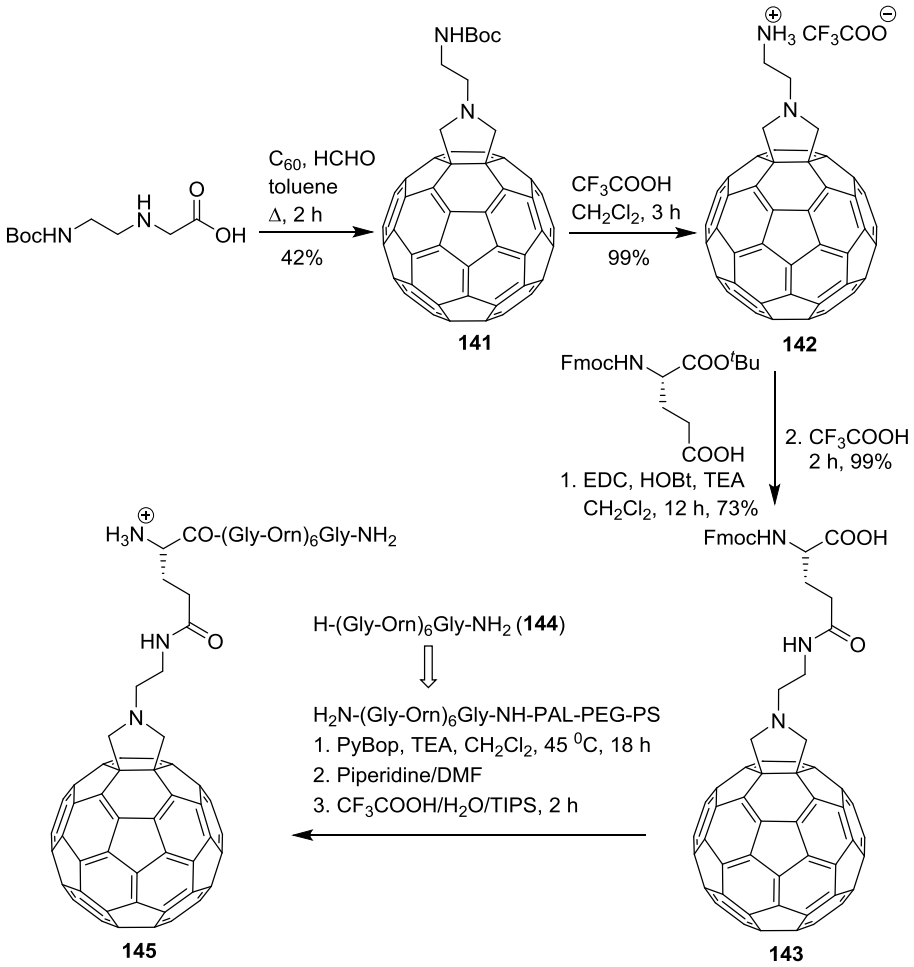
Scheme 1.25. Synthesis of [*60*]fullerenyl amino acids **131**.

[*60*]Fullerene pyrrolidine derivative (**137**), bearing two carboxylic acid functional groups has been reported (Scheme 1.26).[68] The crucial glycine precursor **135**, was synthesised from commercially available starting materials and reacted with [*60*]fullerene and formaldehyde under Prato's reaction conditions to yield the diester **136**. The *tert*-butyl groups of **136** were deprotected under acidic conditions, which efficiently provided the stable bis-acid derivative **137**. This diacid derivative was readily functionalized under solid phase conditions to yield the derivatives dipeptide **138** and PEG **139**. In addition, the reaction of **136** with TFA and TFAA provide an anhydride intermediate, which was used to synthesise [*60*]fullerene derivatives bearing two different amide moieties **140**.



Scheme 1.26. Synthesis of bis-carboxylic acid group functionalized [*60*]fullerene derivatives.

The [*60*]fullerene derivative **141** was deprotected with TFA to give a free amino functional linker containing [*60*]fullerene derivative **142**. This was then reacted with *N*-Fmoc-L-glutamic acid α-*tert*-butyl ester to yield a [*60*]fullerene functionalized amino acid. The *tert*-butyl ester was deprotected under acidic conditions to give the carboxylic acid **143**, which was subjected to solid-phase peptide synthesis with peptide **144**. The product **145** was cleaved from the resin support and was found to be water soluble.[69]



Scheme 1.27. Solid phase synthesis of fullereno peptide **145**.

1.6 Applications of [*60*]fullerene amino acids and peptides

The potential uses of amino acid and peptide derivatives of fullerenes range from the biological to the material sciences. This section will outline the major areas of achievement.

1.6.1 Biological Applications

The fullereopeptide **108** (Scheme 1.18) was biologically active against sera from Mixed Connective Tissue Disease (MCTD) and Systemic Lupus Erythematosus (SLE) patients (ELISA experiments).

The peptide **144** (Scheme 1.27) itself was not active against *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli),* whereas the analogous [*60*]fullerenopeptide **145** showed antimicrobial activity against *S. aureus* and *E. coli* (Table 1.1).[69]

Table 1.1. Antimicrobial activity of peptides **144** and **145**, Reported as MIC (Minimum Inhibitory Concentrations). Results are the mean of a minimum four independent evaluations run in duplicate (na = nonactive).

Peptide	**144**	**145**
S. aureus	na	8 μM
E. coli	na	64 μM

The antioxidant properties of the water soluble amino acid derivatives of the sodium salts of fullerenylaminobutyric acid (C_{60}-γ-ABNa, **146**) and fullerenylaminocaproic acid (C_{60}-ω-ACNa, **147**) as well as that of the hybrid structure based on a *N*-fullerenoproline and the natural oxidant carnosine (C_{60}-Pro-carnosine, **148**) have been studied. Their roles in the inhibition of herpes virus infection have also been described.[70] The amino acid fullerene derivatives **146** and **147** of [*60*]fullerene were found to have significant antioxidant activities and were not cytotoxic (IC_{50} 1000 and 1200 μg mL^{-1} (mln. cells), for **149** and **146**, respectively). These derivatives were also studied as inhibitors of cytomegalovirus (CMV) infection. The introduction of C_{60}-γ-ABNa **146**, into infected human embryonic fibroblasts (HEF) reduced the concentration of virus proteins in the cells to values approaching that in non-infected cells (Table 1.2). The malonic dialdehyde (MDA) concentration in the infected HEF culture also decreased to the concentration in intact HEF. The drug ganciclovir also decreased the lipid peroxidation (LPO) level in the HEF culture but had significantly lower antioxidant and inhibitory effects than **146** (Table 1.2). These results indicated that the antioxidant activity of these fullerene derivatives played an important role in

their antiviral effect against CMV infection. Compound **146** was considered as a potential drug against CMV infections. Its chemotherapeutical index (CTI) was found to be 5000, which fivefold exceeds that of ganciclovir.

Table 1.2. Contents of the protein and MDA in the HEF cultures

Sample	Protein content /mg (mln. cells)$^{-1}$	[MDA].10^{-5} /mol.L^{-1}(mln.cells)$^{-1}$
HEF	0.07±0.02	3.75±0.03
HEF-CMV	0.284±0.03	6.8±0.01
HEF-CMV- gancyclovir	0.22±0.04	5.4±0.03
HEF-CMV-C_{60}-γ-ABNa (**146**)	0.072±0.02	3.8±0.03

Note. The concentration of ganciclovir and C_{60}-γ-ABNa **146**, in the cell culture was 2.10^{-5} mol L^{-1} (mln. cells)$^{-1}$. The average values of five measurements were presented. HEF (human embryonic fibroblasts), CMV (cytomegalovirus infection), MDA (malonicdialdehyde).

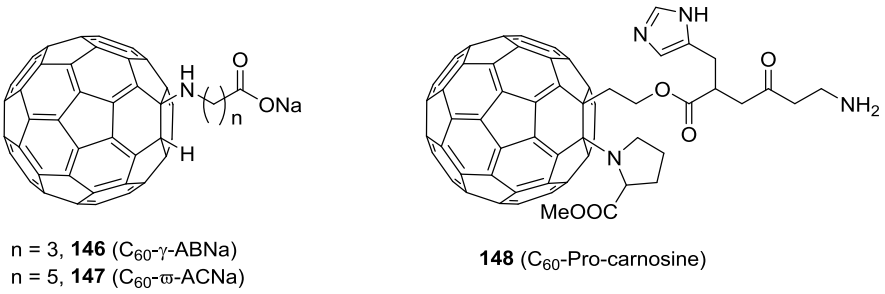
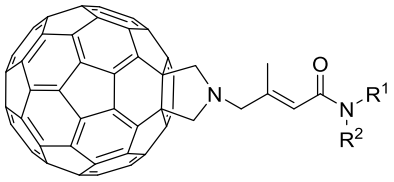


Figure 1.1: [*60*]Fullerene based water soluble amino acids.

The antioxidative/anti-inflammatory activities of the fullerene-polyamine (FUL-PA) conjugates **149-156** have been described.[71] Conjugation of [*60*]fullerene with spermine (SPM) or spermidine (SPD) resulted in a large improvement of both the anti-lypoxygenase (LOX) and the anti-lipid peroxidation activity of the unconjugated molecules. An enhancement in the anti-inflammatory potency of [*60*]fullerene was observed only with the conjugate **150** (42%), which was comparable in activity to the reference compound indomethacin (47%). Most of the FUL-SPD conjugates, and

especially compounds **150** and **152**, were of comparable toxicity, even at the highest concentration tested (50 mM), therefore they could be potentially safely used for possible biomedical applications.[71]



149-156

149; $R^1 = (CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, $R^2 = H$
150; $R^1 = (CH_2)_4NH(CH_2)_3NH_2$, $R^2 = H$
151; $R^1 = (CH_2)_2$-TET-CH_2Ph, $R^2 = H$
152; $R^1 = (CH_2)_3NH_2$, $R^2 = (CH_2)_4NH_2$
153; $R^1 = (CH_2)_3NH(CH_2)_4NH(CH_2)_3NHCO$-FPB, $R^2 = H$
154; $R^1 = (CH_2)_3$-TET-$(CH_2)_4$-TET-$(CH_2)_2NHCO$-FPB, $R^2 = H$
155; $R^1 = (CH_2)_3NH(CH_2)_4NH(CH_2)_3NHCO$-ACl, $R^2 = H$
156; $R^1 = (CH_2)_3NH(CH_2)_4NH(CH_2)_3NHCO$-TRA, $R^2 = H$
TET = tetrazole
FPB = fullerenopyrrolebutenyl

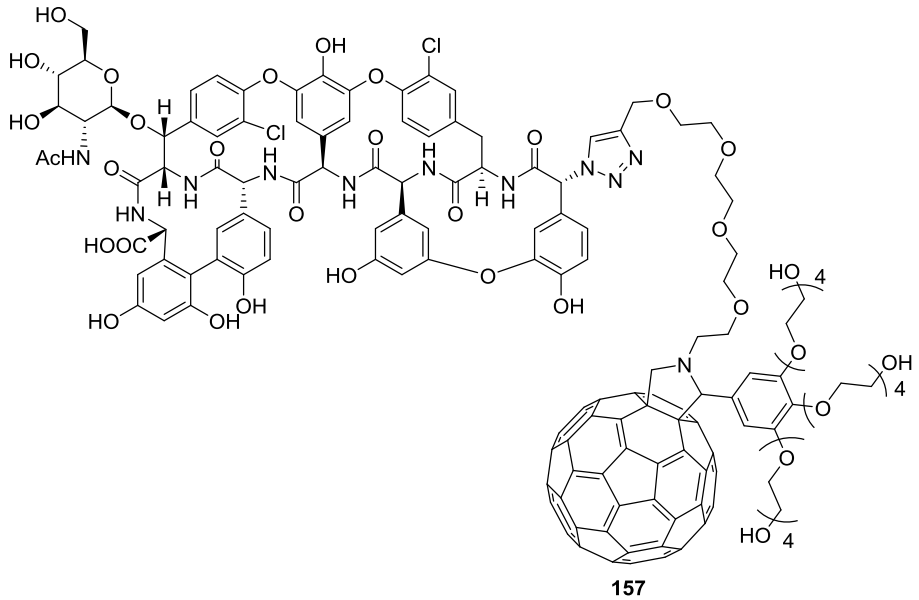
Figure 1.2: Biologically active novel fullerene-polyamine (FUL-PA) conjugates.

The glycopeptide antibiotic derivative teicoplanin ψ-aglycone has been covalently attached to a fullerenopyrrolidine derivative using azide-alkyne "click chemistry". The aggregation of the resulting antibiotic-fullerene conjugate **157** in aqueous solution has been studied resulting in nano-sized clusters.[72] The conjugate exhibited antibacterial activity against Enterococci resistant to teicoplanin (Table 1.3).

Table 1.3. Antibacterial activity of compound **157**.

Bacteria	Teicoplanin	Compound **157**
	MIC (μg/mL)	
Bacillus subtilis ATCC 6633	0.5	16
S. aureus MSSA ATCC 29213	0.5	8
S. aureus MRSA ATCC 33591	0.5	4
S. epidermidis ATCC 35984 biofilm	4	2
S. epidermidis mec A	16	1
E. faecalis ATCC 29212	1	6
E. faecalis 15376 VanA	256	16
E. faecalis ATCC 51299 VanB	0.5	16

Note. MIC: Minimum Inhibition Concentration, ATCC: American Typed Culture Collection, MRSA: Methicillin Resistant *Staphylococcus aureus*, vanA: vanA gene positive, vanB: vanB gene positive.



157

Figure 1.3: Teicoplanin ψ-aglycon-fullerene conjugate **157**.

The water-soluble dendrofulleropyrrolidine **158** (synthesised from **102**) and its bis-analogues **159** and **160** have been prepared (Figure 1.4).[73] These cationic species can efficiently complex plasmid DNA as demonstrated by gel electrophoresis studies.

These molecules showed excellent DNA binding efficiencies and were therefore considered to be potential candidates for DNA binding therapies.

The fulleropyrrolidine peptides **162-172** containing only GABA (γ-aminobutyric) residues, having zero or one glycine moiety were reported (Figure 1.5).[74] The authors claim that these may be used as nanobioparticles, however, no results related to these properties were reported.

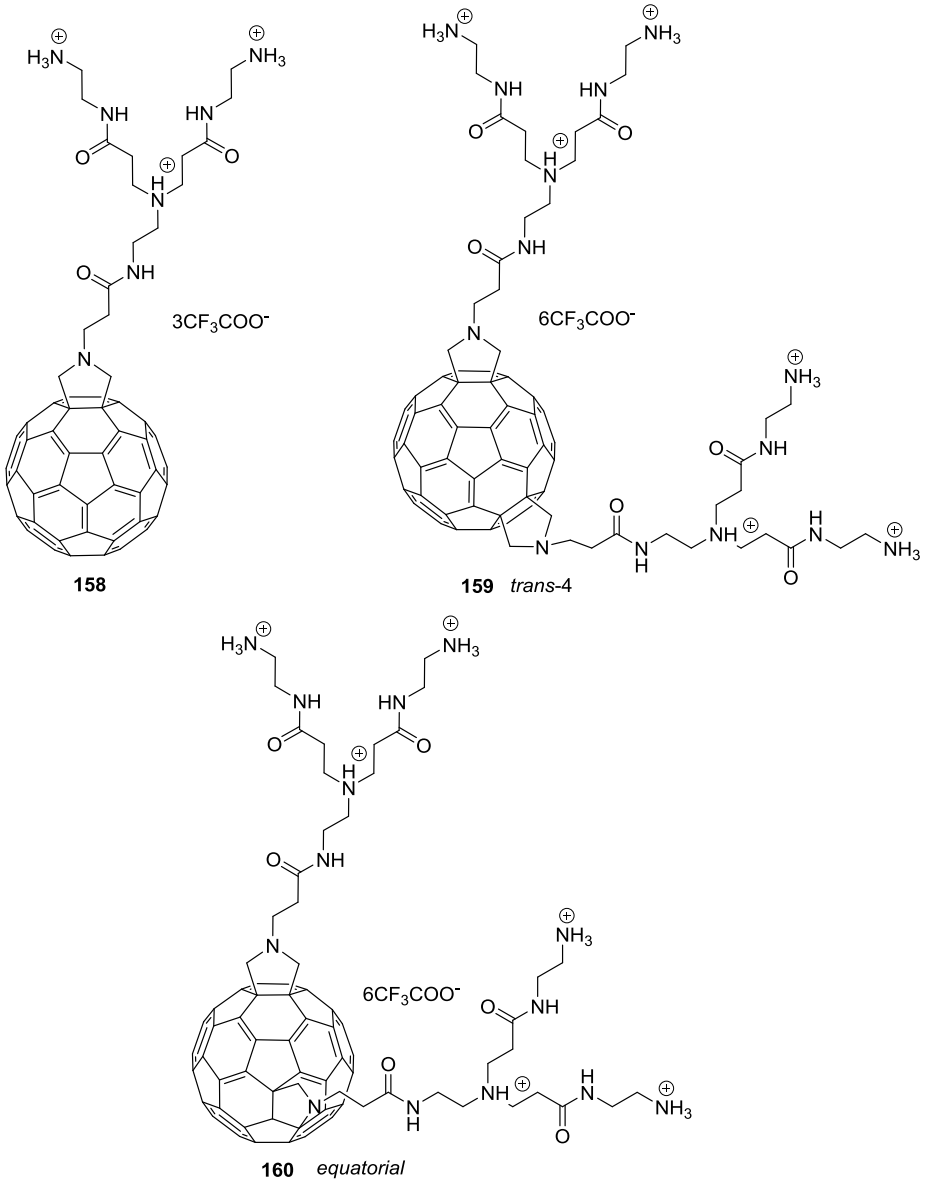


Figure 1.4. Monoadduct **158** and bisadducts **159** and **160** of highly water soluble polycationic [*60*]fullerenes.

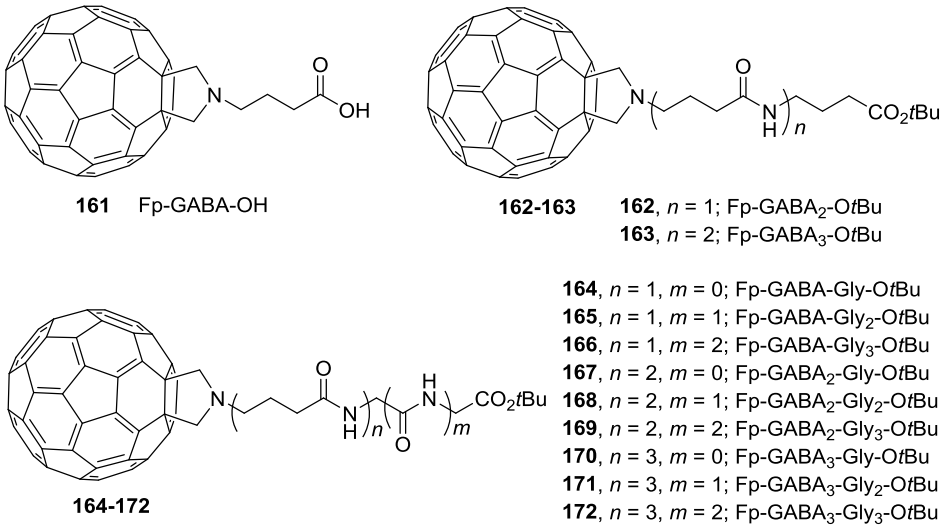


Figure 1.5. Fulleropeptides **162-172** synthesized from fulleropyrrolidinic acid **161**.

Mitsutoshi *et al.* reported the inhibitory effects of [*60*]fullerene derivatives **173** and [*60*]fullerene-ethylenediamine-*N*,*N*′-diacetic acid **174** (Figure 1.6) on acetylcholine-induced (endogenous NO) relaxation in endothelium-intact rabbit thoracic aorta precontracted by phenylephrine (10^{-6} M).[75] These [*60*]fullerene derivatives **173** (10^{-5} M) and **174** (10^{-5} M) reduced the maximum amplitude of the acetylcholine-induced relaxation without significantly changing the pD2 values obtained from the concentration response curves.

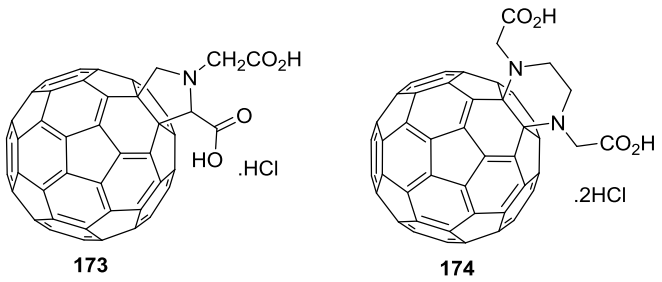


Figure 1.6. [*60*]Fullerene derivatives **173** ([*60*]fullerene-proline-*N*-acetic acid) and **174** ([*60*]fullerene-ethylenediamine-*N*,*N*′-diacetic acid).

Water soluble [*60*]fullerene derivatives **175-177** (WSFD), have been studied to show the potential to prevent NO-induced cytotoxicity without obvious toxicity (Figure

1.7).[76] These fullerene derivatives apply protective activities by direct interaction with NO and neutralization of $O_2^{\cdot-}$. The folacin fullerene derivative **177** (FFD) self-assembles to form spherical aggregates because of hydrogen bonding, and the aggregate ion morphology impacts on the NO-scavenging activities of WSFD. Pretreatment of the cells with WSFD, prior to sodium nitroprusside (SNP) exposure blocks NO-induced cellular events including oxidation of membrane lipids, depolarization of mitochondrial membranes, reduction of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidise (GSH-Px) activities and caspase-mediated apoptotic cell death. Apart from the biological studies, the authors have revealed the synthesis of novel fullerene derivatives with anti-apoptotic properties.[76,77]

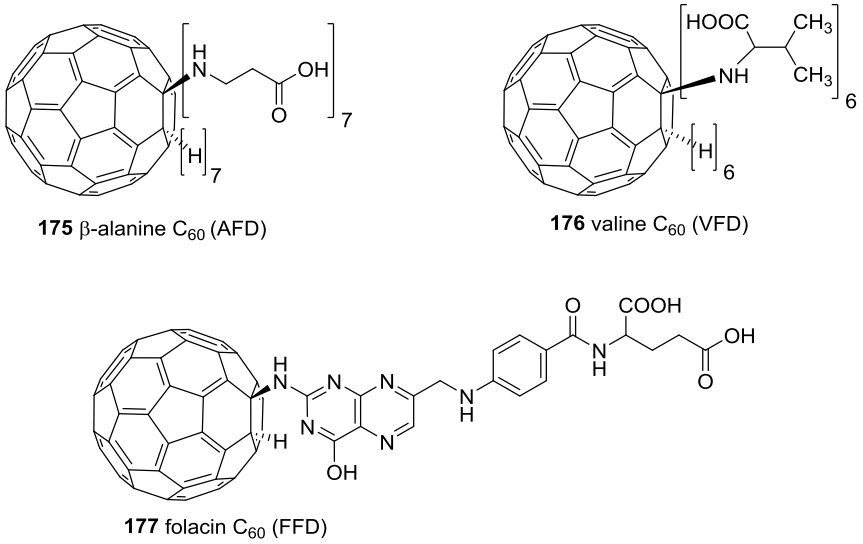
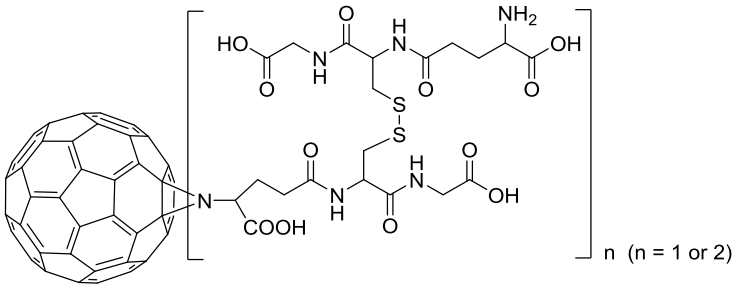


Figure 1.7. The structures of AFD (**175**), VFD (**176**) and FFD (**177**).

Oxidized [*60*]fullerene glutathione derivatives have been synthesized and characterized.[78] The [*60*]fullerene glutathione derivative **178** (Figure 1.8) was soluble in dimethylsulfoxide, dimethylformamide and dimethylacetamide. Rat pheochromocytoma (PC12) cells were treated with hydrogen peroxide and then cytotoxicity and apoptotic death was estimated by a MTT assay, flow cytometry analysis, PI/Hoechst 33342

staining and glutathione assay. These results indicated that the glutathione [*60*]fullerene derivative **178** has the potential to prevent oxidative stress-induced cell death without toxicity.[77]

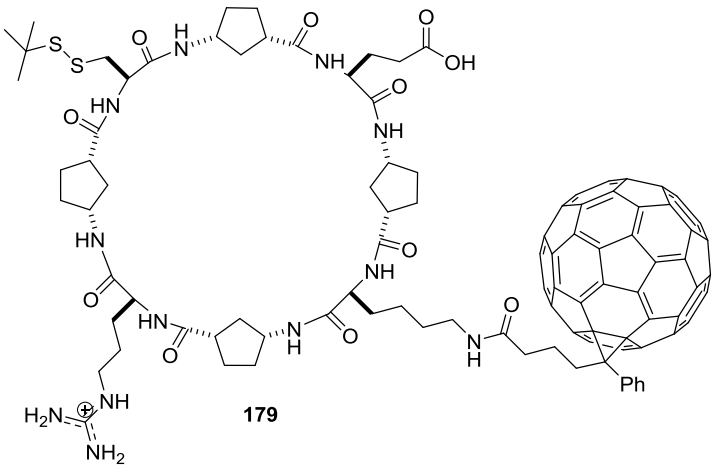


178, oxidised glutathione C_{60} derivative

Figure 1.8. Oxidized glutathione [*60*]fullerene derivative **178**

1.6.2 Materials Chemistry Applications

A new class of peptide nanotubes from α,γ-cyclic peptides (CPs) **179** (Figure 1.9) tethered to a methanofullerene moiety has been reported.[79] These CPs are able to form nanotubes in which the fullerenes point outward from the nanotube on both sides (180° orientation). The fullerenes form two parallel wires separated by the insulating peptide nanotube. The authors suggest that these materials may have applications as nanowire components and/or in optical and electronic devices.



179

Figure 1.9: Structure of tethered α,γ-cyclic peptide (CP) **179**.

Two molecules (**180** and **181**, Figure 1.10) having a pyrrolidino[*60*]fullerene tethered through an intramolecular hydrogen bonding peptide linker to a nitroxide radical have been synthesised.[80] These peptide moieties have a rigid 3_{10}-helical structures that possess a strong molecular dipole. The direction of the molecular dipole moment can be reversed by switching the position of the fullerene and the nitroxide with respect to the peptide nitrogen and carbon terminus. The fullerene-peptide-radical systems **180** and **181** were compared to the behaviours of otherwise identical peptides but without either [*60*]fullerene or the free radical moiety with retention of the number of intramolecular hydrogen bonds.

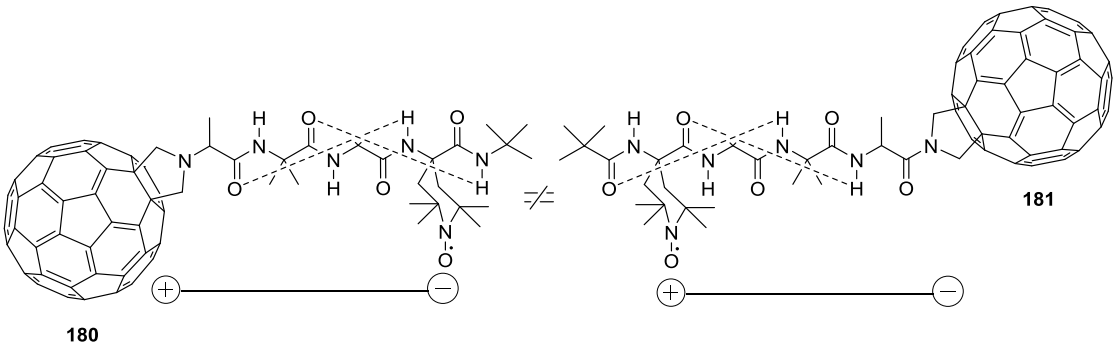


Figure 1.10: Structures of [*60*]fullerene based peptides with intramolecular hydrogen bonds showing their molecular dipoles.

A strategy of photocurrent generation out of thin layers composed by the amphiphilic [*60*]fullerene and pyrene derivatives was investigated by Shunsaku's group.[81,82] Three different types of compounds, namely pyrene derivative **182** (R-Pyr) having diethylene glycol and a long alkyl chain, [*60*]fullerene-PEG conjugate **183** (C_{60}-PEG) without cyclic peptide scaffold and [*60*]fullerene-cyclic peptide-PEG conjugate **184** (cyclo8-C_{60}-PEG), were synthesised (Figure 1.11). The study of photoinduced electron transfer concluded that a bilayer structure with desired orientation of functional

units is important for efficient photoinduced electron transfer and that the cyclic peptide scaffold is useful to locate hydrophobic functional groups in a thin layer.

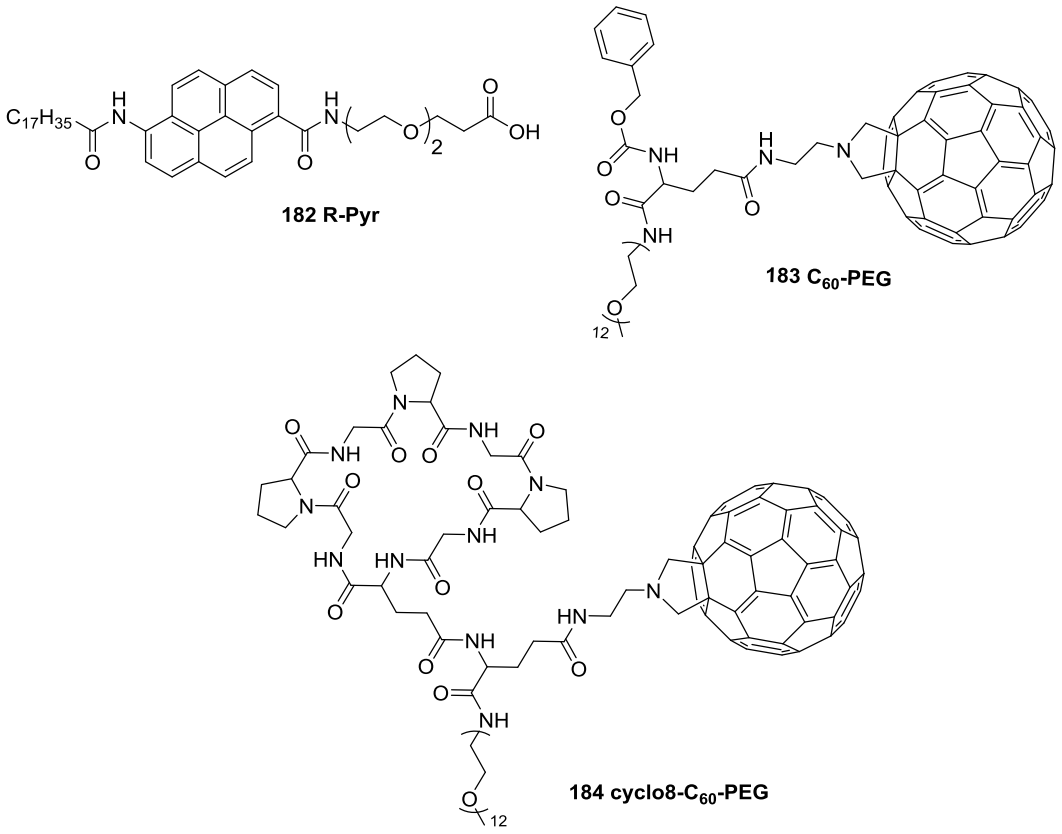


Figure 1.11. The structures of R-Pyr **182**, C_{60}-PEG **183** and cyclo8-C_{60}-PEG **184**.

1.7 Background of this Project

There is much current research interest in the synthesis and design of chemical systems for use in organic solar cells such as in organic photovoltaic applications as bulk heterojunction solar cells.[83-85] Solladie *et al.* have been successful in synthesising such potential systems based on polypeptide-porphyrin structures (Scheme 1.28).[86-88] Porphyrins with their highly conjugated structures and light adsorption features are well suited materials to act as electron donors.[86] Additionally, the design of suitable electron acceptor systems has focussed on the use of fullerenes which can reversibly accept up to

six electrons (Figure 1.12).[89] It has been shown that by interchelation of fullerenes to two porphyrin moieties in polypeptide-porphyrin structures, self-organized helical systems suitable for use in organic solar cells readily form.[86,87] A schematic structure of one potential amino acid/fullerene-porphyrin composite system is shown in Figure 1.12.

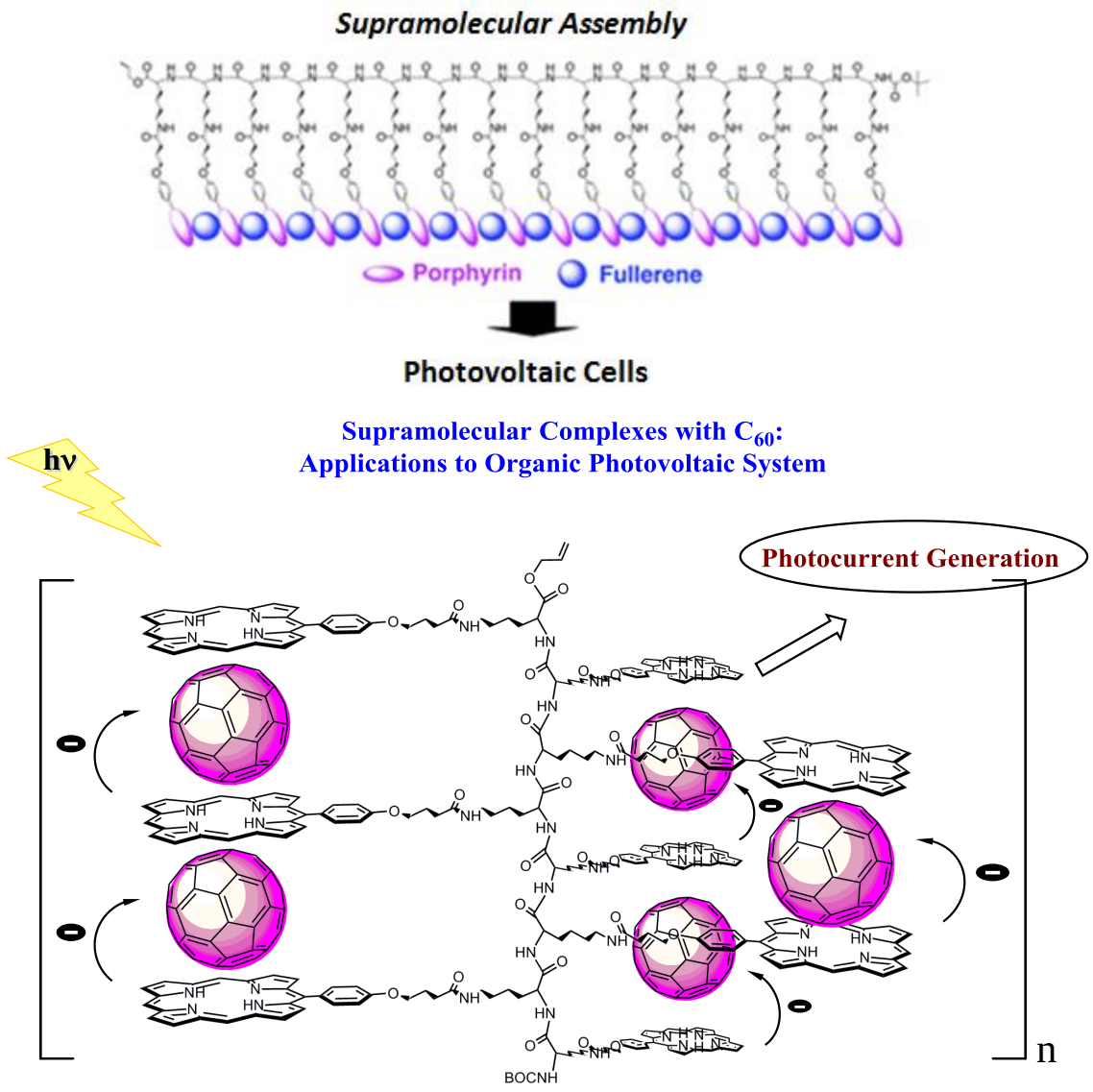
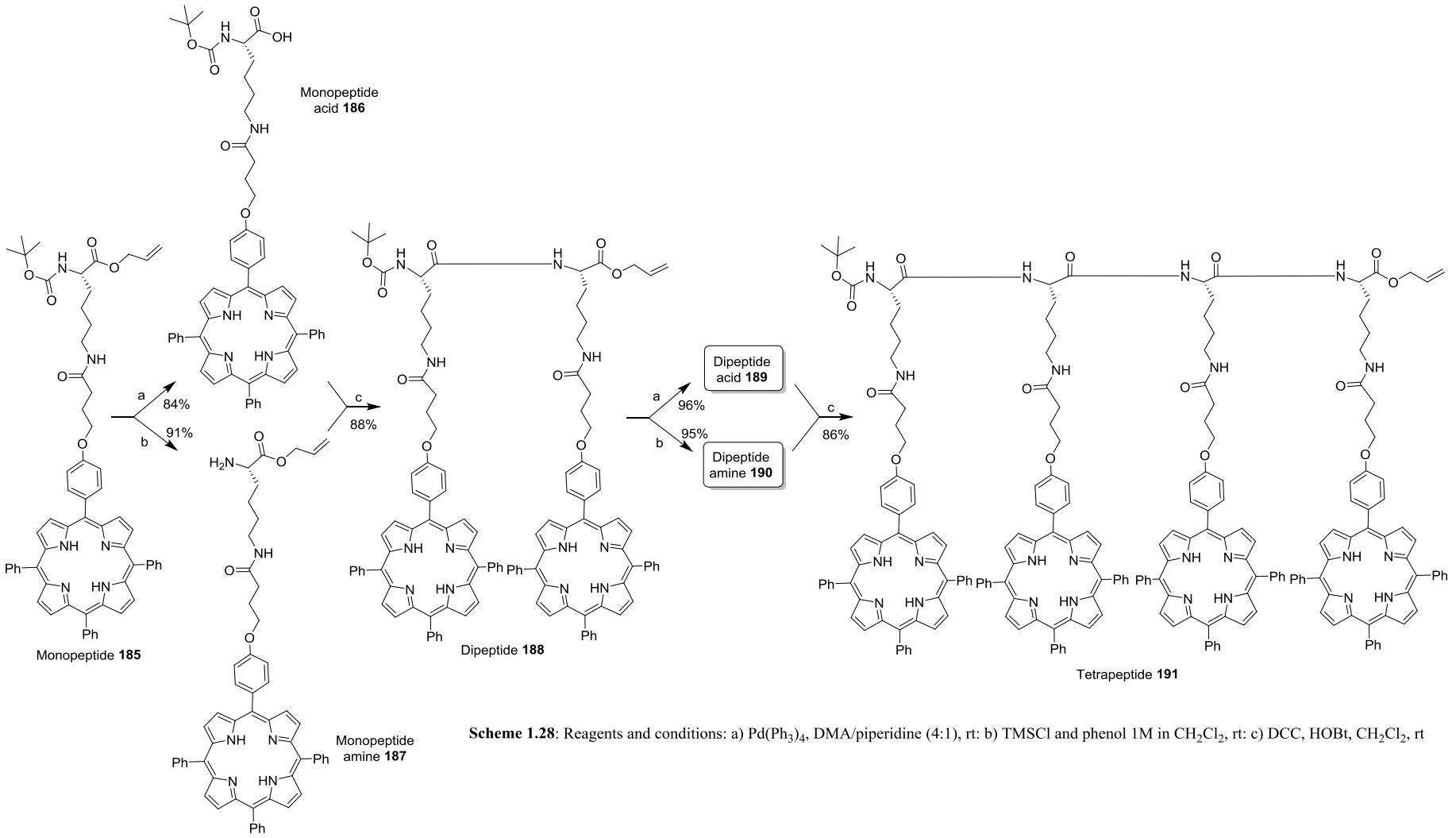


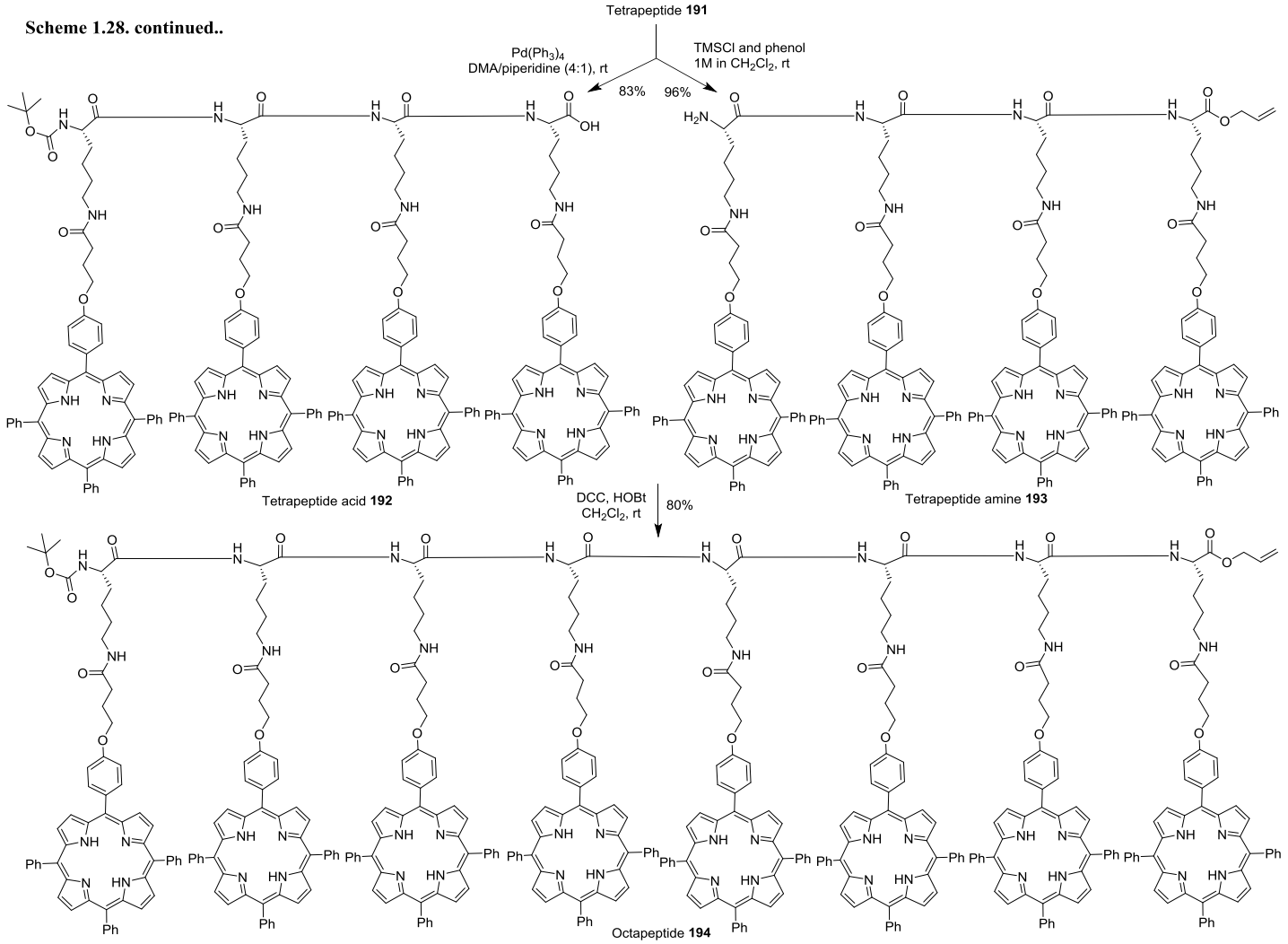
Figure 1.12. Supramolecular complex of **194** with [*60*]fullerene of 3_{10} helix of porphyrin oligomer[86]

The synthesis of the polypeptide-porphyrin structure **194** was realised from the starting material **185** (Scheme 1.28).[86-88] A selective deprotection of allyl ester to the acid **186** was performed under $Pd(PPh_3)_4$/dimethylacetamide/piperidine reaction conditions to give the acid **186** in 84% yield. The Boc group removal to the amine **187** was successfully performed using TMSCl and phenol in 1 M CH_2Cl_2 solution to give amine **187** in 91% yield. An amide coupling reaction of acid **186** and amine **187** under DCC and HOBt conditions gave the dipeptide **188** in 88% yield (Scheme 1.28). By repeating the reaction conditions mentioned above for the synthesis of acid **186**, amine **187** and dipeptide **188**, the polypeptide-porphyrin structure **194** (Scheme 1.28) was achieved which was used to construct a supramolecular complex with [*60*]fullerene (Figure 1.12).[86-88]



Scheme 1.28: Reagents and conditions: a) $Pd(Ph_3)_4$, DMA/piperidine (4:1), rt: b) TMSCl and phenol 1M in CH_2Cl_2, rt: c) DCC, HOBt, CH_2Cl_2, rt

Scheme 1.28. continued..



1.8 Aims of this Project

While the Solladie group are developing helical porphyrin oligomers to intercalate fullerene (Figure 1.12), we plan to build fullerenes into the peptide oligomer structure which may allow for the insertion of porphyrins as shown in Figure 1.13. We planned to prepare octapeptide[88] and hexadecapeptide derivatives from L-lysine which are functionalized with fullerenes at their lysine amino termini. Beyond a certain degree of oligomerisation the development of a secondary structure, such as a 3_{10} helix, may be observed (Figure 1.13). This would force the fullerenes to arrange in a defined spatial arrangement as shown in Figure 1.13. These fullerenyl peptides can be studied for the accommodation of guests like porphyrin rings in between two helices. Preparation of supramolecular complexes using these fullerene-peptide oligomers as hosts and porphyrins as guest molecules could be expected to be potentially useful as organic photovoltaic cells (as shown in Figure 1.13).

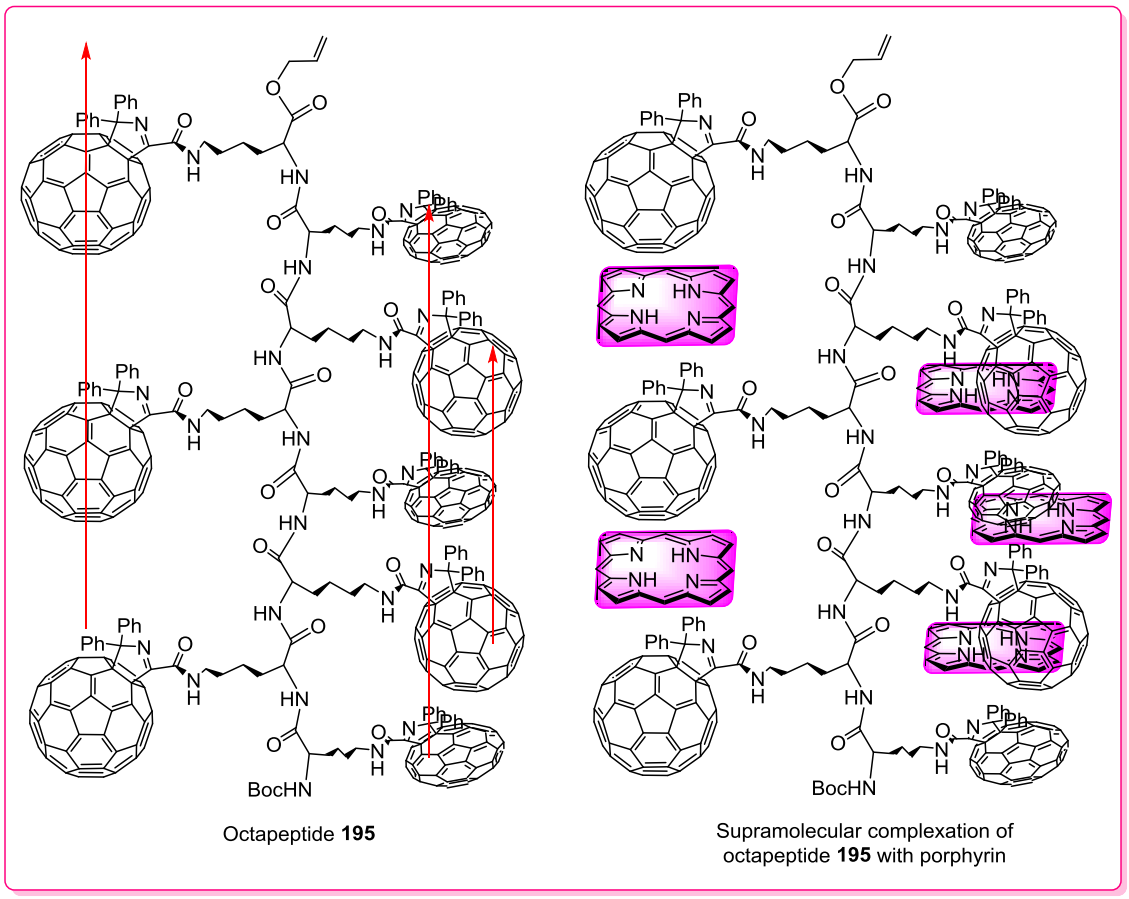


Figure 1.13. Potential structure of a amino acid/porphyrinic-peptide system for use in organic solar cells.

An additional object of this project was to investigate the [*60*]fullerene moiety as a hydrophobic anchor in the synthesis of dicationic peptoids as potential anti-biotic agents. A more comprehensive background to this part of the project is presented at the beginning of Chapter 4.

Therefore, the specific aims of this thesis were:

- The design, synthesis, isolation and characterization of [*60*]fullerene based oligomers (ex. tetrapeptide, octapeptide, dodecapeptide and hexadecapeptide).
- Supramolecular complexation with suitable electron acceptor-donor substrates, including; porphyrins for potential solar cell applications.

- The design, synthesis, isolation and characterisation of [*60*]fullerene based peptides.
- The synthesis of water soluble dicationic [*60*]fullerene peptide salts for anti-bacterial studies.

CHAPTER 2

The Synthesis of [60]Fullerenyl Amino Acids Oligomers

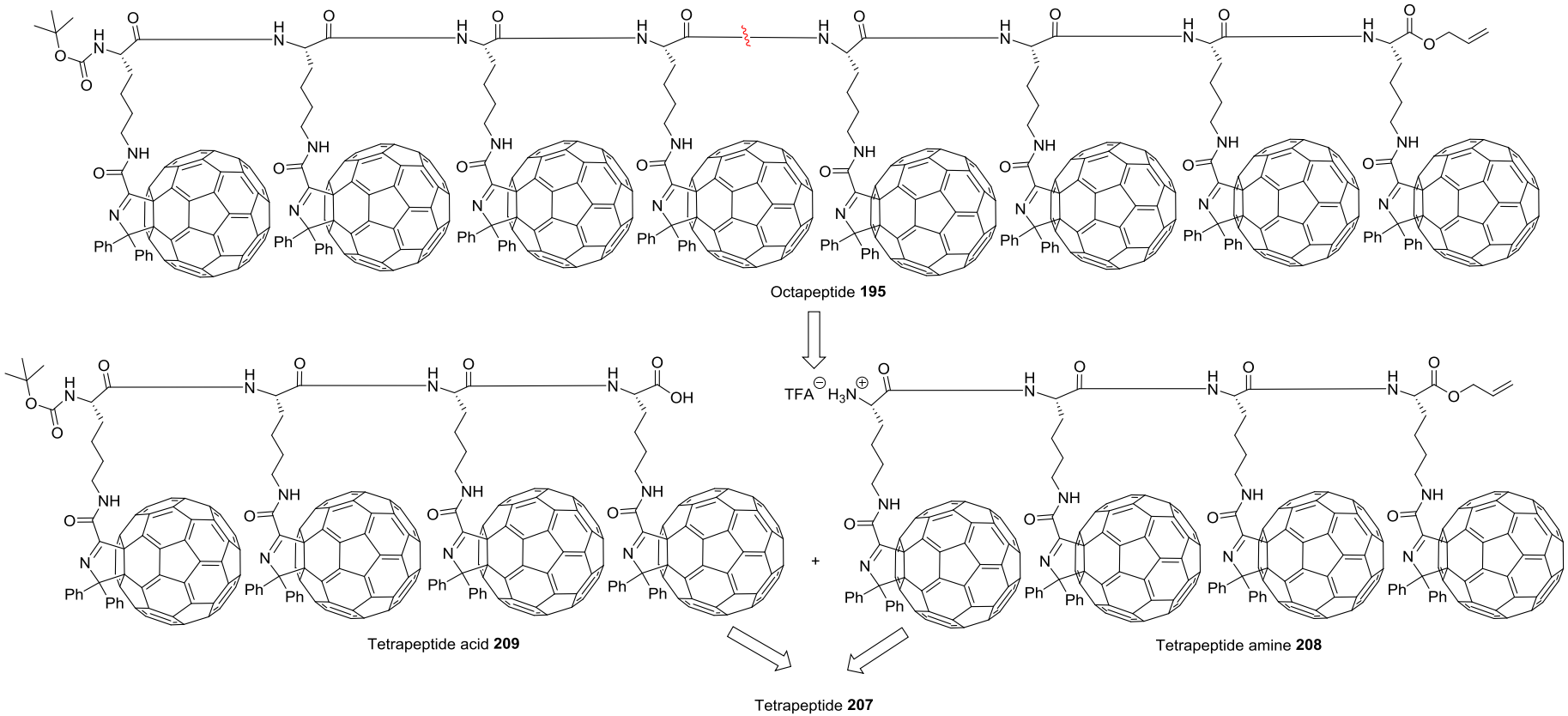
2.1 Applications as Solar Cells in Material Science

The major aim to this project was to build fullerenyl-peptides which may allow the intercalation of porphyrins as shown in Figure 1.13 see page 39. Specifically, we planned to prepare octapeptide and hexadecapeptide derivatives from L-lysine which are functionalized with fullerenes at the lysine side chain amino termini. Beyond a certain degree of oligomerisation the development of a secondary structure, such as a 3_{10} helix, may be observed (Figure 1.13). This would force the fullerenes to arrange in a defined spatial arrangement as shown in Figure 1.13. These fullerenyl-peptides can be studied for the accommodation of guests, including porphyrins, in between two helices. Preparation of supramolecular complexes using these fullerene-peptide oligomers as hosts and porphyrins as guest molecules could be expected to be potentially useful as organic photovoltaic cells as discussed in the previous Chapter (as shown in Figure 1.13).

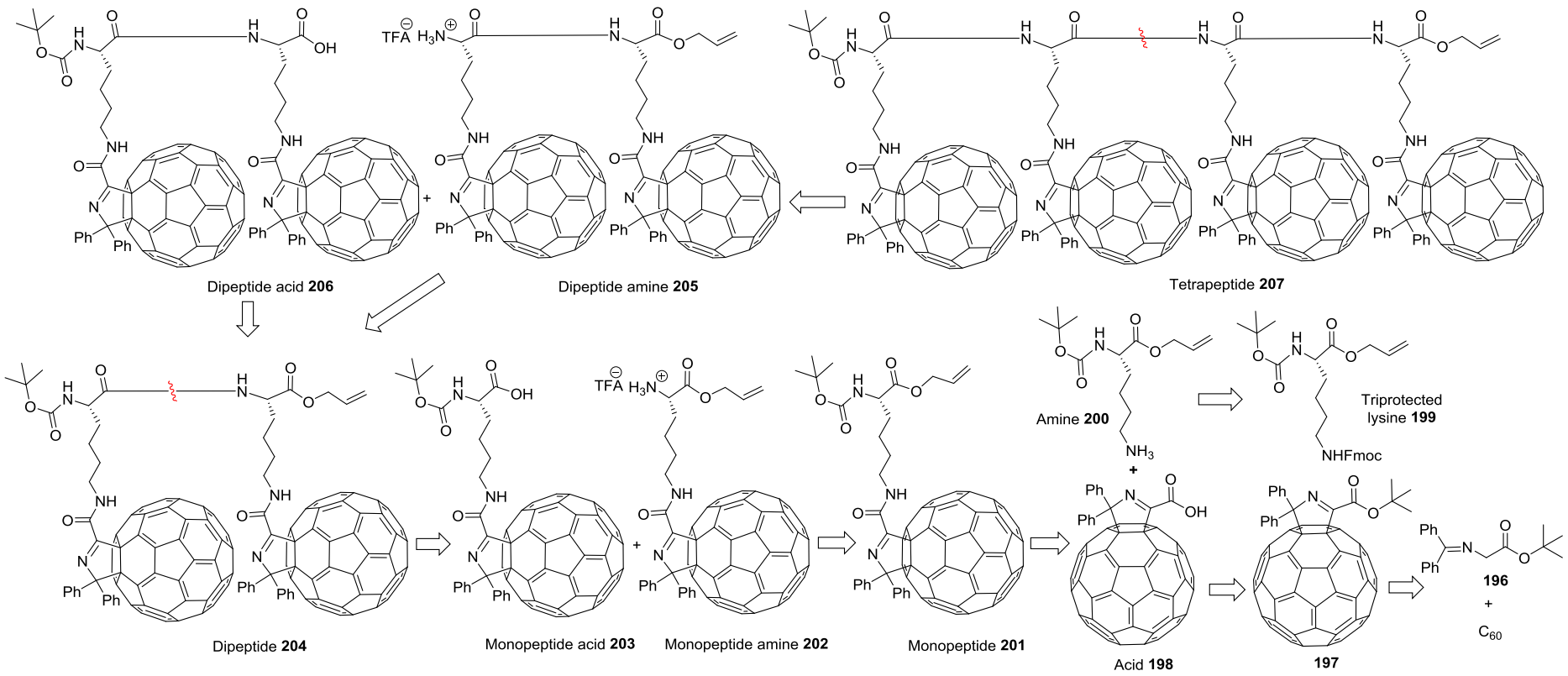
2.2 Strategy 1: Synthesis of [*60*]fullerenolysine monopeptide 201, dipeptide 204, tetrapeptide 207 and octapeptide 195 by sequential coupling

2.2.0 Proposed synthetic strategy for the initial [*60*]fullerenolysine octapeptide 195.

The retro-synthesis of the [*60*]fullerenolysine octapeptide **195**, starting from the previously reported 5,5-diphenylfullerenedihydropyrrole-2-carboxylic acid[33] **198**, is outlined in Scheme 2.1. A key step was the coupling of the fullerenyl acid **198** to the diprotected L-lysine **200** to give the crucial monopeptide **201**. The coupling partner amine **200** could be readily prepared from the known triprotected L-lysine **199** (Scheme 2.1). The monopeptide **201**, could then be deprotected to its amine derivative **202** and acid derivative **203** and these could then be coupled under standard peptide coupling reaction conditions to yield the dipeptide **204**. By repeating the deprotection and coupling reaction protocol the targeted octapeptide **195** could then be realised (Scheme 2.1).



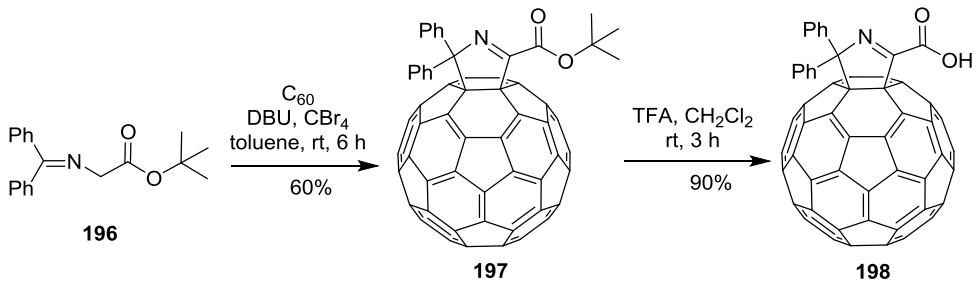
Scheme 2.1 Retrosynthetic analysis for the synthesis of [*60*]fullerenolysine octapeptide **195** from the tetrapeptide **207**.



Scheme 2.1 continued: Retrosynthetic analysis for the synthesis of [*60*]fullerenolysine tetrapeptide **207** from the known diprotected lysine **200**, and known [*60*]fullerenoproline acid **198.**

2.2.1 Addition of *tert*-butyl 2-((diphenylmethylene)amino)acetate to [*60*]fullerene under Bingel conditions followed by acidolytic cleavage of the *tert*-butyl group

Slightly modified literature synthetic procedures were adopted to synthesize the [*60*]fullerene adducts **197** and **198**.[33,90] To a solution of [*60*]fullerene, carbon tetrabromide and the imine **196** in toluene was added DBU dropwise. The reaction mixture was stirred at rt for 6 h before the crude material was subjected to flash silica gel chromatography. Elution with CH_2Cl_2/hexanes (1:1) led to the isolation of compound **197** in 60% yield, which was carried forward for the acidolytic cleavage of the *tert*-butyl group with TFA and CH_2Cl_2 (1:4 v/v) to form the acid **198** in 90% yield (Scheme 2.1). The spectroscopic data of **198** were in agreement with that previously reported.[90] Examination of the ^{1}H NMR spectrum of **198** revealed the loss of the *tert*-butyl resonance at δ 1.60 (s, 9H) when compared to that of **197**, along with the appearance of a broad singlet, with a relative integration of 1H, at δ 8.63 attributed to the carboxylic acid proton. Analysis of the mass spectrum of **198** showed a base peak at *m/z* 958 assigned to the protonated molecular ion $(M+H)^+$.

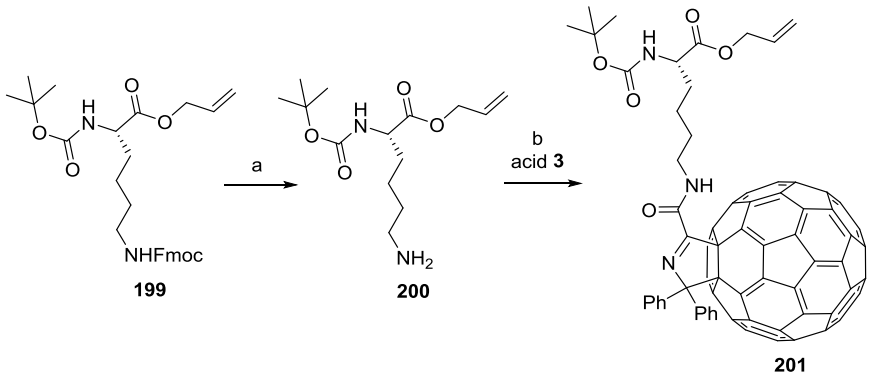


Scheme 2.2: Synthesis of the fullerenyl amino acid **198**.

2.2.2 Base-promoted *N*-Fmoc removal for the triprotected L-lysine 199, followed by amide coupling to give the monopeptide 201

The Fmoc protecting group is widely utilized in the synthesis of peptides [91, 92] as it can be cleaved from the desired amine by the use of a simple base – most commonly piperidine.[93] Therefore, in a typical procedure[94] a solution of triprotected L-lysine **199** in piperidine/acetonitrile (1:9 v/v) was stirred overnight at rt to produce the free amine **200**, in 100% crude yield, which was carried forward to the successive reactions without additional purification (Scheme 2.3).

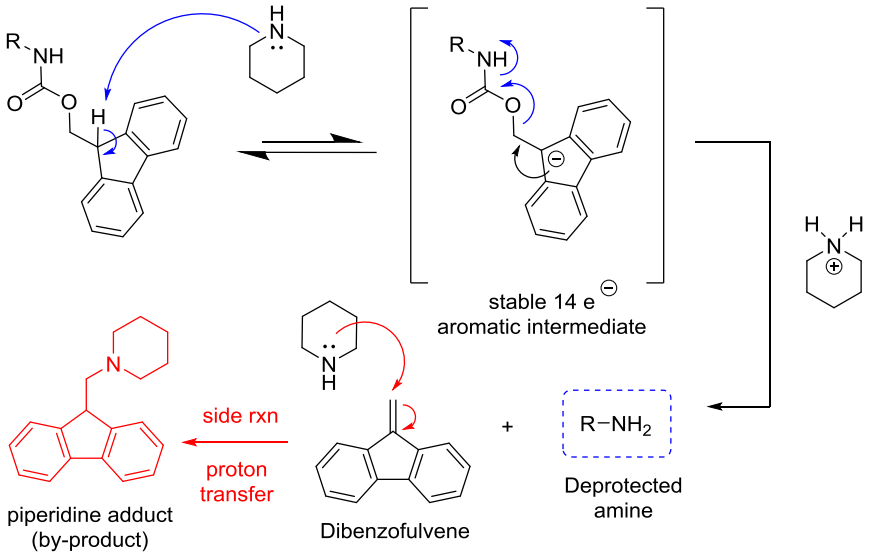
The molecular structure of this salt was verified by the appearance of an ion at *m/z* 287 in the ESIMS, which was assigned to the protonated molecular ion ($[M+H]^+$, $C_{14}H_{27}N_2O_4$).



Scheme 2.3: **Reagents and conditions**: (a) (a) piperidine, CH_3CN, rt, 4 h; (b) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 62%.

The increased lability of the Fmoc group is attributable to its aromatic fluorenyl structure and the presence of the sp^3 methine carbon bridging the two benzene rings. As a result, this methine proton is susceptible to deprotonation by moderate bases (pKa ~25) and this produces a delocalized 14-electron aromatic carbanion (Scheme 2.4).[95] The aromatic carbanion then rapidly decomposes to generate dibenzofulvene (DBF), CO_2, and the desired free amine. The pKa of piperidine is much lower (pKa ~11)[35] than

that of the Fmoc methine proton and only a minute percentage of the molecules will ever be deprotonated at any one time. However, any aromatic carbanion that forms will immediately undergo an irreversible decomposition, the released CO_2 gas escapes the reaction mixture and this continually pushes the reaction to the side of deprotection.



Scheme 2.4: Mechanism of base-promoted Fmoc deprotection by piperidine with the dibenzofulvene scavenging pathway (red) shown.

While high yielding, the piperidine-promoted reaction also produces copious quantities of an insoluble, white by-product, i.e. the dibenzofulvene (DBF)-piperidine adduct. This side reaction (Scheme 2.4 – red pathway) results from interaction of the piperidine base with DBF and it is known to occur with most amine bases.[92,93] The excess piperidine thus functions as a scavenger as well as preventing the desired product amine from reacting with the dibenzofulvene.[92,93] The DBF-piperidine adduct is soluble in DMF and hence, solid-phase peptide synthesis allows the adduct to be washed away *via* filtration.[93] Since solution-phase synthesis was employed in this study, DMF was avoided as a reaction solvent due to its relatively high boiling point. The DBF-piperidine adduct was found to be partially soluble in acetonitrile and therefore could not be removed by simple filtration. Thus, chromatography was necessary to isolate the

pure amine **200**, which was the most polar compound in the reaction product mixture. This problem could have been avoided by employing an amine base that produces an adduct with DBF that is soluble in aqueous phosphate buffer, such as 4-(aminomethyl)piperidine or tris(2-aminoethyl)amine.[93] Therefore, an aqueous extractive work-up could be utilized in the future and chromatography possibly avoided.

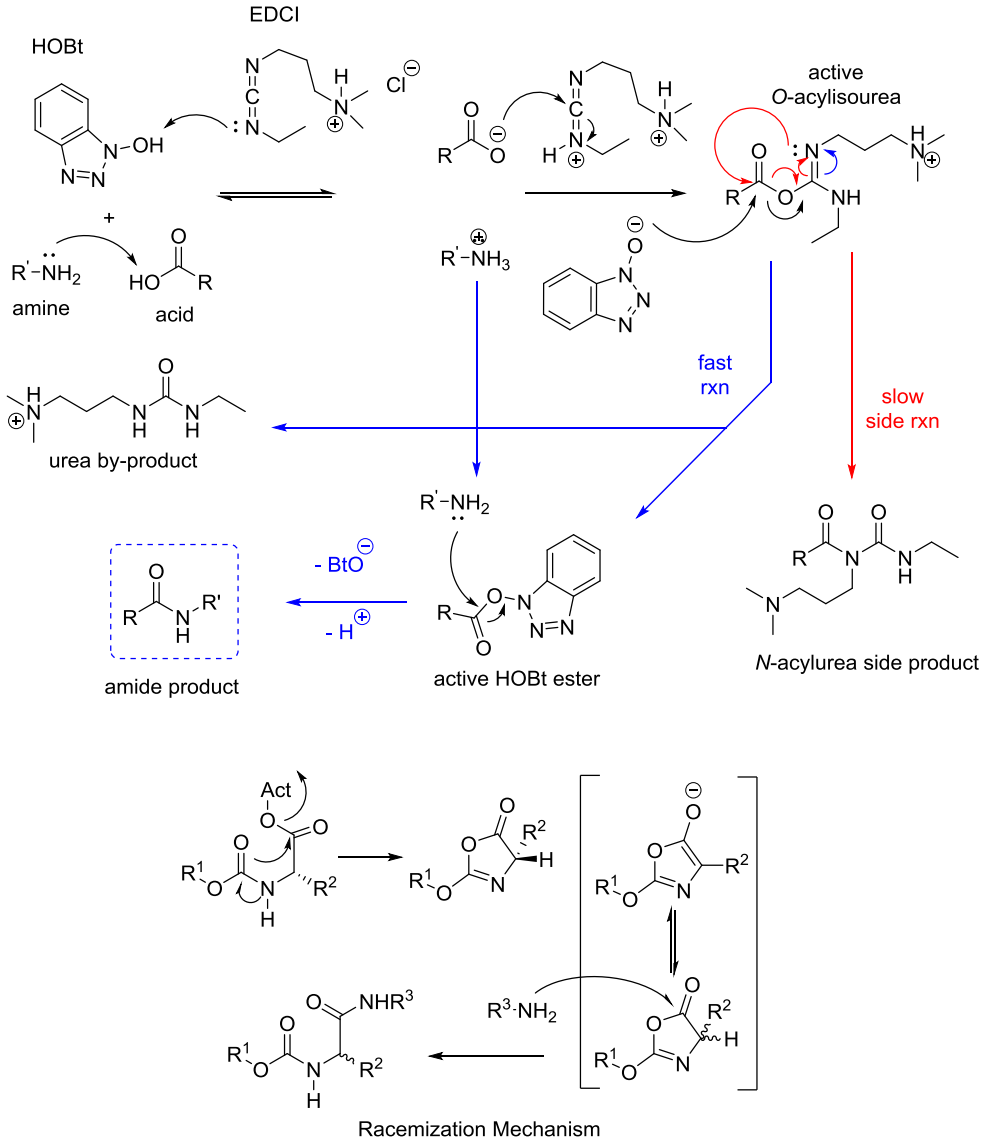
Amide coupling

The formation of amide bonds from carboxylic acids and amines (e.g. amino acids) was a key reaction in the synthesis of the target [*60*]fullerenolysine derivatives. The transformation was employed for coupling of *C*-terminal amino acid onto the *N*-terminal amino acid for the synthesis of dipeptide scaffold. Previous syntheses of peptides have utilized the common EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride), HOBt (1-hydroxy-1*H*-benzotriazole) peptide coupling reagent system in dichloromethane/chloroform to form the necessary peptide bonds.[94,96,97] Therefore, a general procedure was developed and utilized in all subsequent amide coupling reactions (see Scheme 2.3). For example, the synthesis of the crucial monopeptide **201** (Scheme 2.3) was realized in 62% yield by stirring in dichloromethane, a solution of previously synthesised amine **200** and the 5,5-diphenylfullerenedihydropyrrole-2-carboxylic acid[33] **198** with the coupling reagents EDCI and HOBt at rt for 4 h. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired monopeptide **201** in 62% yield (Scheme 2.3).

The broad singlet at δ 8.55 (-COO<u>H</u>) in the ^{1}H NMR spectrum of acid **198** (see Appendix) was no longer observed in the spectrum of **201** but two signals appeared at δ

3.52-3.58 (m, 2H, C$\underline{H_2}$N (Lys)) and δ 7.88 (bs, 1H, NH), assigned to the methylene and a newly formed amidic proton of monopeptide **201**. This evidence indicated that the methylene protons, adjacent to the primary amine in precursor **200**, experienced a downfield shift as a result of the newly installed amide bond. Additionally, the ^{13}C NMR spectrum showed resonance at δ 162.1, assigned to the new amide carbonyl group. The molecular structure of the product **201** was verified by the appearance of a peak at *m*/*z* 1248.2494 in the HRMS, that was assigned to the sodiated molecular formula ($[M + Na]^+$ $C_{89}H_{35}N_3O_5Na$).

The synthesis of the amide bond in **201**, and the large number of amide bonds (i.e. iterative amide coupling) in later compounds in this study, were made feasible due to the reliable and mild conditions afforded by the amide coupling reagents EDCI and HOBt. The uncatalysed reaction between the carboxylic acid and amine components generates an ammonium carboxylate salt; their subsequent amide condensation would require intense heat, which would be incompatible with such multi-functionalized molecules.[98] Therefore, peptide coupling reagents (or 'activators') are utilized to increase the electrophilicity of the acyl moiety, which allows amide bond formation *via* aminolysis of an activated acyl species under much milder conditions.[98-100] Carbodiimides (e.g. EDCI) are employed to generate an active *O*-acylisourea intermediate (Scheme 2.5) by reaction with the carboxylate anion. This intermediate can undergo aminolysis by the precursor amine to give the desired product amide.[98] In a possible side reaction, the highly activated *O*-acylisourea intermediate can undergo intramolecular acyl transfer to give an inactive *N*-acylurea by-product (Scheme 2.5 – red pathway),[98,100] thus consuming the precursor acid without formation of the desired amide product.



Scheme 2.5 Mechanism of EDCI/HOBt promoted amide coupling: both the product pathway (blue) and the undesired side reaction (red) are displayed.

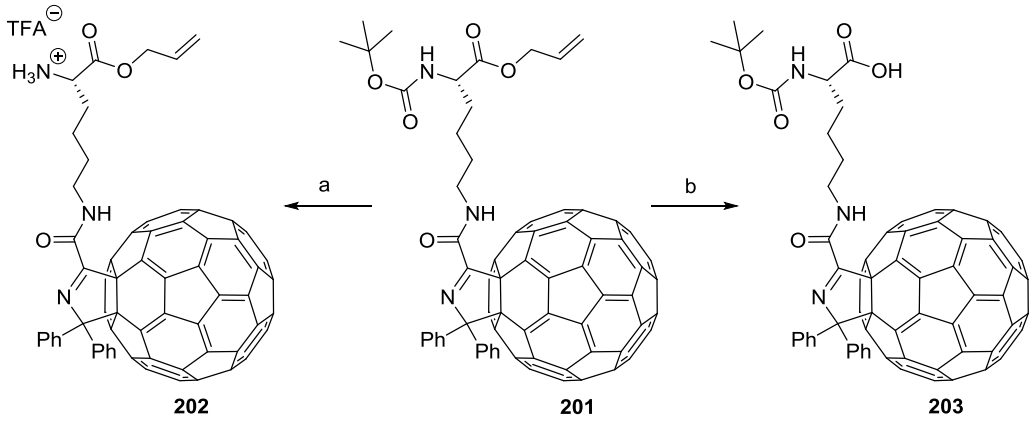
To avoid this deleterious side reaction and the possibility of racemization, nucleophilic additive (e.g. HOBt) was added to the reaction.[98-100] HOBt reacts with the active *O*-acylisourea intermediate to generate an activated HOBt ester with the key acyl moiety (Scheme 2.5 – blue pathway). Crucially, this reaction occurs faster than the intramolecular acyl transfer, thus preventing the side reaction but still generating an activated acid derivative for subsequent aminolysis to the product amide.[98] Epimerization of the stereogenic α-carbon on the *O*-acylisourea intermediate is another

possible side reaction and it can occur through two mechanisms – simple enolate formation by deprotonation of the α-hydrogen and then re-protonation (resulting in loss of chirality) or *via* formation of the planar 5(4*H*)-oxazolone intermediate, in which the α-hydrogen is readily deprotonated due the resonance stabilization of the resulting carbanion. HOBt helps to prevent racemization as the intermediate HOBt ester that is preferentially formed is less activated than the *O*-acylisourea, i.e. the -OBt functionality is less electron-withdrawing than the corresponding isourea substituent and therefore the adjacent α-hydrogen is less prone to enolization.[100] Furthermore, the use of the Fmoc group (and other carbamates) for protection of the α-nitrogen during coupling is known to reduce the prevalence of 5(4*H*)-oxazolone mediated racemization by induced destabilization of the resultant oxazolone carbanion. The lack of epimerization observed during the performed amide coupling reactions in this study was a result of these combined factors (e.g. HOBt and carbamate protection). Such an epimerization process would result in the formation of diastereomeric products that could be detected by ^{1}H NMR and/or TLC analysis. Based on such analyses, the isolated amide products were obtained as single diastereomers. Based on these findings and the analysis that dipeptide **204** has a single diastereomer we conclude that product **201** is a single enantiomer.

2.2.3 *N*-Boc and *tert*-butyl ester removal *via* acidolysis, allyl ester hydrolysis by using $(CH_3)_3SnOH$

The acidolytic cleavage of the Boc and *tert*-butyl ester protecting groups in this study could be easily achieved with a variety of acids. However, trifluoroacetic acid (TFA) was used most often in this study.[91,101] The ability of these groups to be cleaved separately or simultaneously under relatively mild acidic conditions was an essential feature of this general reaction. As such, a previously published general procedure[97] was

employed, with modifications, for the deprotection *N*-Boc alone and/or *tert*-butyl ester group at rt for 4 h. The N-Boc derivative **201** was dissolved in a mixture of TFA and CH_2Cl_2 (1:1 v/v) and stirred at rt for 4 h which gave a 90% yield of the TFA salt of the target amine **202** (Scheme 2.6).

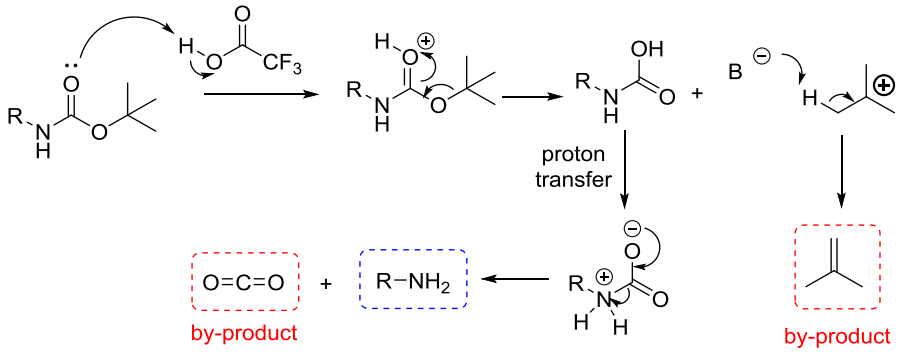


Scheme 2.6: **Reagents and conditions**: (a) TFA: CH_2Cl_2 (1:1), rt, 4 h, 90%. (b) 1,2-DCE, $(CH_3)_3SnOH$, 80 ^{0}C, 6 h, 88%.

The ^{1}H NMR spectrum of compound **202** (see Appendix) was lacking the prominent 9H resonance at δ 1.44 that was present in the spectrum of carbamate **201** and was assigned to the *tert*-butyl methyl protons. ESIMS analysis of compound **202** showed a peak at *m*/*z* 1126 which was assigned to the protonated molecular ion ($[M + H]^+$, $C_{84}H_{28}N_3O_3$).

In this chapter the Boc and *tert*-butyl ester protecting groups were cleaved using TFA. The cleavage mechanism is similar for both substituents (Schemes 2.7) and proceeds *via* protonation followed by heterolytic cleavage and release of a relatively stable *tert*-butyl carbocation. The tertiary nature of the *tert*-butyl cation stabilizes the positive charge by donating electron density onto the tertiary carbon (Scheme 2.7). Release of the cationic species from the *N*-protected substrate results in a carbamic acid (Schemes 2.7), which decomposed by the loss of CO_2 and gave the product amine as the TFA salt.

The simple acidolytic conditions required for removal of the *N*-Boc protecting group allowed for the isolation of the crude TFA salts by simple solvent removal. Due to the volatility of the Boc deprotection by-products, no resultant impurities were observed. Overall, it was the relative ease, speed, and resultant product purity of the acidolytic deprotection method that allowed for the efficient turn-over of TFA salt of the corresponding amine or free amine.



Scheme 2.7. Mechanism of *tert*-butylcarbamate acidolysis by TFA to give the desired amine (blue) and by-products (red)

Allyl ester cleavage

$(CH_3)_3SnOH$ had been previously used by Mascaretti and co-workers[102-105] to cleave phenacyl ester anchored amino acids and peptides from a polystyrene resin and to hydrolyze methyl phenylacetate (Figure 2.1). By utilizing this reagent, K. C. Nicolaou and co-workers developed a method to hydrolyse esters on highly epimerization sensitive systems[106] (Figure 2.2). This method remarkably resulted in quantitative conversion of a dipeptide allyl ester to the corresponding acid with negligible erosion of the stereochemical integrity (Figure 2.2).

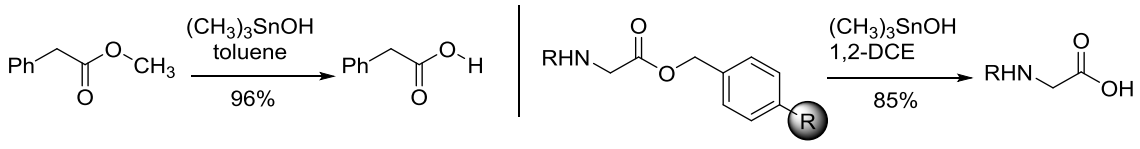


Figure 2.1. Literature precedent for $(CH_3)_3SnOH$ cleavage of esters.

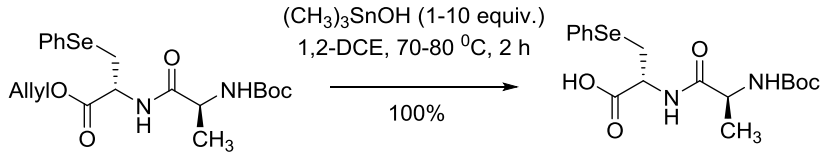


Figure 2.2. Mild and selective cleavage of an ester with $(CH_3)_3SnOH$.

A probable mechanistic interpretation can be seen in Figure 2.3. The hydroxide moiety of the reagent engages the generic ester to create a polar tetracoordinated transition state. This results in the formation of a tin ester intermediate (confirmed by ^{1}H, ^{13}C, and ^{119}Sn NMR spectroscopy)[104] which is easily hydrolysed upon aqueous workup to the desired acid. Additionally, the Sn atom most likely facilitates the reaction by participating as a weak Lewis acid and coordinating to the carbonyl oxygen of the ester starting material.

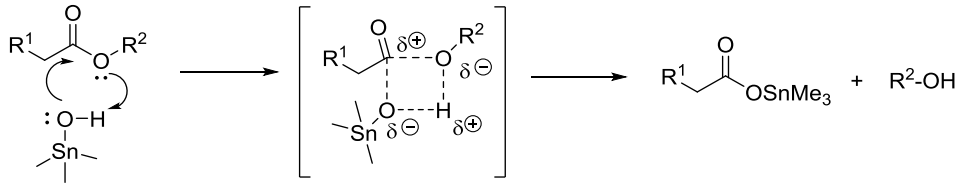


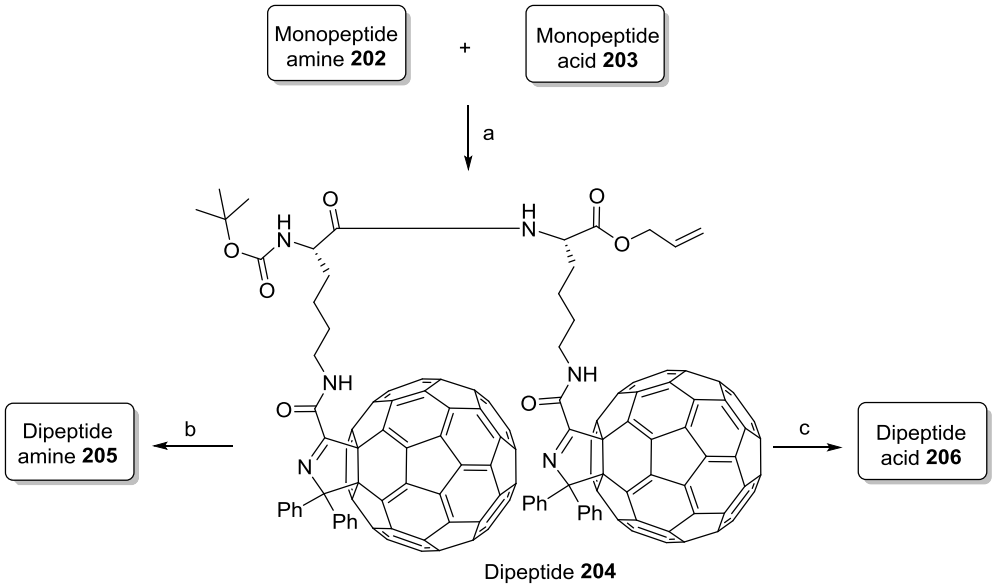
Figure 2.3. A proposed mechanism of $(CH_3)_3SnOH$ mediated hydrolysis of esters.

The [*60*]fullerenolysine monopeptide **201** was treated with $(CH_3)_3SnOH$ under the above mentioned reaction conditions to selectively hydrolyse the allyl ester to give the acid **203** in 88% yield (Scheme 2.6).

The ^{1}H NMR spectrum of compound **203** (see Appendix) was lacking the prominent allyl ester resonances at δ 4.58 (m, 2H), 5.28 (d, J = 10.0 Hz, 1H), 5.30 (d, J = 17.0 Hz, 1H) and 5.84 (m, 1H), that were present in the spectrum of ester **201**. HRMS analysis of compound **203** contained a peak at m/z 1208.2145 that was assigned to the sodiated molecular ion ($[M + Na]^+$, $C_{86}H_{31}N_3O_5Na$). This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

2.2.4 Amide coupling to synthesize dipeptide 204, followed by deprotection reactions to obtain 205 and 206

The synthesis of the dipeptide **204** (Scheme 2.8) was realized in 57% yield by stirring in dichloromethane, a solution of previously synthesised monopeptide amine **202** and the monopeptide acid **203** under the coupling conditions described in Section 2.2.2. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired dipeptide **204** in 57% yield (Scheme 2.8). Subsequent deprotection of dipeptide **204** yielded the dipeptide amine **205** (in 90%), and the dipeptide acid **206** (in 86%), using the reaction conditions adopted from Section 2.2.3.



Scheme 2.8: Synthesis of a dipeptide **204**, dipeptide amine **205** and dipeptide acid **206**. **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 57%; (b) TFA: CH_2Cl_2 (1:1), rt, 4 h, 90%. (c) 1,2-DCE, $(CH_3)_3SnOH$, 80 ^{0}C, 6 h, 86%.

The ^{1}H NMR spectrum of dipeptide **204** (see Appendix) showed the prominent allyl ester resonances [at δ 4.58 (m, 2H), 5.28 (d, *J* = 10.0 Hz, 1H), 5.30 (d, *J* = 17.0 Hz, 1H) and 5.84 (m, 1H)], that were present in the spectrum of amine **202** and the Boc methyl

resonances (δ 1.45, s, 9H), that was present in the spectrum of acid **203**. Additionally, an amidic proton was also present at δ 6.68 (bs, 1H, NH), due to the newly formed amide bond. Furthermore, the ^{13}C NMR spectrum showed a resonance at δ 162.7, which was assigned to the newly formed amide carbonyl group. The molecular structure of the product **204** was verified by the appearance of the peak at *m*/*z* 2315.4114 in the HRMS, that was assigned to the sodiated molecular ion ($[M+Na]^+$ $C_{170}H_{56}N_6O_7Na$).

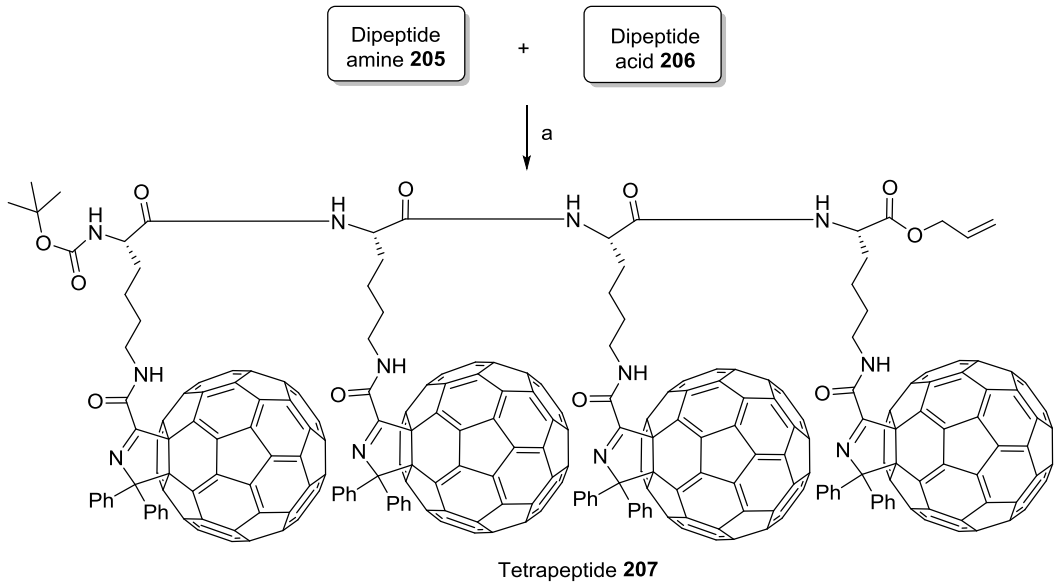
The dipeptide amine **205** showed the disappearance of a prominent Boc group resonance at δ 1.45 (s, 9H), that was present in the ^1H NMR spectrum of dipeptide **204**. The molecular structure of the product **205** was verified by the appearance of the peak *m*/*z* 2193.3764 in the HRMS, that was assigned to the protonated molecular ion ($[M+H]^+$ $C_{165}H_{48}N_6O_5$).

The deallylation of dipeptide **204** to its acid **206** was supported by the loss of resonances [at δ 4.58 (m, 2H), 5.28 (d, *J* = 10.0 Hz, 1H), 5.30 (d, *J* = 17.0 Hz, 1H) and 5.84 (m, 1H)], in the ^1H NMR spectrum, that were present in the dipeptide **204**, which were assigned to the allyl protons. The molecular structure of the product **206** was verified by the appearance of the peak at *m*/*z* 2276 in the ESIMS, that was assigned to the sodiated molecular ion ($[M+Na]^+$ $C_{167}H_{51}N_6O_7Na$).

2.2.5 Coupling of amine 205 and acid 206 to the tetrapeptide 207

The general synthetic procedure was employed to synthesise tetrapeptide **207**, was as described in section 2.2.2. The synthesis of the tetrapeptide **207** (Scheme 2.9) was realized by stirring in chloroform, a solution of previously synthesised dipeptide amine **205** and the dipeptide acid **206** under the coupling conditions mentioned in Section 2.2.2. TLC analysis of tetrapeptide **207** showed the presence of dipeptide acid **206** along

with a new spot (R_f = 0.6), which was assumed to be the tetrapeptide **207**. Further base (sat. $NaHCO_3$) treatment did not completely remove the acid **206** from the crude mixture. Purification of the crude product by flash column chromatography followed by simple trituration from diethyl ether did not yield the desired tetrapeptide **207**, in satisfactory purity. Further purification by preparative thin layer chromatography (PTLC) followed by trituration from dichloromethane/diethyl ether (1:1) resulted in 51% yield of tetrapeptide **207** (Scheme 2.9), which we believe to have a small amount of acid **206** impurity remaining in it. In a subsequent reaction, a change of solvent from chloroform to chloroform/pyridine (1:1) for the coupling reaction of compounds **205** and **206** yielded tetrapeptide **207** in 50% crude yield. Unfortunately subsequent base (sat. $NaHCO_3$) wash, flash column chromatography and PTLC could not remove all the acid **206** impurity from the tetrapeptide **207**.



Scheme 2.9: Synthesis of the tetrapeptide **207**. **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, $CHCl_3$, rt, 4 h, ~51%; or EDCI, HOBt, Et_3N, $CHCl_3$/Pyridine (1:1), rt, 4 h, ~50%.

The ^1H NMR spectrum of tetrapeptide **207** showed the prominent allyl ester resonances [at δ 4.56 (m, 2H), 5.25 (d, *J* = 9.0 Hz, 1H), 5.27 (d, *J* = 17.0 Hz, 1H) and 5.82 (m, 1H)], that were present in the spectrum of amine **205** and the Boc methyl resonances (δ 1.44, s, 9H), that was present in the spectrum of acid **206**. Additionally, an amidic

proton was also appeared at 7.80 (bs, 1H, NH), due to the newly formed amide bond. Unfortunately, the products from both coupling procedures were not sufficiently soluble in most of the organic solvents to generate an adequate ^{13}C NMR spectrum and were not sufficiently soluble in MeOH to generate an adequate ESI-MS. Thus we could not verify if this reaction was successful.

2.2.6 Optimization of coupling reaction to synthesise tetrapeptide 207

To optimize the coupling reaction of dipeptide amine **205** and dipeptide acid **206** to provide the tetrapeptide **207** in purer form and perhaps better solubility, several different reaction conditions were tried. These included changes to the coupling reagent, solvent, base and the reaction temperatures. Unfortunately, none of these attempts could evolve as suitable conditions to synthesise tetrapeptide **207** in purer form, instead an inseparable mixture was the end result (Table 2.1).

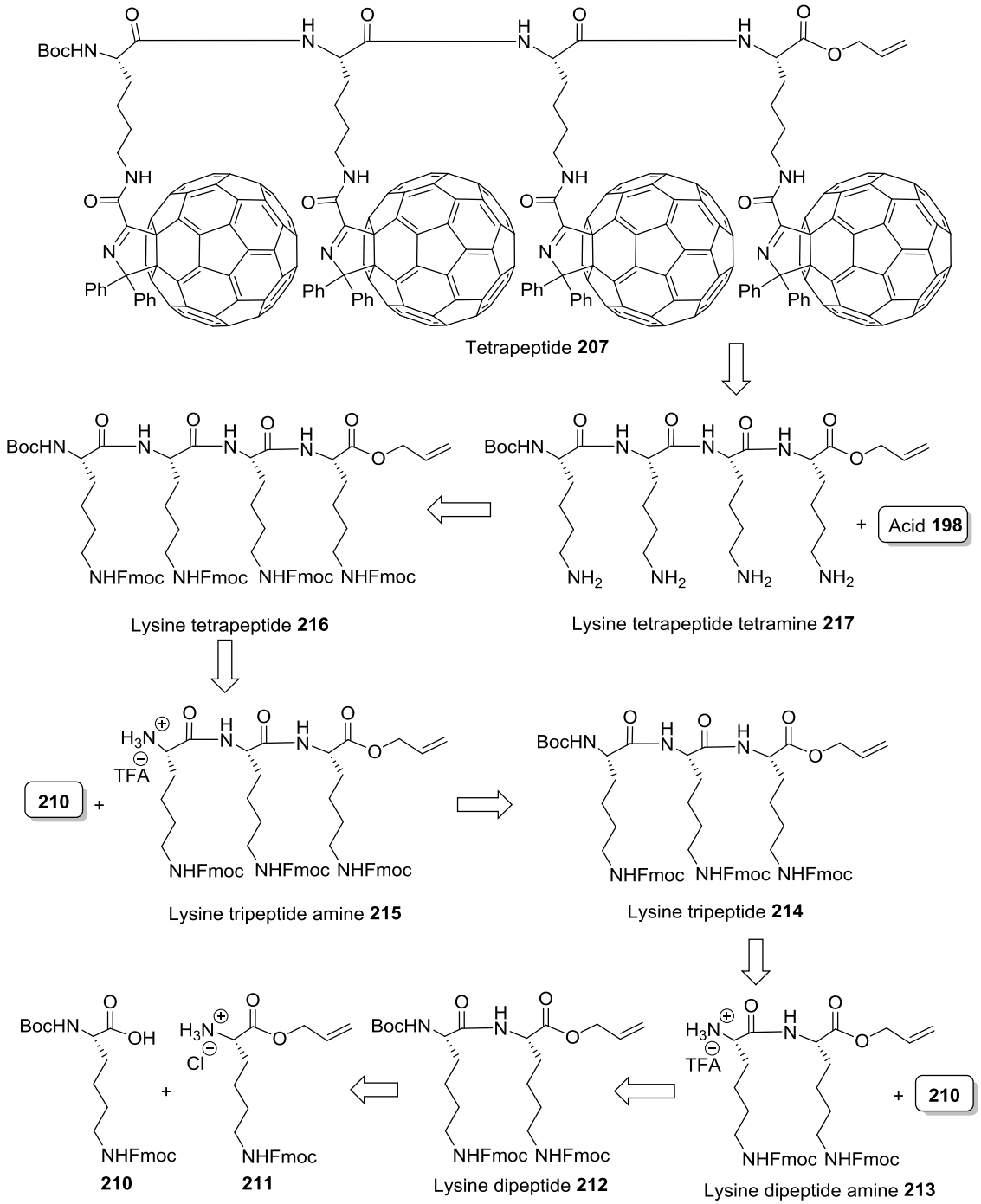
Table 2.1 Attempted optimisation reaction conditions:

Entry	Solvent	Coupling reagent	Base	Reaction conditions[a]	Result[b]
1	CH_2Cl_2	DCC, HOBt	Et_3N	rt	mixture
2	CH_2Cl_2	EDCI, HOBt	Et_3N	rt	mixture
3	CH_2Cl_2	HBTU	Et_3N /DIPEA	rt-40 ^0C	mixture
4	$CHCl_3$	DCC, HOBt	Et_3N /DIPEA	rt	mixture
5	1,2-DCE	DCC, HOBt	Et_3N	rt-80 ^0C	mixture
6	1,2-DCE	EDCI, HOBt	Et_3N /DIPEA	rt-80 ^0C	mixture
7	1,2-DCE	HBTU	Et_3N /DIPEA	rt-80 ^0C	mixture
8	1,2-DCE	HATU	Et_3N /DIPEA	rt-80 ^0C	mixture
9	1,2-DCB	EDCI, HOBt	Et_3N /DIPEA	rt-80 ^0C	mixture
10	1,2-DCB	HBTU	Et_3N /DIPEA	rt-80 ^0C	mixture
11	1,2-DCB	HATU	Et_3N /DIPEA	rt-80 ^0C	mixture

(a). All the reactions were tried on 15 mg scale and verified by the TLC analysis. (b). Possible compounds in the mixture are tetrapeptide **207** along with dipeptide acid **206**.

2.2.7 Strategy 2: Synthesis of tetrapeptide 207, by synthesising a lysine tetramine 217 first and then coupling with acid 198.

In an alternative strategy, the synthesis of the tetrapeptide **207** was proposed from the previously unknown lysine tetrapeptide tetramine **217** and the previously known [*60*]fullerenoproline acid **198**. Compound **207** was proposed in seven synthetic steps (Scheme 2.10) starting from the known lysine acid **210** and amine **211**. This strategy was adopted due to the relative ease of handling peptides in terms of solubility, purification and the size of the molecule. The previous attempts (Sections 2.2.5 and 2.2.6) were unsuccessful in providing pure samples of **207**, perhaps because of steric hinderance due to the steric bulk of the incoming dipeptides **205** and **206**. This strategy could possibly overcome these adverse steric effects, with the reaction between the relatively smaller peptide **217** and [*60*]fullerenoproline acid **198**.

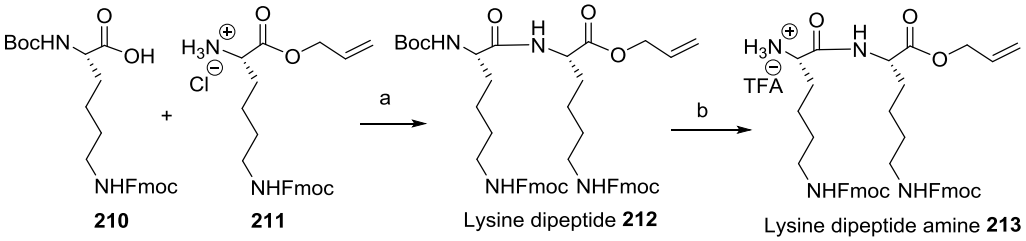


Scheme 2.10 Retrosynthetic analysis for the synthesis of [*60*]fullerenolysine tetrapeptide **207** from the unknown lysine tetrapeptide tetramine **217** and a known acid **198**.

2.2.8 Synthesis of lysine dipeptide 212 and its amine 213

The synthesis of the lysine dipeptide **212** (Scheme 2.11) was realized in 85% yield by stirring a solution of commercially available acid **210** and the amine **211** in

dichloromethane under the coupling conditions mentioned in section 2.2.2. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired dipeptide **212** in 85% yield (Scheme 2.11). Subsequent Boc deprotection of dipeptide **212** with TFA realised the amine salt **213** in 90%, by the conditions adopted from Section 2.2.3.

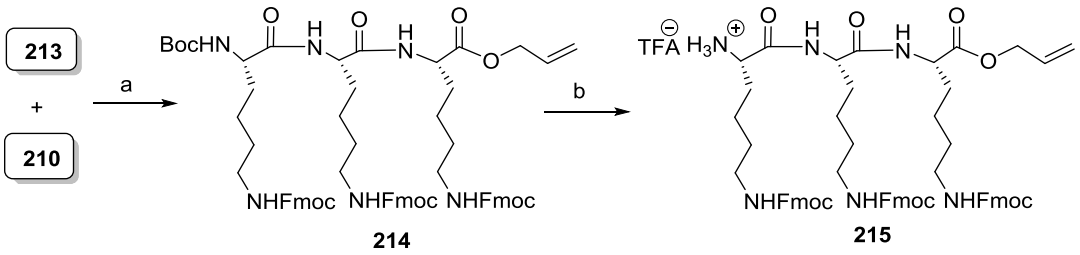


Scheme 2.11: **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 85%; (b) TFA: CH_2Cl_2 (1:1), rt, 4 h, 90%.

The broad singlet at δ 8.55 ($CHN\underline{H}_3^+$) in the ^1H NMR spectrum of **211** was no longer observed in the spectrum of **212** but two signals appeared at δ 4.12 (m, 1H) and δ 4.18 (m, 1H), assigned to the two different lysine α-methine protons in **212**. This evidence indicated that the α-proton, adjacent to the primary amine in precursor **211**, experienced a downfield shift as a result of the newly installed amide bond. Additionally, the molecular structure of the product **212** was verified by the appearance of the peak at *m*/*z* 881.7 in ESI-MS, that was assigned to the sodiated molecular ion, $[M + Na]^+$. The ^1H NMR spectrum of compound **213** was lacking the prominent 9H resonance at δ 1.42 that was present in the spectrum of carbamate **212** and was assigned to the *tert*-butyl methyl protons. ESIMS analysis of compound **213** indicated peak at *m*/*z* 759 that was assigned to the protonated molecular ion ($[M + H]^+$, $C_{45}H_{50}N_4O_7$).

2.2.9 Synthesis of lysine tripeptide 214 and its amine 215

The synthesis of the lysine tripeptide **214** (Scheme 2.12) was realized in 83% yield by stirring in dichloromethane, a solution of previously synthesised dipeptide amine **213** and the monopeptide acid **210** under the coupling conditions mentioned in section 2.2.2. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired dipeptide **214** in 83% yield (Scheme 2.12). Subsequent Boc deprotection of tripeptide **214** in TFA realised the amine salt **215** in 98%, by the conditions adopted from Section 2.2.3.



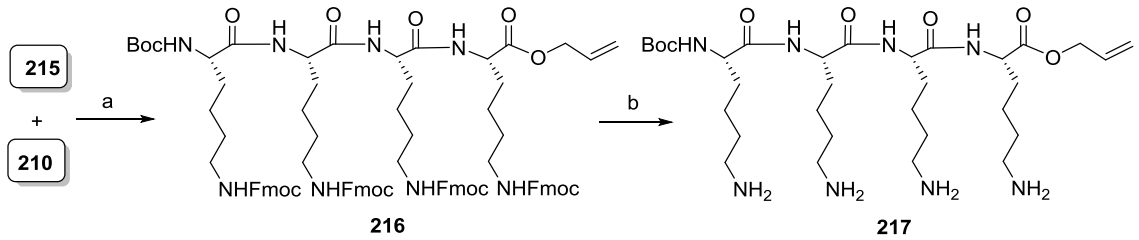
Scheme 2.12: Synthesis of the lysine tripeptide **214** and amine **215**. **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 83%; (b) TFA: CH_2Cl_2 (1:1), rt, 4 h, 98%.

Analysis of the ^{1}H NMR spectrum of **214** showed a loss of the resonance attributable to the carboxylic acid proton in **210** and the presence of resonances for the characteristic protons of both the carboxylic acid **210** and amine **213**. A multiplet at δ 3.43-3.44 (-CH<u>N</u>H$_2$) in the ^{1}H NMR spectrum of **213** was no longer observed in the spectrum of **214** but a multiplet at δ 4.14-4.15 with an integration of 6H was assigned to the three lysine α-methine protons and three Fmoc methine protons. This evidence indicated that the α-proton, adjacent to the primary amine in precursor **213**, experienced a downfield shift as a result of the newly installed amide bond. This sample turned into a gum over a period of time, so without measuring the ^{13}C NMR spectrum, the material was carried forward to the subsequent reaction. Additionally, the molecular structure of the product was verified by the appearance of the peak at *m*/*z* 1231 in the ESI MS, that was assigned

to the sodiated molecular ion ($[M + Na]^+$ $C_{71}H_{80}N_6O_{12}Na$). Compound **215** was characterised by the appearance of the peak at *m/z* 1109 in the ESI MS, that was assigned to the protonated molecular ion ($[M + H]^+$ $C_{66}H_{73}N_6O_{10}$).

2.2.10 Synthesis of lysine tetrapeptide 216 and its tetramine 217

The synthesis of the lysine tetrapeptide **216** (Scheme 2.13) was achieved in 70% yield by stirring in dichloromethane, a solution of lysine tripeptide amine **215** and the monopeptide acid **210** under the coupling conditions described in section 2.2.2. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired tetrapeptide **216** (Scheme 2.13). To remove the Fmoc groups in **216** a solution of tetrapeptide **216** in piperidine/acetonitrile (1:9 v/v) was stirred overnight at rt to produce the lysine tetramine **217**, which was carried forward to the next reaction.



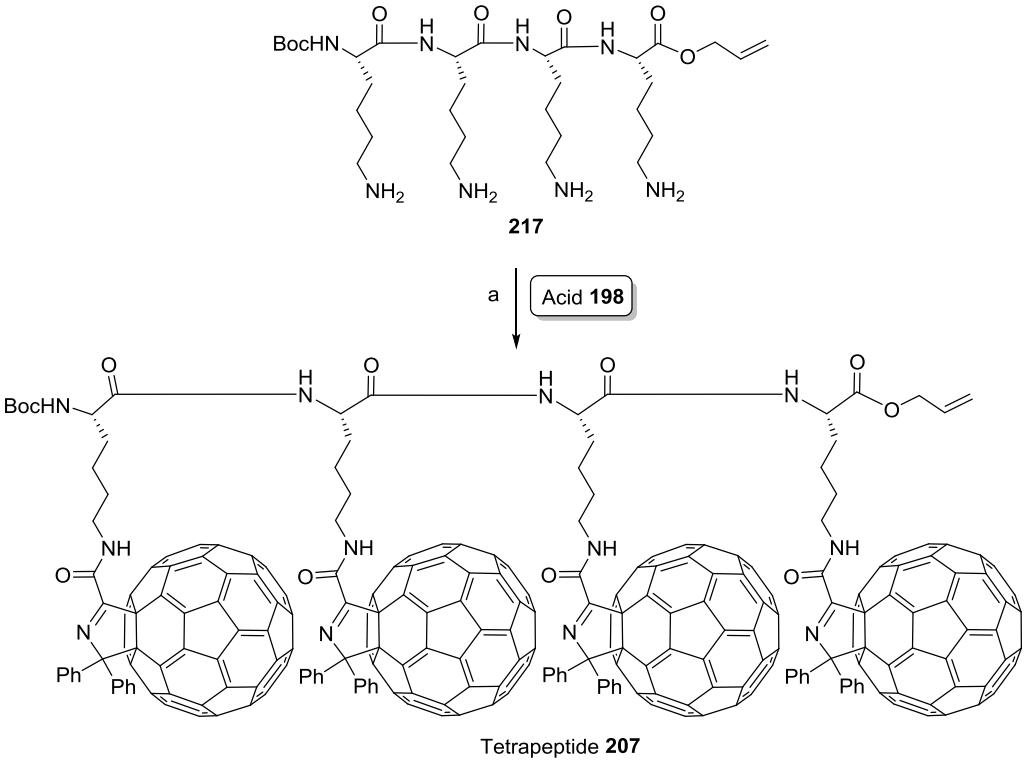
Scheme 2.13: Synthesis of the lysine tetrapeptide **216** and tetramine **217**. **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 70%; (b) piperidine, CH_3CN, rt, 4 h, 100%.

The ^1H NMR spectrum of tetrapeptide **216** showed the prominent allyl ester resonances [at δ 4.46 (bs, 2H), 5.11 (d, *J* = 10.5 Hz, 1H), 5.21 (d, *J* = 17.5 Hz, 1H) and 5.78 (m, 1H)], that were present in the spectrum of amine **215** and Boc methyl resonances (δ 1.29, s, 9H), that were present in the spectrum of acid **210**. Additionally, an amidic proton was also appeared at δ 8.18 (bs, 1H, NH), this was due to the newly formed amide bond. Additionally, the ^{13}C NMR spectrum of **216** showed resonances at δ 155.5, assigned to the Boc carbonyl and δ 172.3 that was assigned to the ester carbonyl. This

indicated the presence of carbonyl signals from both the reactants. The molecular structure of the product **216** was verified by the appearance of the peak at *m/z* 1581.7362 in the HRMS, that was assigned to the sodiated molecular ion ($[M+Na]^+$ $C_{92}H_{102}N_8O_{15}Na$). Compound **217** was characterised by the appearance of the peak at *m/z* 671 in the ESI MS, that was assigned to the protonated molecular ion ($[M+H]^+$ $C_{32}H_{62}N_8O_7$).

2.2.11 Coupling of lysine tetrapeptide tetramine 217 and acid 198 to synthesise tetrapeptide 207

The general synthetic procedure was employed to synthesise tetrapeptide **207** using two different combinations of solvents (chloroform and 1:1 mixture of chloroform/pyridine) for better solubility. The attempted synthesis of the tetrapeptide **207** (Scheme 2.14) produced a mixture of products in both the cases; three significant compounds (10 mg, 14 mg and 56 mg) were separated by column chromatography and the ESIMS showed molecular ions at *m/z* 1167, 1387 and 1417, respectively. Unfortunately, none of these MS data matched with the possible fullerene substituted peptides, including the mono-fullerene, di-fullerene, tri-fullerene or the desired tetra-fullerene coupled tetrapeptide **207**. This could possibly be due to steric hindrance between the incoming [*60*]fullerenoproline acid **198** units. Therefore, a less sterically hindered cyclic peptide was considered.

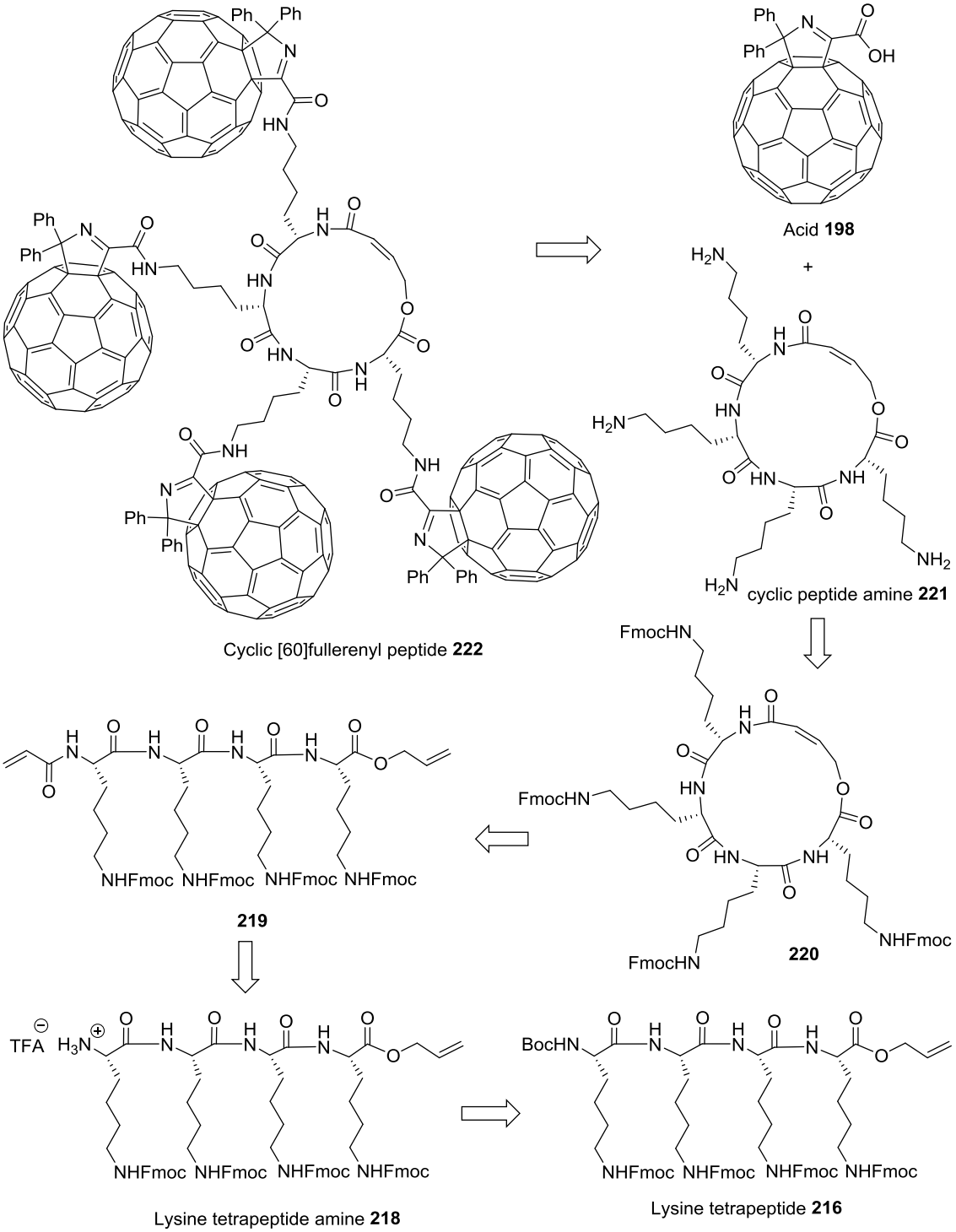


Scheme 2.14: Attempted synthesis of the tetrapeptide **207**. **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, $CHCl_3$, rt, 8 h, mixture of products; or EDCI, HOBt, Et_3N, $CHCl_3$/Pyridine (1:1), rt, 8 h, mixture of products.

2.2.12 Strategy 3: Synthesis of [*60*]fullerenolysine cyclic tetrapeptide 222, by synthesising a lysine cyclic tetrapeptide amine 221 first and then coupling with acid 198.

The synthesis of the cyclic tetrapeptide **222** was proposed from the unknown cyclic tetrapeptide tetramine **221** and the [*60*]fullerenoproline acid **198**. Compound **221** was proposed in four synthetic steps (Scheme 2.15) starting from previously synthesised lysine tetrapeptide **216**. This strategy was adopted to reduce the steric hindrance between the incoming fullerenyl units on to the cyclic peptide. The unsuccessful results described in Sections 2.2.5, 2.2.6 and 2.2.11 were thought to arise from the steric bulk of the dipeptides **205** and **206**, and therefore the less hindered cyclic peptide **221** might

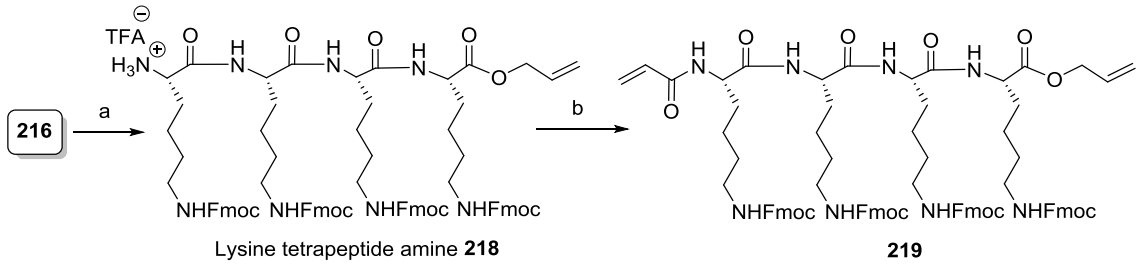
have a higher reactivity towards acid **198** due to less steric hindrance as shown for the proposed synthesis of the fullerenyl peptide **222** in Scheme 2.15.



Scheme 2.15: Retrosynthetic analysis for the synthesis of [*60*]fullerenolysine cyclic tetrapeptide **222** starting from the tetrapeptide **216**.

2.2.13 Synthesis of lysine tetrapeptide amine salt 218 and acrolyl coupled tetrapeptide 219

The synthesis of the lysine tetrapeptide amine salt **218** (Scheme 2.16) was realized in 98% by using TFA (1:1) in dichloromethane as solvent by following the procedure described in Section 2.2.3. This product was carried forward to the subsequent coupling reaction with acrylic acid under the coupling conditions mentioned in Section 2.2.2. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired product **219** in 75% yield (Scheme 2.16).

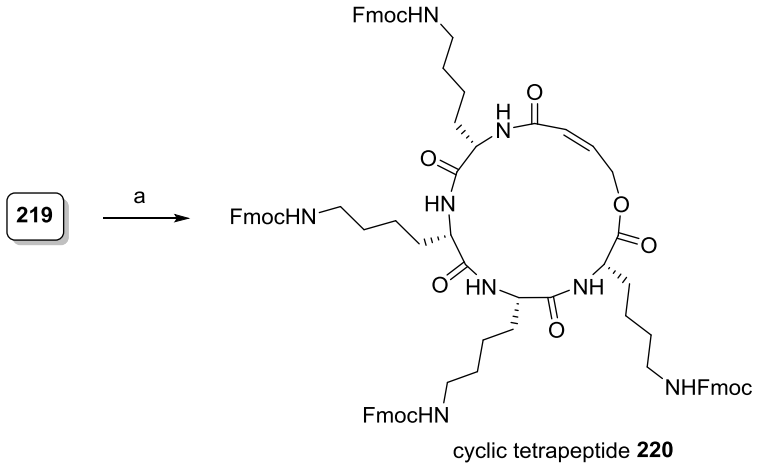


Scheme 2.16: Synthesis of compound **218** and **219**. **Reagents and conditions**: (a) TFA: CH_2Cl_2 (1:1), rt, 4 h, 98%. (b) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 75%.

Compound **218** was characterised by the appearance of the peak at *m/z* 1459 in the ESIMS, that was assigned to the protonated molecular ion ($[M+H]^+$ $C_{87}H_{94}N_8O_{13}$). The ^1H NMR spectrum of compound **219** showed the prominent allyl ester resonances [at δ 4.56 (bs, 2H), 5.20-5.33 (m, 2H), and 5.63-5.65 (m, 1H)], and acrylyl group resonances [at δ 5.81-5.83 (m, 1H, NHCOCH=CHH), 6.06-6.20 (m, 1H, NHCOCH=CHH)]. Additionally, the molecular structure of the product **219** was verified by the appearance of the peak at *m/z* 1535 (100%) in the ESIMS, that was assigned to the sodiated molecular ion ($[M+Na]^+$ $C_{90}H_{96}N_8O_{14}Na$).

2.2.14 Synthesis of cyclic tetrapeptide 220 by ring closing metathesis (RCM)

A solution of **219** in CH_2Cl_2 (100 mL) was heated at reflux then Grubbs' II catalyst[107] was added in one portion. The colour became pink then purple and then darkened to orange. The reaction was heated at reflux for 4 h, after which TLC analysis showed complete consumption of **219**. The crude reaction mixture was then subjected to flash silica chromatography, elution with $MeOH/CH_2Cl_2$ (1:25) provided the cyclic compound **220** as an off-white solid (Scheme 2.17).



Scheme 2.17: Synthesis of compound **220**. **Reagents and conditions**: (a) CH_2Cl_2, Grubb's II catalyst, reflux, 4 h, 19%.

The cyclic peptide **220** was characterised by the appearance of the peak at *m/z* 1486 (80%) in the ESIMS, that was assigned to the protonated molecular ion ($[M+H]^+$ $C_{88}H_{93}N_8O_{14}$). This compound was not sufficiently soluble to generate an adequate ^1H or ^{13}C NMR spectrum.

This strategy was not pursued any further because of the difficulty of characterising compounds due to their poor solubilities and the low chemical yields.

2.2.15 Material Science Applications of compounds 201 and 204 as Fullerene van der Walls Oligomers

The results from this section were generated by collaborators in Germany (Prof. Timothy Clark and Prof Dirk M. Guldi, University of Erlangen-Nuremberg). [*60*]Fullerenes are used extensively as electron acceptors, for instance in phenyl-C_{60}-butyric acid methyl ester (PCBM) based organic solar cells and in devices based on polycrystalline fullerene films. In most cases, van der Waals interactions between fullerenes are present. An exciting possibility for low-voltage organic field-effect transistors (FETs) is to combine the dielectric and the semiconductor functionalities in a single molecule that can form a self-assembled monolayer (SAM).[108] When the semiconductor moiety in such molecules is a substituted fullerene, an n-type semiconductor layer results that can become negatively charged under conductance conditions. This results in considerable hysteresis in the voltage-current characteristics of the device.[109] When our collaborators in Erlangen were analyzing semiempirical molecular orbital calculations on such SAMs,[110] they noticed that the local electron affinity[111,112] indicated electron traps in the interstitial volumes between adjacent fullerenes in the semiconductor layer and that these traps were particularly deep when three fullerenes were involved.[110] A literature survey revealed that several groups have studied covalently linked fullerenes, their radical anions and dianions,[113-123] but that little attention has been paid to non-bonded van der Waals aggregates of fullerenes.[124] The covalently bonded C_{60}-dimer radical anion/dianion, $\mathbf{A}^{-\bullet/2-}$ can be considered to be the result of an addition to a C_{sp2}-C_{sp2} bond in the fullerene cage, whereas a reduced van der Waals dimer, **B** can form one-electron bonds[125] between fullerene "super atoms" (Figure 2.4).[126] A third possibility is the dimer adduct formed by a formal [2+2] cycloaddition of two 5,6-bonds, **C** (Figure 2.4).[113]

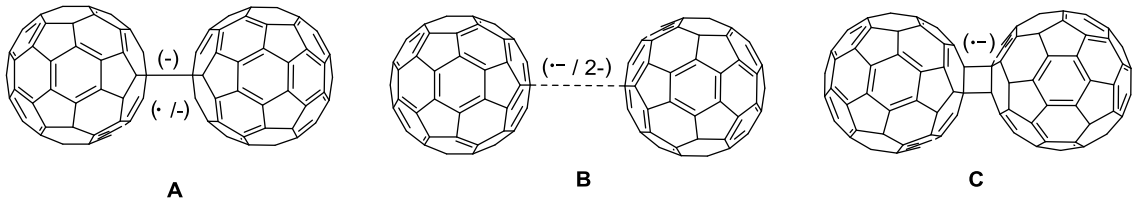


Figure 2.4: Structures of fullerene dimers **A**, **B** and **C**.

The "super atom" picture of fullerenes is interesting in this context because the lowest unoccupied molecular orbitals of C_{60} are triply degenerate. This means that additional electrons can occupy low-lying intermolecular molecular orbitals, leading to the possibility that larger van der Waals C_{60}-oligomer dianions can form multi-center two-electron bonds to give trimers (**D**) analogous to H_3^+ [127] and tetramers (**E**) analogous to the 1,3-dehydro-5,7-adamatanediyl dication.[128]

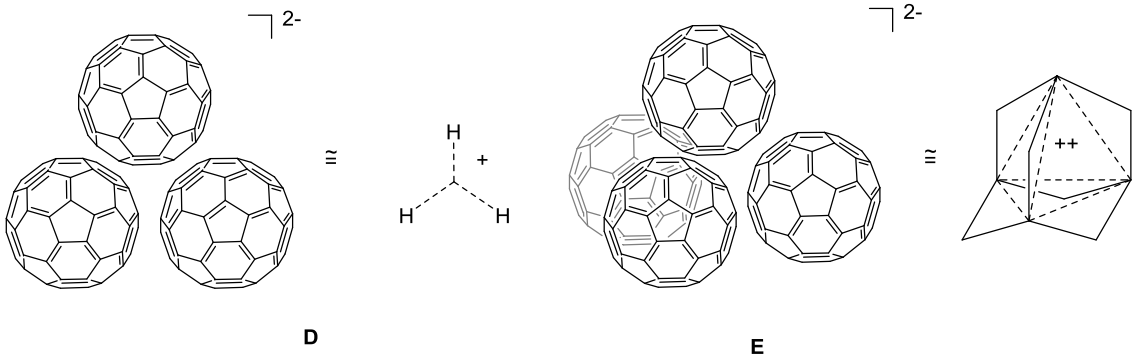


Figure 2.5: Structures of fullerene trimer **D** and tetramer **E**.

Table 2.2 shows the results obtained by Prof. T. Clark *et al*. from density-functional theory (RI-BP86[129,130]/TZV) calculations on fullerene dimers, trimers and tetramers and one- and two-electron reduced forms. They used an empirical correction to account for dispersion,[131] although the correctness of such treatments for fullerenes has recently been placed in doubt.[132] They have investigated the three structures **A**-**C** in Figure 2.4 for $(C_{60})_2^{-\bullet}$ and $(C_{60})_2^{2-}$. **A** has a long covalent bond of 1.6 to 1.7 Å, whereas **B** features a shortened van der Waals contact distance of approximately 2.6 Å between the fullerenes

(the van der Waals radius of carbon is 1.7 Å). Surprisingly the non-bonded structure **B** is found to be more stable than the covalently bonded isomer **A** (by up to 11.3 kcal mol^{-1} for the radical anion and 4.8 kcal mol^{-1} for the singlet dianions). The D3-dispersion correction lowers these values (stabilizes the dimers) by approximately 5 kcal mol^{-1}.

Table 2.2 Relative energies (E_{Rel}, kcal mol^{-1}) of C_{60} oligomers 1-5, the corresponding anions and dianions, relevant Electron Affinities (EA, eV) and reaction energies (ΔE_R, kcal mol^{-1}) calculated at the RI-BP86/TZV level of theory.

Species	E_{Rel}	EA	Dissociation	
			Reaction	ΔE_R
C_{60}		2.82		
B	0.0 (0.0)	3.24	$\rightarrow 2C_{60}$	+11.9
C	+1.2 (–5.4)	3.25	$\rightarrow 2C_{60}$	+17.3
D		3.49	$\rightarrow 3C_{60}$	+34.2
E		3.62	$\rightarrow 4C_{60}$	+66.9
$C_{60}^{-\cdot}$		–0.21		
$A^{-\cdot}$	+11.3 (+6.0)	2.98	$\rightarrow C_{60}+C_{60}^{-\cdot}$	+15.8
$B^{-\cdot}$	0.0 (0.0)	1.11	$\rightarrow C_{60}+C_{60}^{-\cdot}$	+21.7
$C^{-\cdot}$	+1.53 (–5.6)	1.03	$\rightarrow C_{60}+C_{60}^{-\cdot}$	+27.3
$D^{-\cdot}$		1.58	$\rightarrow 2C_{60}+C_{60}^{-\cdot}$	+49.7
$E^{-\cdot}$		1.86	$\rightarrow 3C_{60}+C_{60}^{-\cdot}$	+85.6

Because this result could easily be caused by the self-interaction error in DFT, Prof. T. Clark *et al*. also calculated the energies of $\mathbf{A}^{-\bullet}$ and $\mathbf{B}^{-\bullet}$ using increasing amounts (0 to 50%) of Hartree-Fock exchange in a modified hybrid B3LYP functional[133,134] and performed additional calculations using the long-range corrected CAM-B3LYP[135] functional. The results are shown in Figure 2.6.

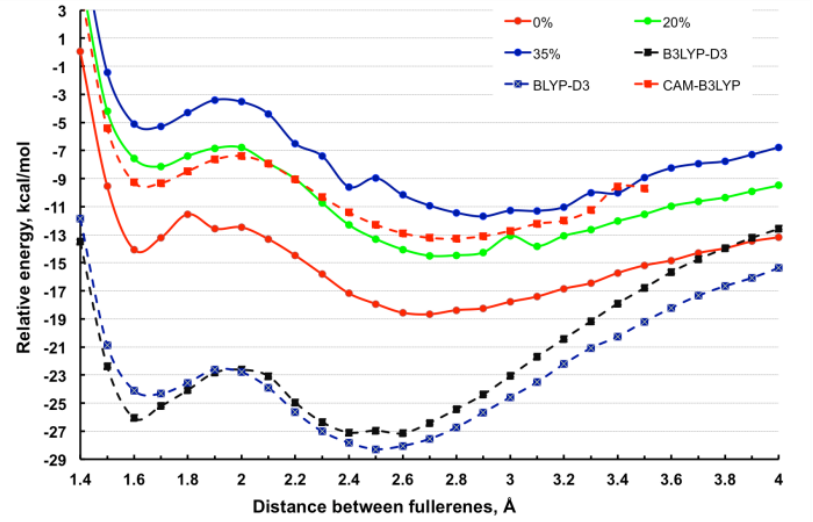


Figure 2.6. Calculated potential energy diagrams for the dissociation of $(C_{60})_2^{-\bullet}$.

The data shown in Table 2.2 and Figure 2.6 demonstrate that the van der Waals dimer radical anion and dianion are probably more stable than the covalently bound isomer **B** investigated previously or comparable to the [2+2] adduct **C** in the same oxidation states. These values lie between those obtained at the RI-BP86-D3/TZV level (–15.8 and –21.7 kcal mol^{-1}, for $\mathbf{A}^{-\bullet}$ and $\mathbf{B}^{-\bullet}$, respectively). All calculations find the deepest minimum to be 2 kcal mol^{-1}, with a significantly shortened van der Waals contact (between 2.4 and 2.9 Å). Thus, the range of DFT functionals tested agrees that $\mathbf{B}^{-\bullet}$ is more stable than $\mathbf{A}^{-\bullet}$. HF-TPSS and HF-PBE calculations with the def2-SVP basis set also predict $\mathbf{B}^{-\bullet}$ to be more stable than $\mathbf{A}^{-\bullet}$ by 15 kcal mol^{-1}, with a barrier of ca. 3 kcal mol^{-1} between them.

They also performed CASSCF[136]/CASPT2[137,138](5,4) calculations on DFT-optimized geometries to verify the DFT and HF-DFT results (Figure 2.7). CASPT2(5,4) calculations also show that $\mathbf{A}^{-\bullet}$ and $\mathbf{B}^{-\bullet}$ are close in energy, with a barrier of 6.2 kcal mol^{-1} between them. Unfortunately, the size of the system limits calculations to the CASPT2(5,4)/ANO-L-VDZ level.[139,140]

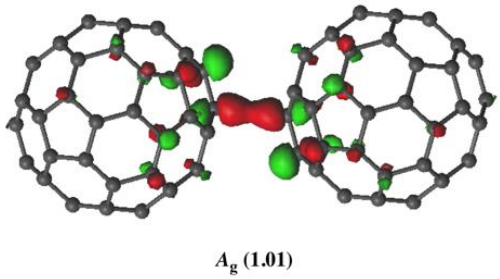
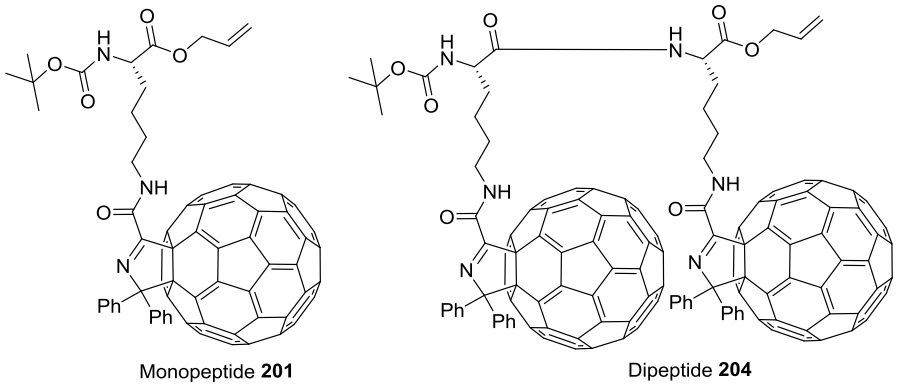


Figure 2.7. CAS (5,4) formally singly occupied orbital of $\mathbf{B}^{-\bullet}$. The calculated occupancy is given in parentheses. This orbital corresponds to a one-electron σ-bond between the two fullerenes.

The calculated IR and UV-Vis spectra suggest that $\mathbf{A}^{-\bullet}$ and $\mathbf{B}^{-\bullet}$ can be distinguished easily. Structure $\mathbf{2}^{-\bullet}$ is characterized by very intense absorptions at 1430-1486 and 1600 cm^{-1} that are far weaker in the separated monomers and $\mathbf{A}^{-\cdot}$. Once again, the van der Waals dimer radical anion $\mathbf{B}^{-\bullet}$ is clearly distinguished by a strong absorption in the infrared at 1760 nm (5672 cm^{-1}). This absorption is far more intense than that found at slightly longer wavelength (1848 nm) for $\mathbf{A}^{-\bullet}$, which also exhibits a second weaker absorption at 1441 nm. Separated fullerene and its radical anion do not absorb in this region.

To investigate the proposed effect experimentally, they performed cyclic voltammetry and spectroelectrochemical experiments on the fullerene-substituted peptides **201** and **204**. Both the monomer **201** and dimer **204** are soluble in chloroform, chlorobenzene and ortho dichlorobenzene. Monomer **201** is more soluble in these organic solvents than the dimer **204** possibly because of the absence of interactions between the fullerene balls, whereas this effect is more possible dimer **204**. These two compounds offer the opportunity to observe and compare a system in which interactions between non-bonded fullerenes are possible (**204**) with one in which they are not (**201**). The redox properties of the mono- and dipeptides were studied by means of cyclic voltammetry (CV). The measurements were performed at room temperature in chlorobenzene with

tetrabutylammonium hexafluorophosphate as supporting electrolyte. Chlorobenzene, an aprotic solvent, was chosen to avoid protonation of the charged species. Figure 2.8 shows the reductive scans between -0.2 to -1.6 V of **201** and **204**. They compared the spectra obtained upon fast and slow scanning. Both compounds show a reduction, which is assigned to the one-electron reduction of C_{60}. At fast scan rates (0.2 Vs^{-1}), the reduction appeared to be quasi-reversible and occured at nearly the same potential for both **201** and **204**. In contrast, slowing down the scan rate to 0.005 Vs^{-1} lead to an irreversible reduction of **204**, while **201** remains reversible. Moreover, at slower scan rates **204** was more easily reduced than **201**. The change to an irreversible reduction of **204** at slower scan rates is consistent with the formation of a van der Waals dimer of the fullerenes in **204**.



Monopeptide **201** Dipeptide **204**

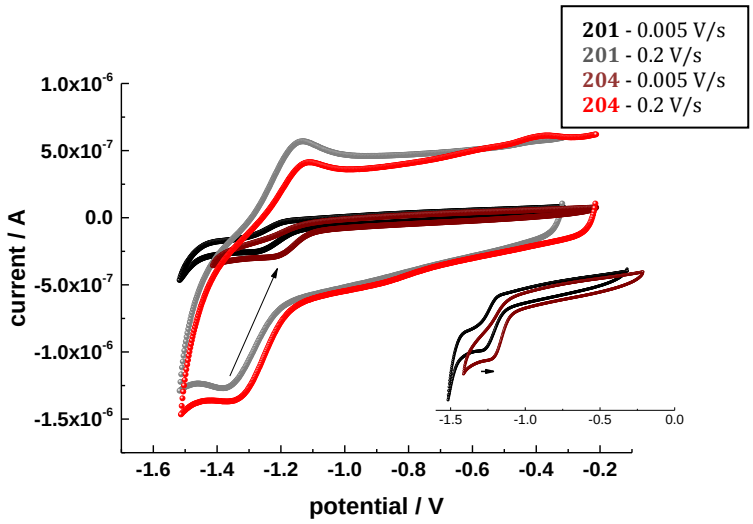


Figure 2.8. Cyclic voltammograms of **201** and **204** with a scan rate of 0.0050 Vs^{-1} and 0.200 Vs^{-1}. Measurements were performed in chlorobenzene containing 0.05 M $TBAPF_6$ as supporting electrolyte with a glassy carbon working electrode, a platinum counter electrode, and a silver pseudo-reference electrode. Corrected for ferrocene as an internal standard.

With this information in hand, they carried out spectroelectrochemical experiments to gather information about the absorption features of the reduced species. A differential absorption spectra for **204** in chlorobenzene solution is shown in Figure 2.9. The corresponding spectrum for **201** shows the features known from the literature for *N*-methylfulleropyrrolidine.[141] In particular, a sharp peak with a 1028 nm maximum is observed for the one electron reduced C_{60}. In contrast, the differential spectrum of **204** is characterized by a broad absorption between 700 and 1200 nm and with a local maximum at 860 nm. These spectral attributes are assigned to a van der Waals dimer of **204** and are in sound agreement with the calculated spectrum shown for the relevant conformation of **204**$^{\bullet-}$.

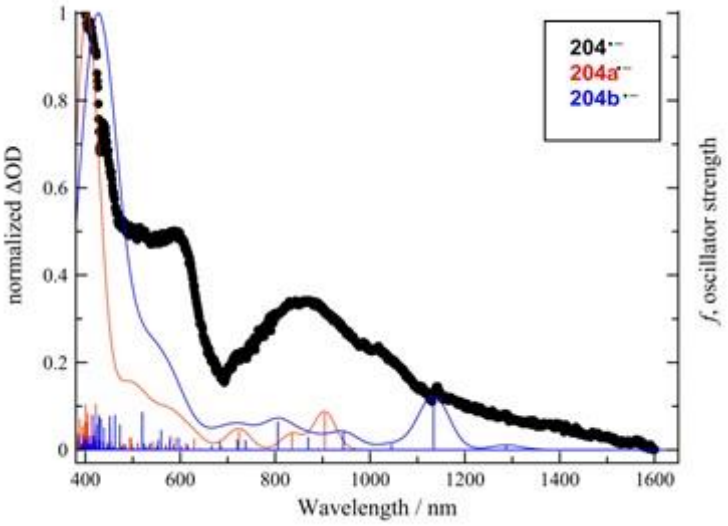
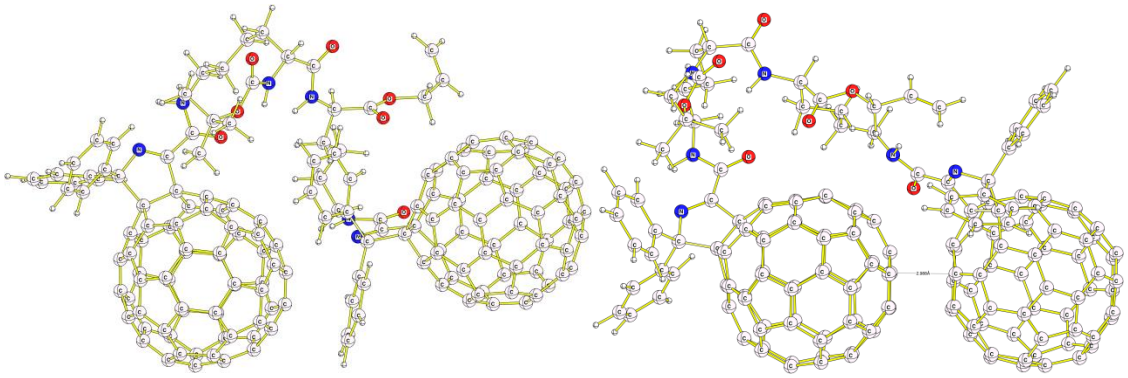


Figure 2.9. Normalized differential absorption spectrum of **204** with an applied voltage of −0.85 V. Measurements were performed in chlorobenzene containing 0.05 M TBAPF6 as supporting electrolyte with a platinum gauze as working electrode, a platinum plate as counter electrode, and a silver wire as pseudoreference electrode. Calculated spectra for **204a**•− and **204b**•− (**204a**•− has separated fullerenes, whereas **204b**•− has fullerenes in van der Waals contact) shown in red and blue, respectively.



204a•− **204b•−**

The calculations presented are on a system for which it is difficult to obtain definitive results because of its size and the electronic characteristics. However, standard functionals with and without dispersion corrections, HF-DFT calculations, modified functionals with increasing amounts of HF-exchange and long-range corrected functionals all gave consistent results that indicate that fullerene can function as a "super-atom" that forms a one-electron intermolecular bond, resulting in an electron trap that is even more stable than the isolated fullerene, which is itself an unusually strong electron acceptor.

These experimental results were all consistent with **204** existing as at least two conformations in solution, with a minority conformation having a van der Waals contact between the two fullerenes (e.g. **204b**). At slow CV scan rates, **204b** can be formed by rearrangement and stabilized by the inter-fullerene one-electron bond. At fast scan rates, only the majority conformations without inter-fullerene contact are observed. The observed spectrum of $\mathbf{204}^{-\cdot}$ corresponds to a superposition of those of the majority non-contact conformation and $\mathbf{204b}^{-\cdot}$ or a similar conformation.

Although definitive calculations are not yet possible, they used standard density-functional theory to calculate the electron affinities of fullerene van der Waals

oligomers. These results are shown in Figure 2.10. The first electron affinity increases from 2.7 eV for the monomer to 3.6 eV for the tetramer, which exhibits a second electron affinity of almost 2 eV. The tetramer is also calculated to just be able to bind a third electron. Van der Waals fullerene oligomers can thus exhibit first electron affinities as much as one electron volt more strongly binding than the isolated fullerene and second electron affinities up to approximately ~2 eV. These interstitial electron traps represent an unexpected feature of non-covalently bonded aggregates of fullerenes that can affect the performance of electronic devices strongly. Deep electron traps can be expected in amorphous and crystalline fullerene layers or in self-assembled monolayers of fullerene-substituted organic molecules, all of which are often used as n-type semiconductor components in organic electronics.[142]

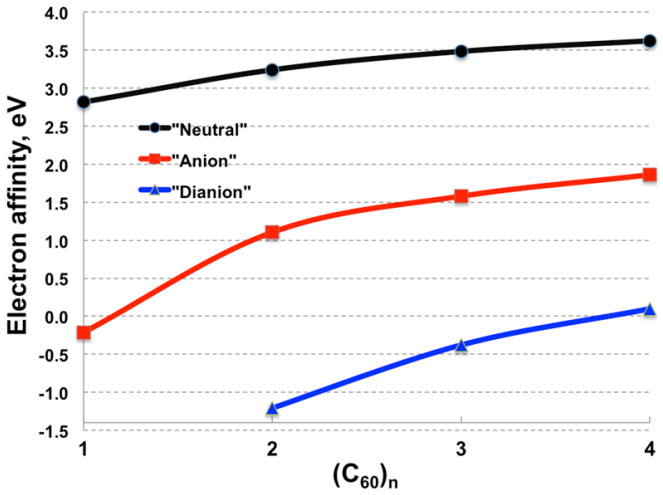


Figure 2.10. Calculated (RI-BP/TZV) electron affinities (eV) of $(C_{60})_n$.

2.2.16 Future directions and conclusions

The synthesis of [*60*]fullerenoproline monopeptide **201**, dipeptide **204** and a lysine tetrapeptide **216** were achieved for the first time and were successfully characterised.

Unfortunately, the tetrapeptides **207** and **220** which were thought to be prepared, could not be fully characterised due to their poor solubilities.

Because of steric hindrance problems in some of our peptide coupling reactions, it was thought that the inclusion of a spacer between two fullerenyl units could be better option. Hence, a new strategy was adopted towards the synthesis of a different hexa and dodecapeptide oligomer (Chapter 3).

The [*60*]fullerene amino acid monomer **201** and the bis-fullerene **204** were studied experimentally using spectroelectrochemical measurements. The bis-fullerene-substituted peptide **204** provided experimental support for density-functional theory (DFT) calculations which indicate that van der Waals fullerene dimers can form between adjacent fullerenes in semiconductor layers resulting in interstitial electron traps. The fullerenes behave like "super atoms" and the interstitial electron traps represent one-electron intermolecular σ-bonds. The proposed electron traps are relevant for all organic electronics applications in which non-covalently linked fullerenes in van der Waals contact with one another are used as n-type semiconductors.[142]

CHAPTER 3

The Synthesis of [*60*]Fullerenyl Amino Acids Oligomers With Spacers

3.1 Material Science Applications as Solar Cells

With the aim of resolving the identified steric hindrance problems in Chapter 2, it was thought that the inclusion of a spacer between two fullerenyl units could be a better option (Figure 3.1 and 3.2). Hence, a new strategy was adopted towards the synthesis of two different hexa and dodecapeptide oligomers (Scheme 3.1 and 3.4).

The aim of this chapter was to synthesise two different combinations of [*60*]fullerenyl based oligomers **223** (Figure 3.1) and **230** (Figure 3.2) having spacers (e.g. lysine and phenylalanine) in between the fullerenyl units. The first target dodecapeptide **223**, which could be prepared from the peptide coupling reaction of hexapeptide acid **228** and hexapeptide amine **229** is shown in Scheme 3.1. Similarly, the second target dodecapeptide **230** (Figure 3.2) could be prepared from the acid and amine precursors of the hexapeptide **239** and **240** under peptide coupling conditions mentioned in section 2.2.2.

These targeted dodecapeptides **223** and **230** showing every fourth lysine unit bearing [*60*]fullerene on the peptide. Beyond a certain degree of oligomerisation the development of a secondary structure, such as a 3_{10} helix, may be observed (Figure 3.1 and 3.2). This would force the fullerenes to arrange in a defined spatial arrangement as shown in Figure 3.1 and 3.2. These fullerenyl peptides can be studied for the accommodation of guests like porphyrin rings in between two helices. Preparation of

supramolecular complexes using these fullerene-peptide oligomers as hosts and porphyrins as guest molecules could be potentially useful as organic photovoltaic cells (as shown in Figure 3.1 and 3.2).

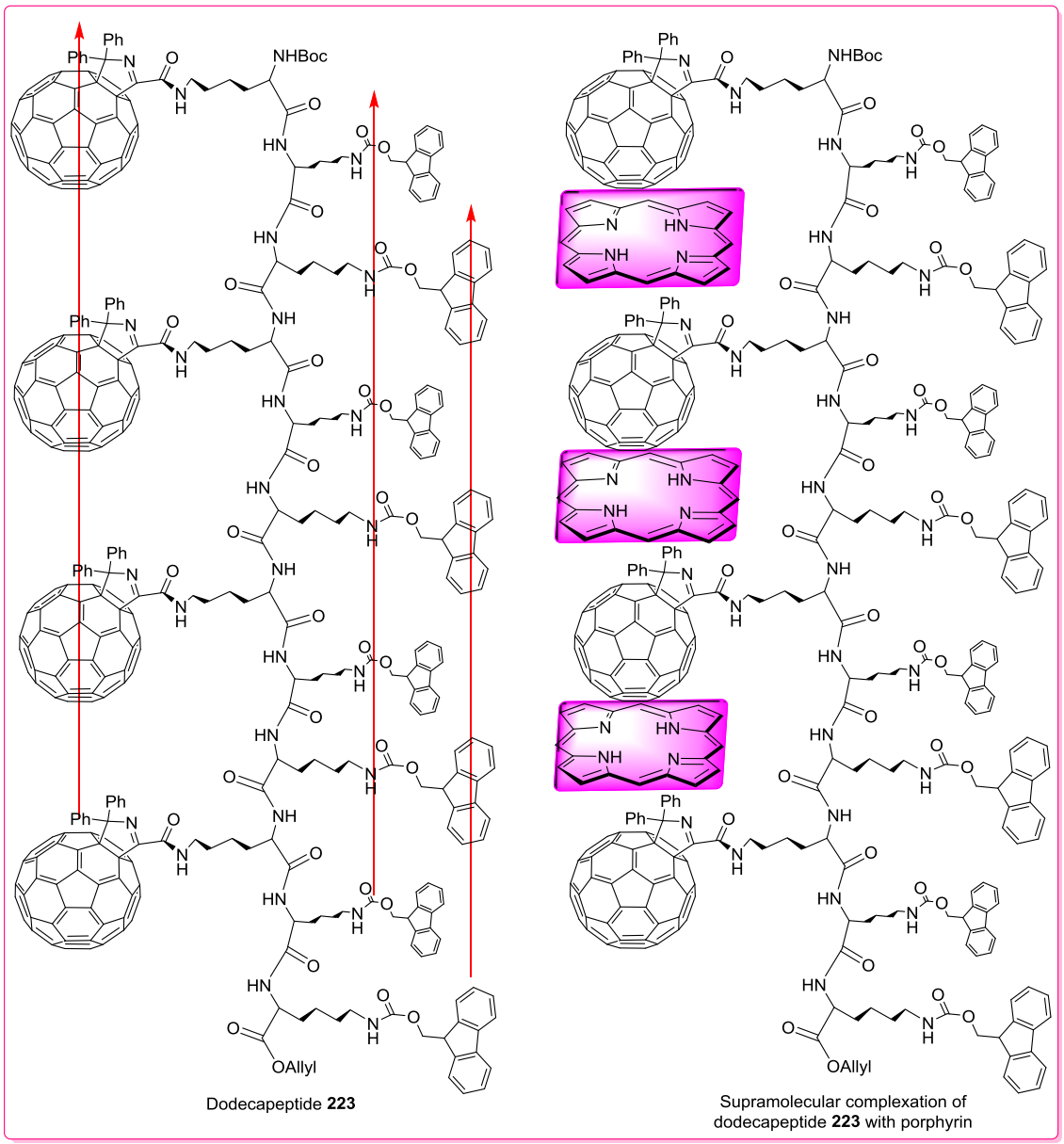
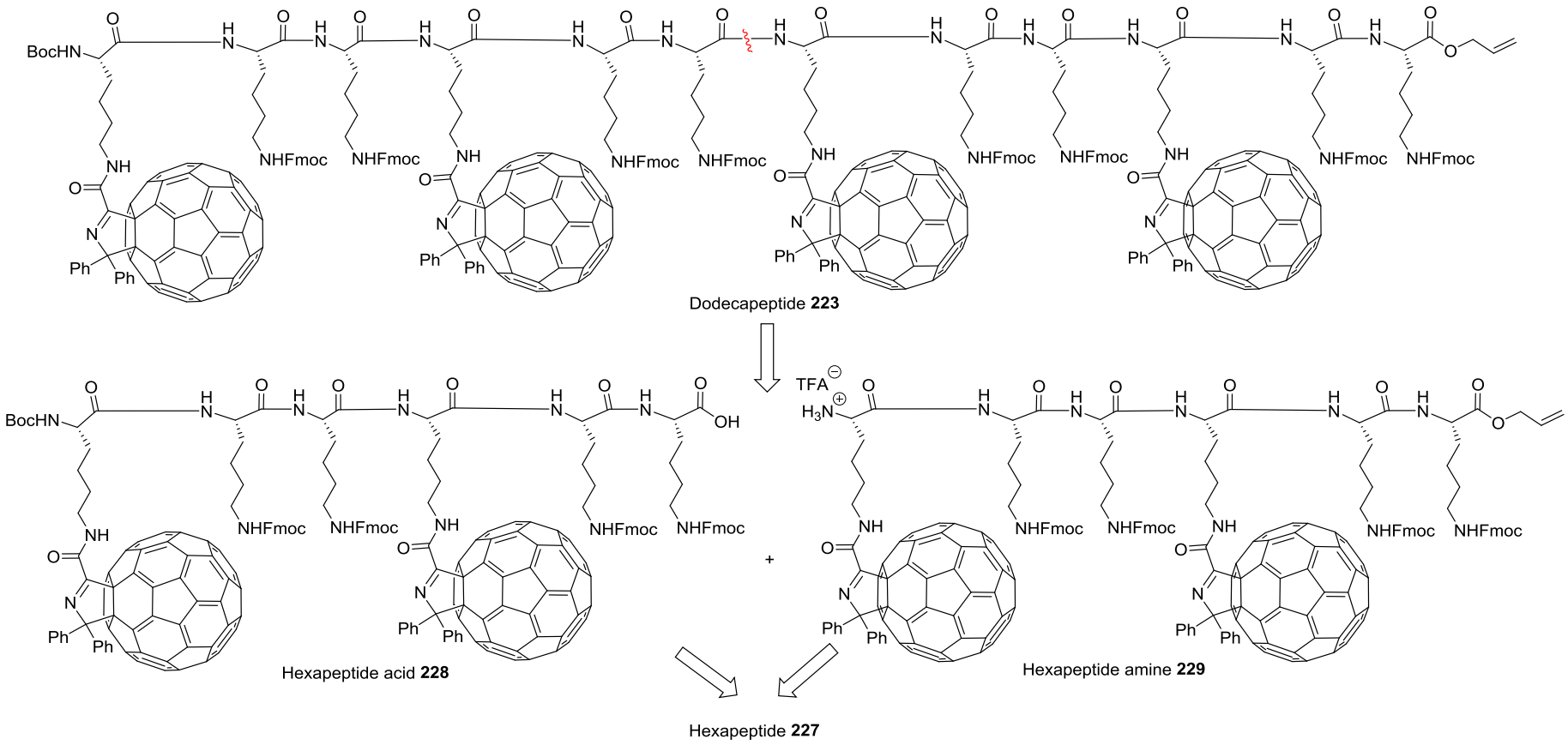


Figure 3.1. Potential structure of a fullerene-peptide system for use in organic solar cells.

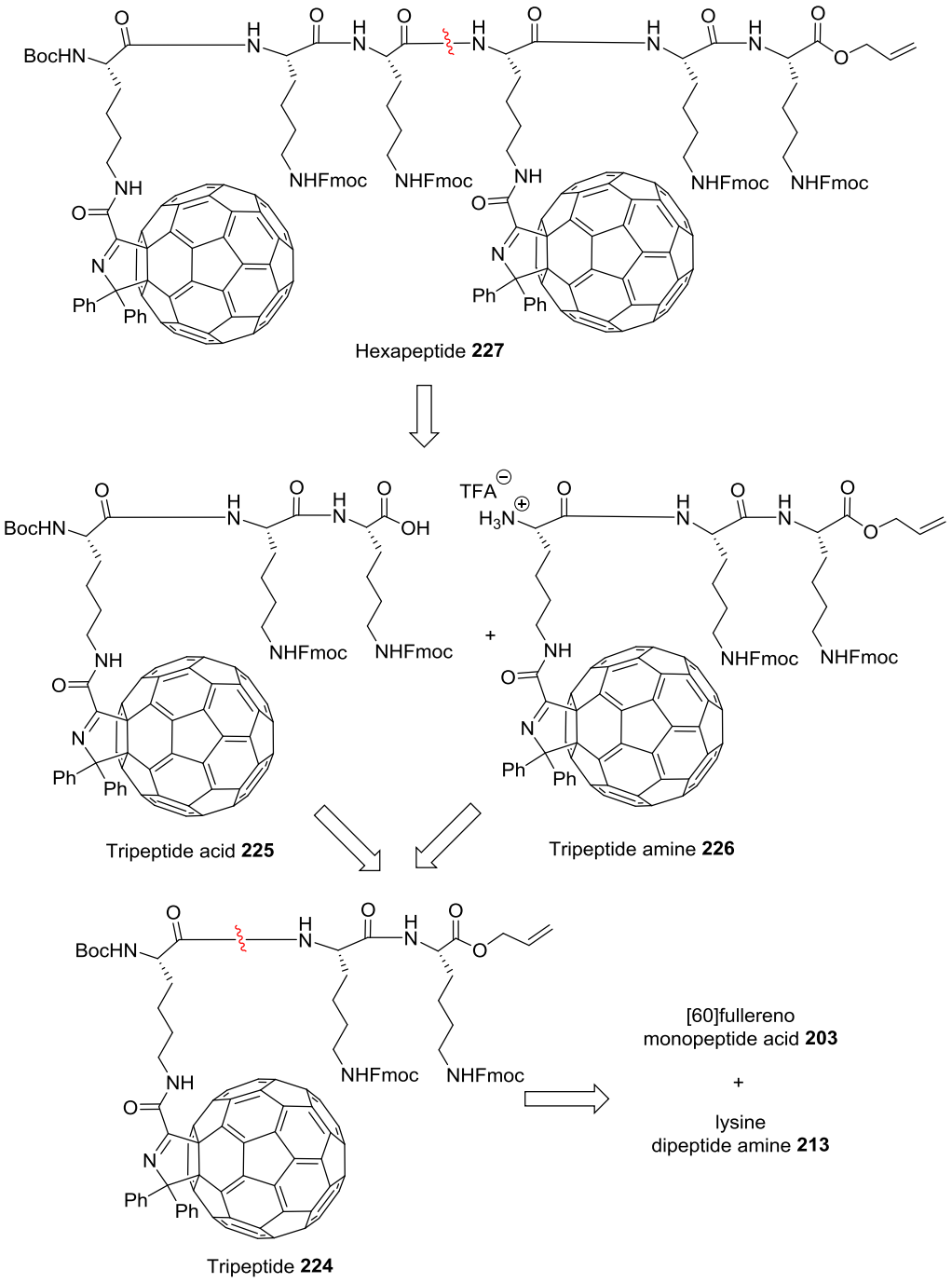
3.2 Target 1: Synthesis of [*60*]fullerenolysine dodecapeptide 223, by the multiplication of coupling reactions.

3.2.1 Proposed synthetic strategy for the [*60*]fullerenolysine dodecapeptide 223.

The retro-synthesis of the [*60*]fullerenolysine dodecapeptide **223**, starting from the previously synthesised [*60*]fullerenomonopeptide acid **203**, is outlined in Scheme 3.1. A key step was the coupling of the acid **203** to the lysine dipeptide amine **213**, to give the crucial tripeptide **224**. The tripeptide **224**, could then be deprotected to its acid derivative **225** and amine derivative **226** and these could then be coupled under standard peptide coupling reactions to yield the hexapeptide **227**. By repeating the deprotection and coupling reaction protocol the targeted dodecapeptide **223** could then be potentially realised (Scheme 3.1).



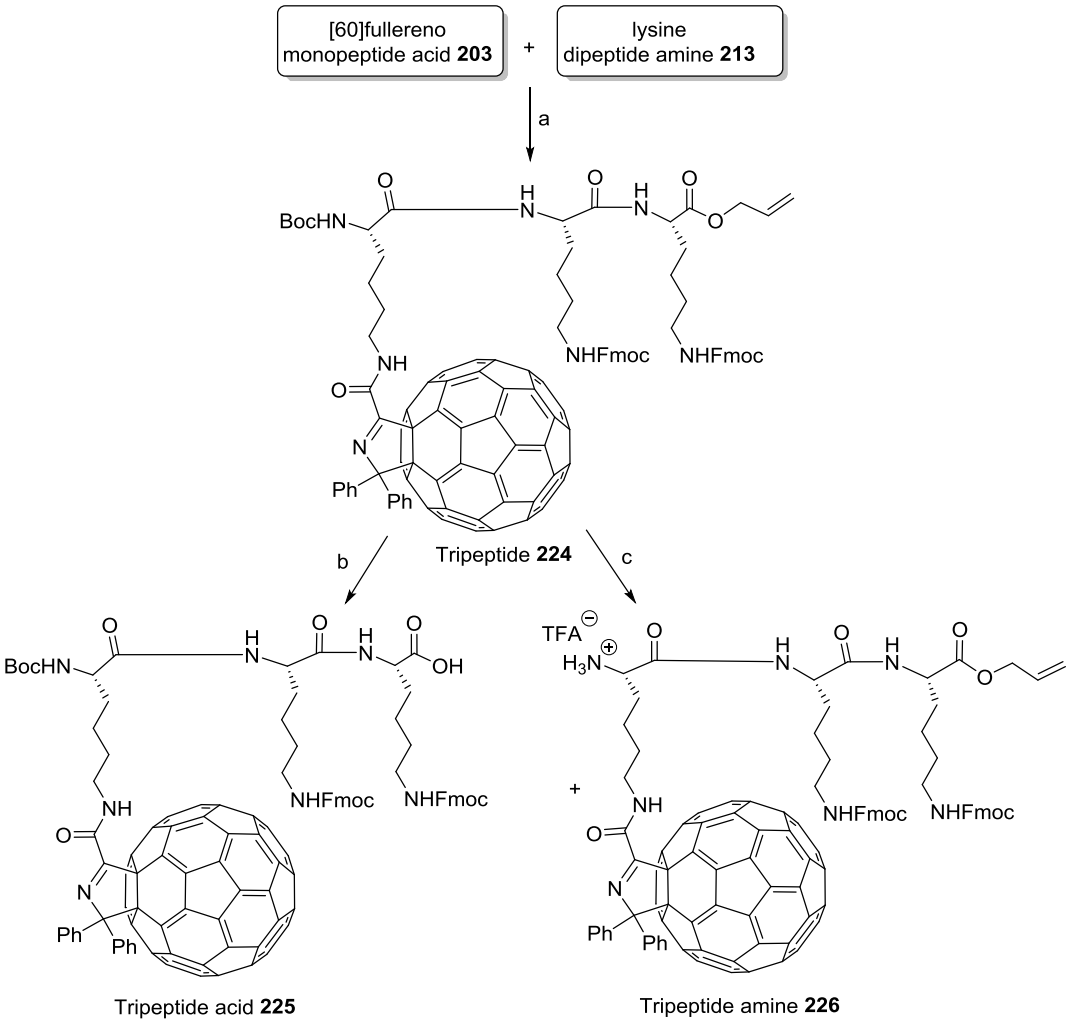
Scheme 3.1 Retrosynthetic analysis for the synthesis of [*60*]fullerenolysine dodecapeptide **223** from the hexapeptide **227**.



Scheme 3.1 continued. Retrosynthetic analysis for the synthesis of [*60*]fullerenolysine hexapeptide **227** from the previously synthesised lysine dipeptide amine **213**, and [*60*]fullerenomonopeptide acid **203.**

3.2.2 Synthesis of tripeptide 224 from acid 203 and amine 213, followed by deprotection to its acid 225 and the amine salt 226

The synthesis of the tripeptide **224** (Scheme 3.2) was realized in 62% yield by stirring in dichloromethane, a solution of previously synthesised lysine dipeptide amine **213** and the [*60*]fullerenomonopeptide acid **203** under standard coupling conditions described in Section 2.2.2. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired tripeptide **224** in 62% yield (Scheme 3.2). Subsequent deprotection reactions of tripeptide **224** yielded the tripeptide acid **225** (in 77%), and the tripeptide amine **226** (in 89%), using the reaction conditions adopted from Section 2.2.3 (Scheme 3.2).



Scheme 3.2: Synthesis of a tripeptide **224**, tripeptide acid **225** and tripeptide amine **226**. **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 62%; (b) 1,2-DCE, $(CH_3)_3SnOH$, 80 ^{0}C, 6 h, 77%; (c) TFA: CH_2Cl_2 (1:1), rt, 4 h, 89%.

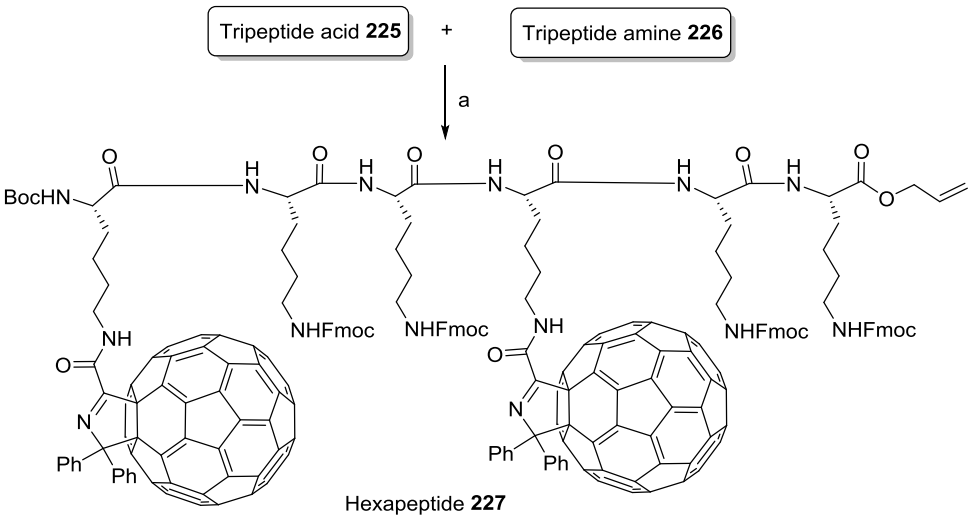
The ^{1}H NMR spectrum of tripeptide **224** showed the prominent allyl ester resonances at δ 4.37-4.43 (m, 2H), 5.22-5.26 (m, 2H), and 5.82-5.88 (m, 1H), that were present in the spectrum of amine **213** and the Boc methyl resonance (δ 1.40, s, 9H), which was present in the spectrum of acid **203**. Additionally, an amidic proton resonance also appeared at δ 6.74-6.82 (m, 1H, NH) which was associated with the newly formed amide bond. Furthermore, the molecular formula of product **224** was verified by the appearance of the peak at *m*/*z* 1948.5831 in the HRMS, that was assigned to the sodiated molecular ion ($[M+Na]^+$ $C_{131}H_{79}N_7O_{11}Na$).

The deallylation of tripeptide **224** to its acid **225** was supported by the loss of resonances at δ 4.37-4.43 (m, 2H), 5.22-5.26 (m, 2H), and 5.82-5.88 (m, 1H), in the ^{1}H NMR spectrum, that were present in the tripeptide **224**, which were assigned to the allyl protons. The molecular formula of product **225** was verified by the appearance of the peak at *m*/*z* 1909.5359 in the HRMS, that was assigned to the sodiated molecular ion ($[M+Na]^{+}$ $C_{128}H_{75}N_7O_{11}Na$).

Tripeptide amine salt **226** showed the disappearance of a prominent Boc group resonance (δ 1.40, s, 9H) that was present in the ^{1}H NMR spectrum of tripeptide **224**. The molecular formula of product **226** was verified by the appearance of the peak at *m*/*z* 1826.5789 in the HRMS, that was assigned to the protonated molecular ion ($[M+H]^{+}$ $C_{126}H_{71}N_7O_9$).

3.2.3 Coupling of acid 225 and amine 226 to the hexapeptide 227

The general synthetic procedure stated in Section 2.2.2 was employed to synthesise the hexapeptide **227**. The synthesis of the hexapeptide **227** (Scheme 3.3) was realized from the coupling reaction of tripeptide acid **225** and the tripeptide amine **226**. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired hexapeptide **227** in 44% yield (Scheme 3.3).



Scheme 3.3: Synthesis of a hexapeptide **227**. **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 44%.

The ^{1}H NMR spectrum of the hexapeptide **227** showed the prominent allyl ester resonances [at δ 4.50-4.62 (m, 2H), 5.20-5.22 (m, 2H), and 5.81-5.86 (m, 1H)], that were present in the ^{1}H NMR spectrum of amine **226** and the Boc resonance (δ 1.39, s, 9H), that was present in the ^{1}H NMR spectrum of acid **225**. Additionally, an amidic proton resonance also appeared at δ 6.83-6.84 (m, 1H, NH) which was associated with the newly formed amide bond. This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum and not sufficiently soluble in MeOH to generate an adequate ESI-MS.

This strategy was not pursued any further because of the difficulty of characterising compounds due to their poor solubilities and the relatively low chemical yields.

3.3 Target 2: Synthesis of [*60*]fullerenolysine phenylalanine dodecapeptide 230, by the multiplication of coupling reactions.

3.3.1 Proposed synthetic strategy for the [*60*]fullerenolysine phenylalanine dodecapeptide 230.

The retro-synthesis of the [*60*]fullerenolysine dodecapeptide **230**, starting from the previously synthesised [*60*]fullerenomonopeptide amine **202**, is outlined in Scheme 3.4. A key step was the coupling of the amine **202** to the L-phenylalanine dipeptide acid **234**, to give the crucial tripeptide **235**. The tripeptide **235**, could then be deprotected to its acid derivative **236** and amine derivative **237** and these could then be coupled under standard peptide coupling reactions to yield the hexapeptide **238**. By repeating the deprotection and coupling reaction protocol the targeted dodecapeptide **230** could then be realised (Scheme 3.4).

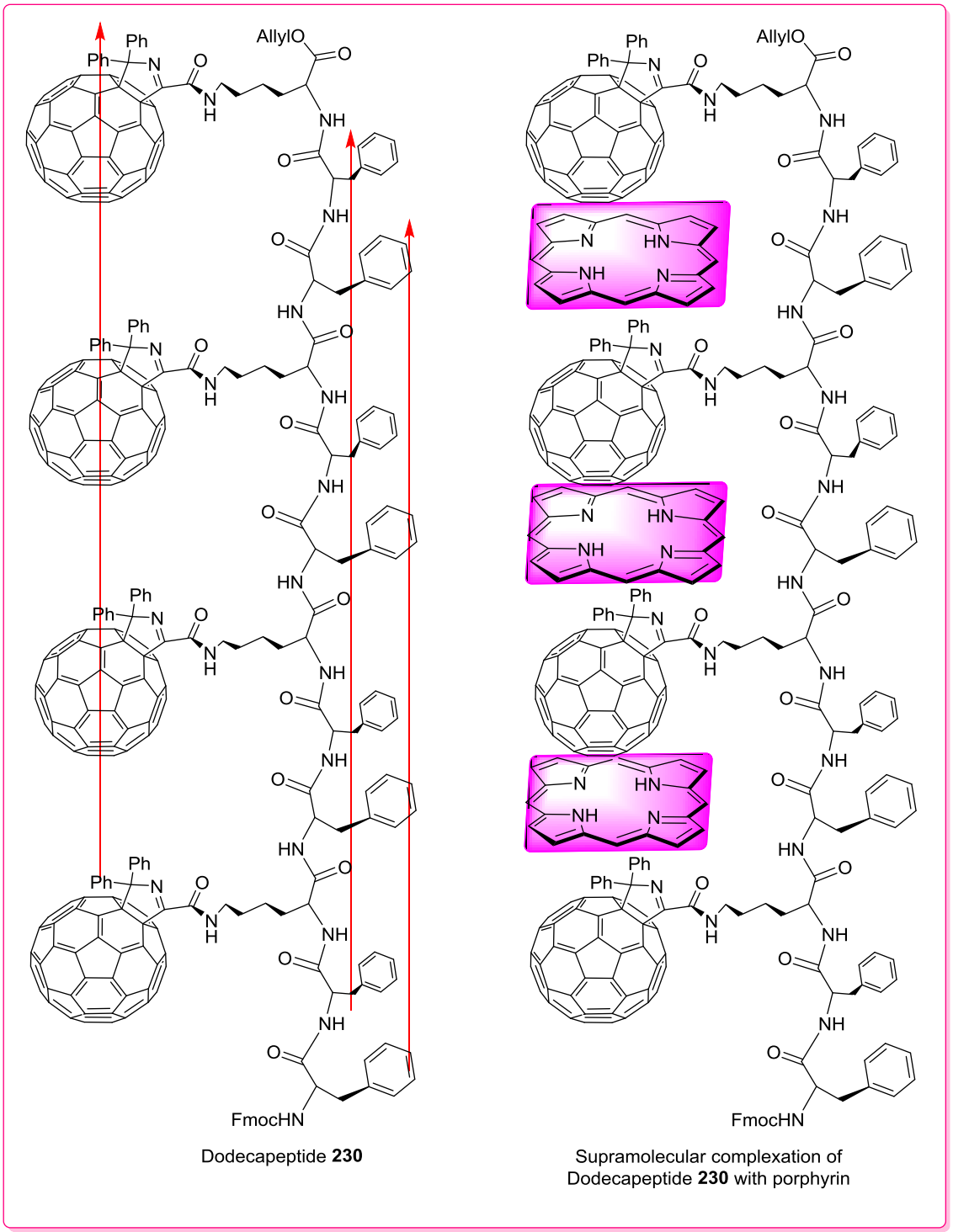
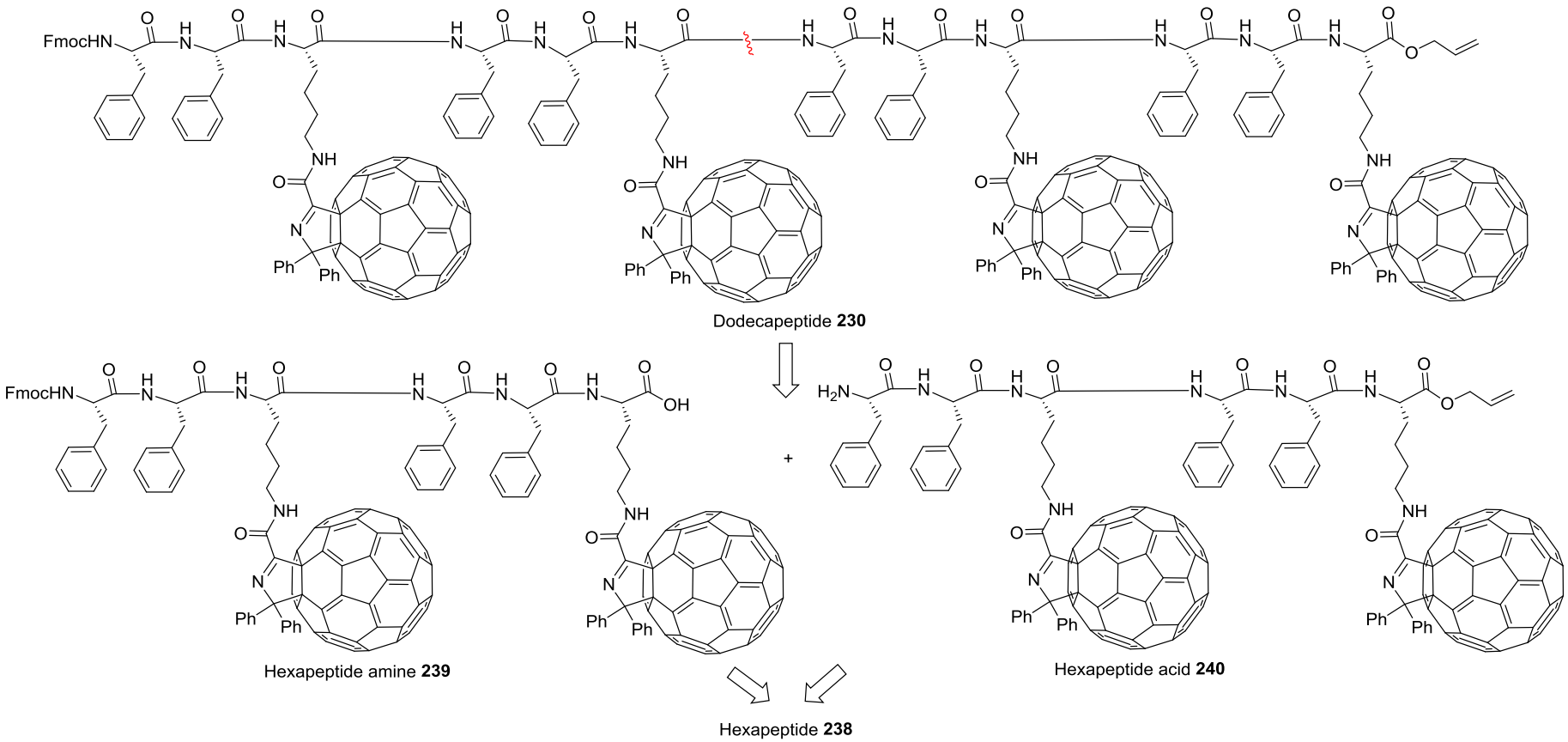
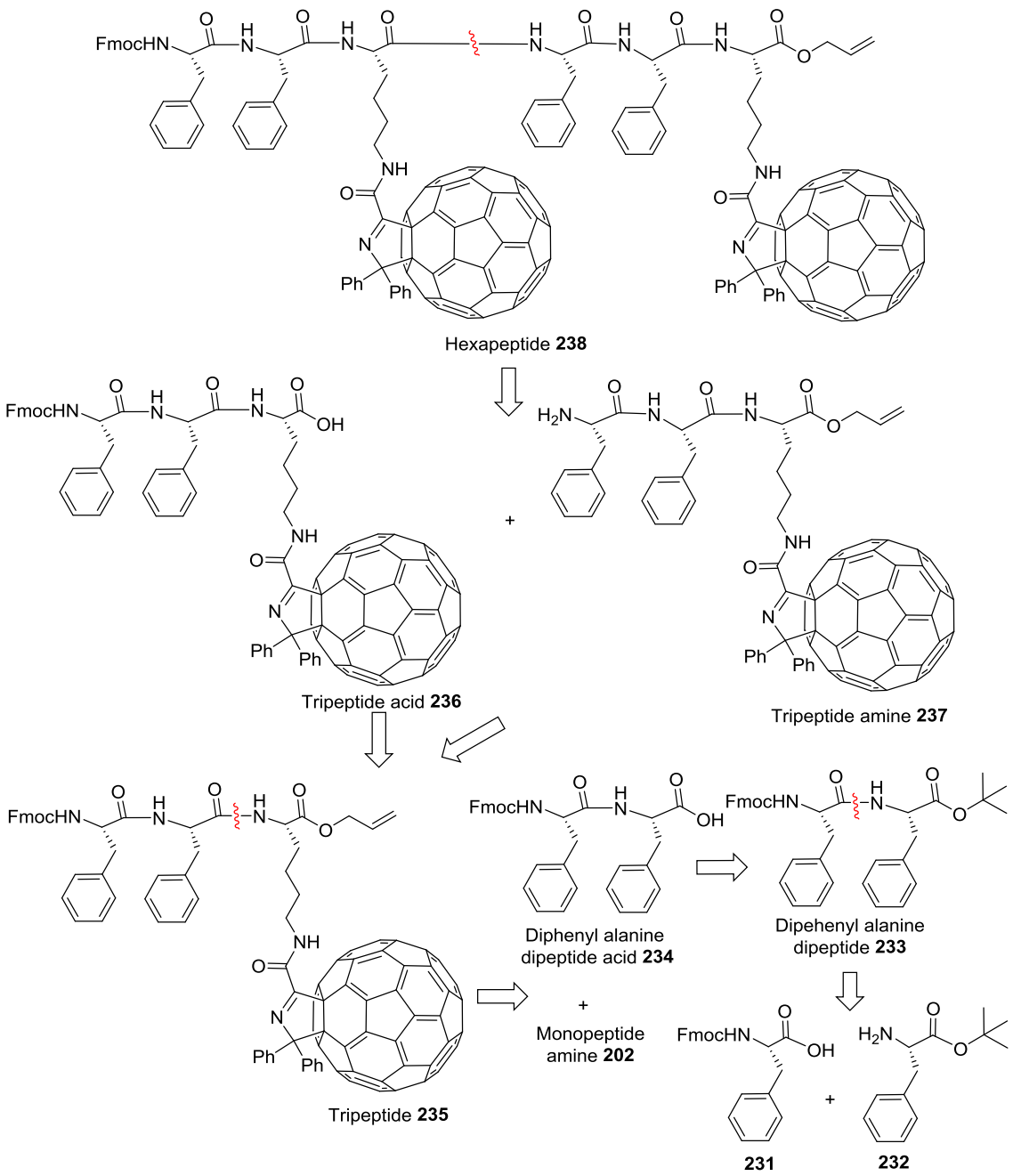


Figure 3.2. Potential structure of a amino acid/porphyrinic-peptide system for use in organic solar cells.



Scheme 3.4 Retrosynthetic analysis for the synthesis of [*60*]fullerenolysine phenylalanine dodecapeptide **230** from the hexapeptide **238**.



Scheme 3.4 continued. Retrosynthetic analysis for the synthesis of [*60*]fullerenolysine phenylalanine hexapeptide **238** from previously synthesised dipehnylalanine dipeptide acid **234**, and [*60*]fullerenomonopeptide amine **202.**

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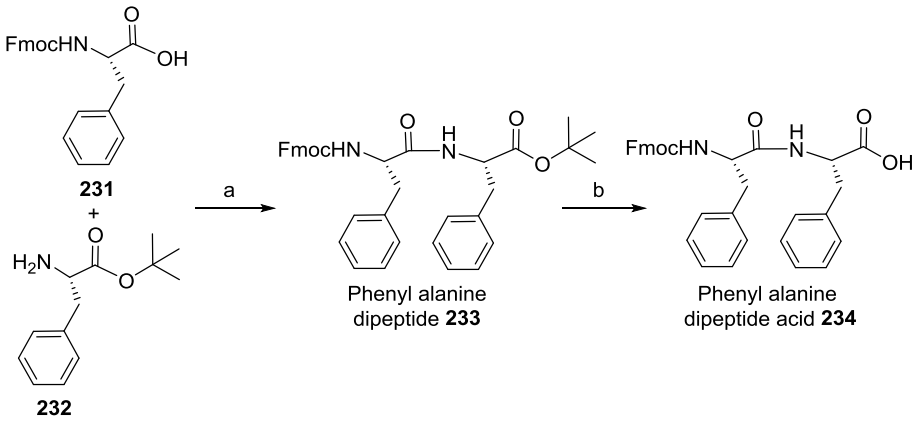
3.3.2 Amide coupling of acid 231 and amine 232 to synthesize a dipeptide 233, followed by ester hydrolysis to its acid 234

The synthesis of the phenylalanine dipeptide[143] **233** (Scheme 3.5) was realized from the peptide coupling reaction of commercially available phenylalanine acid **231** and the amine **232**. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired tripeptide **233** in 92% yield (Scheme 3.5). Subsequent removal of *tert*-butyl group of dipeptide **233** yielded the dipeptide acid **234** (in 90%) using the reaction conditions adopted from Section 2.2.3.



Scheme 3.5: **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 62%; (b) TFA: CH_2Cl_2 (1:1), rt, 4 h, 90%.

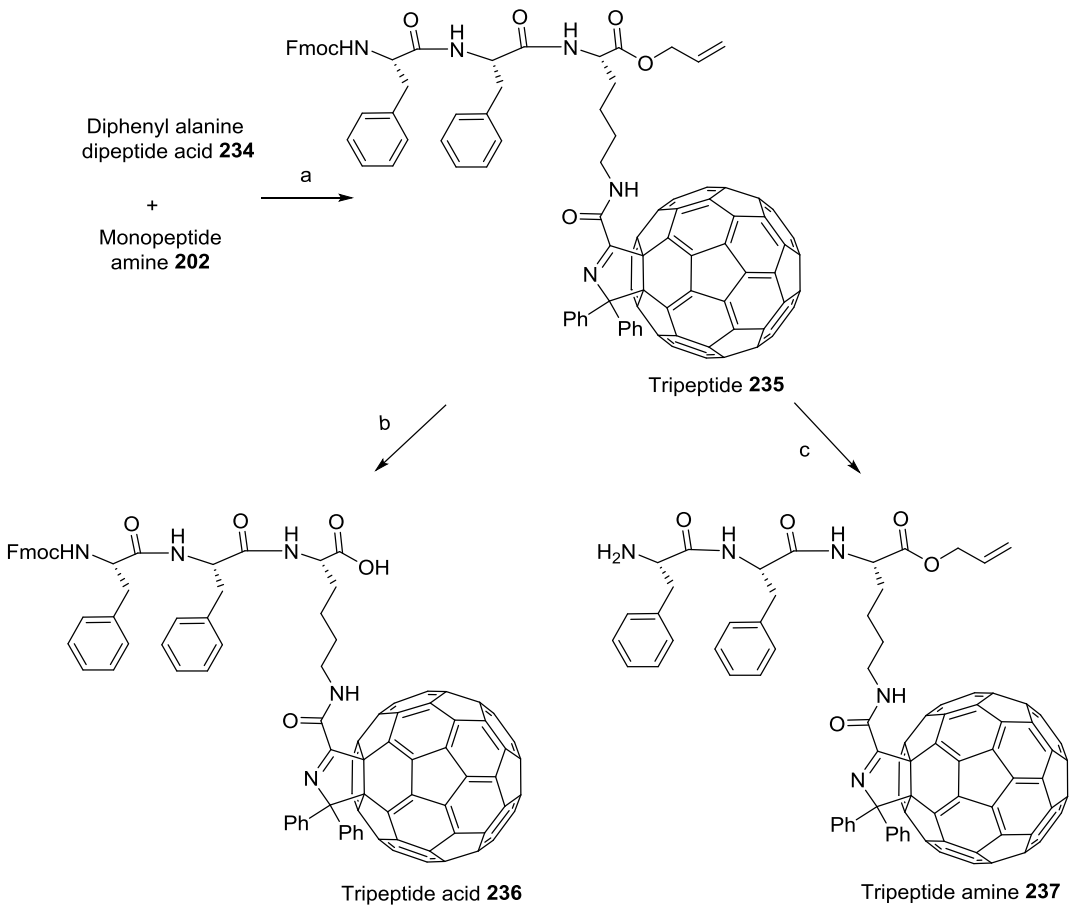
The ^{1}H NMR spectrum of dipeptide **233** showed the prominent *tert*-butyl ester resonance at δ 1.36 (s, 9H), that was present in the ^{1}H NMR spectrum of amine **232** and the Fmoc protecting group methine and methylene resonances at δ 4.28 (bs, 1H, CH), 4.40-4.43 (m, 2H, CH_2), that were present in the ^{1}H NMR spectrum of acid **231**. Additionally, an amidic proton resonance was evident at δ 6.31 (bs, 1H, NH) that was associated with the newly formed amide bond. Additionally, the ^{13}C NMR spectrum of **233** showed resonances at δ 155.9, assigned to the Fmoc carbonyl, and δ 170.2, that was assigned to the ester carbonyl. This indicated the presence of carbonyl resonances from both the reactants. Furthermore, the molecular structure of the product **233** was verified by the appearance of the peak at *m*/*z*

591.2865 in the HRMS, that was assigned to the protonated molecular ion ($[M+H]^+$ $C_{37}H_{38}N_2O_5$).

The ester hydrolysis of dipeptide **233** to its acid **234** was supported by the loss of the *tert*-butyl ^1H NMR resonance at δ 1.36 (s, 9H) that were present in the dipeptide **233**. The molecular structure of the product **234** was verified by the appearance of the peak at *m*/*z* 535.2238 in the HRMS, that was assigned to the protonated molecular ion ($[M+H]^+$ $C_{33}H_{30}N_2O_5$).

3.3.3 Amide coupling of acid 234 and amine 202 to synthesize a tripeptide 235, followed by ester hydrolysis to its acid 236 and Fmoc removal to the amine 237

The synthesis of the [*60*]fullerenyl tripeptide **235** (Scheme 3.6) was realized from the peptide coupling reaction of the previously synthesised phenylalanine dipeptide acid **234** and the monopeptide amine **202**. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired tripeptide **235** in 60% yield (Scheme 3.6). Subsequent allyl ester hydrolysis of tripeptide **235** yielded the tripeptide acid **236** (in 75% yield) using the reaction conditions adopted from Section 2.2.3, and the tripeptide amine **237** (in 99% yield), using the reaction conditions adopted from Section 2.2.2.



Scheme 3.6: Synthesis of a tripeptide **235**, tripeptide acid **236** and tripeptide amine **237**. **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 60%; (b) 1,2-DCE, $(CH_3)_3SnOH$, 80 ^{0}C, 6 h, 75%; (c) piperidine, CH_3CN, rt, 4 h, 99%.

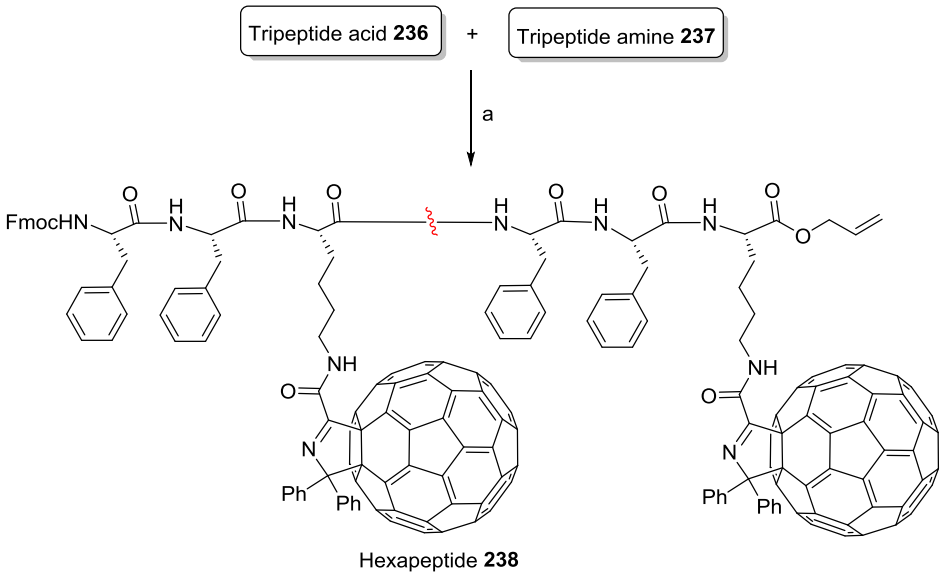
The ^{1}H NMR spectrum of tripeptide **235** showed the prominent allyl ester resonances at δ 4.39-4.59 (m, 2H), 5.19-5.25 (m, 2H) and 5.84-5.90 (m, 1H), that were present in the ^{1}H NMR spectrum of the monopeptide amine **202** and the Fmoc protecting group methine and methylene resonances [at δ 4.06-4.11 (m, 1H, CH), 4.39-4.59 (m, 2H, CH_2)], that were present in the ^{1}H NMR spectrum of acid **234**. Additionally, an amidic proton resonance was observed at δ 6.57 (bs, 1H, NH), that was associated to the newly formed amide bond. Furthermore, the ^{13}C NMR spectrum showed a resonance at δ 162.4, which was assigned to the newly formed amide carbonyl group. The molecular formula of the product **235** was verified by the appearance of the peak at *m*/*z* 1641.4235 in the HRMS, that was assigned to the protonated molecular ion ($[M+H]^+$ $C_{117}H_{55}N_5O_7$).

The deallylation of tripeptide **235** to its acid **236** was carried forward to the subsequent reaction without any spectroscopic analysis, because of its poor solubility.

The molecular formula of the amine product **237**, from removal of the Fmoc protecting group from **235** was verified by the appearance of the peak at *m*/*z* 1419.3529 in the HRMS, that was assigned to the protonated molecular ion ($[M+H]^+$ $C_{102}H_{45}N_5O_5$).

3.3.4 Amide coupling between tripeptide acid 236 and amine 237 to synthesize a hexapeptide 238

The synthesis of the [*60*]fullerenyl hexapeptide **238** (Scheme 3.7) was realized from the peptide coupling reaction of the previously synthesised tripeptide acid **236** and the tripeptide amine **237**. Purification of the crude reaction product by flash column chromatography followed by simple trituration from diethyl ether yielded the desired hexapeptide **238** in 40% yield (Scheme 3.7).



Scheme 3.7: **Reagents and conditions**: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 4 h, 40%.

The ^{1}H NMR spectrum of hexapeptide **238** showed the prominent allyl ester resonances [at δ 4.37-4.61 (m, 2H), 5.17-5.25 (m, 2H) and 5.86-5.92 (m, 1H)], that were present in the ^{1}H NMR spectrum of the tripeptide amine **237** and the Fmoc protecting group methine and

methylene resonances [at δ 4.04-4.15 (m, 1H, CH), 4.37-4.61 (m, 2H, CH_2)], that were present in the ^{1}H NMR spectrum of acid **236**. Additionally, an amidic proton was also appeared at δ 6.65 (bs, 1H, NH), that was associated with the newly formed amide bond. This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum and not sufficiently soluble in MeOH to generate an adequate ESIMS.

3.3.5 Future directions and conclusions

The synthesis of [*60*]fullerenolysine/phenylalanine tripeptides **224** and **235**, and their amine/acid derivatives **225**, **226** and **236**, **237** respectively, were achieved for the first time and characterised. Attempts were made to prepare the hexapeptides **227** and **238** from the coupling reactions of **225** and **226**, and **236** and **237**, respectively. The products of these reactions, however, could not be fully characterised due to their poor solubilities.

Currently, some of these molecules (**224**, **227**, **235** and **238**) are under study to form supramolecular complexes between the [*60*]fullerenes and porphyrin, and photoinduced charge separation experiments in Prof. Fukuzumi's laboratory at Osaka University, Japan.

CHAPTER 4

Synthesis of Mono and Bis[*60*]fullerene Based Di-Cationic Peptoids as Potential Anti-Bacterial Agents

4.1 Introduction

There are significant health care issues posed by multidrug resistant human pathogenic bacteria.[144-147] Of particular concern is the emergence of Gram-positive bacteria, *e.g.* *Staphylococcus aureus* and *Enterococcus faecium*, resistant to the glycopeptide antibiotic, vancomycin.[148,149] Recent work undertaken within our research laboratories has revealed a new peptide class of antibiotics, exemplified by binaphthyl-anchored peptide (Figure 4.1)[96]. These dicationic binaphthyl-templated linear peptides possess some similarity to structural aspects of vancomycin (Fig. 4.1) and could potentially act in a similar way but have added flexibility for interacting strongly with the changed peptido-glycan cell wall moiety[150] in vancomycin-resistant bacteria.[151] Also, the significant antimicrobial activity in some biaryl-based cyclic β–hairpin cationic peptidomimetics is noteworthy.[152] While medicinal chemistry investigations of binaphthyl-anchored peptide as antibacterial agents made significant progress, with the evolution of the amino acids required[94,97,153] and the better termini,[96] early investigations into alternatives to the binaphthyl unit were restricted to macrocycles anchored by hydrophobic scaffolds based on 3,3′-amino acid linked 1,1′-binaphthyls,[154] carbazoles,[155,156] indoles,[157] benzenes[107] and benzo[*b*]thiophene.[158] Within these studies,

acyclic versions of the hydrophobic anchored peptide derivatives were tested with little success. Considering that all of these approaches investigated reducing the size of the hydrophobic anchoring unit, we thought that alternative hydrophobic units could match the space occupied by the binaphthyl unit. Given the diameter of [*60*]fullerenes is 6.8 Å and the distance from distal points of the binaphthyl moiety is 8.3 Å, we considered the possibility of replacing the binaphthyl unit with a [*60*]fullerene. Structure Activity Relationships (SAR) will be compared with the replacement of hydrophobic binaphthyl moiety with [*60*]fullerene unit. This chapter describes the synthesis of the [*60*]fullerene based peptoids, **243**, **245**, **247**, **272-279** and **285** and their antibacterial activities.

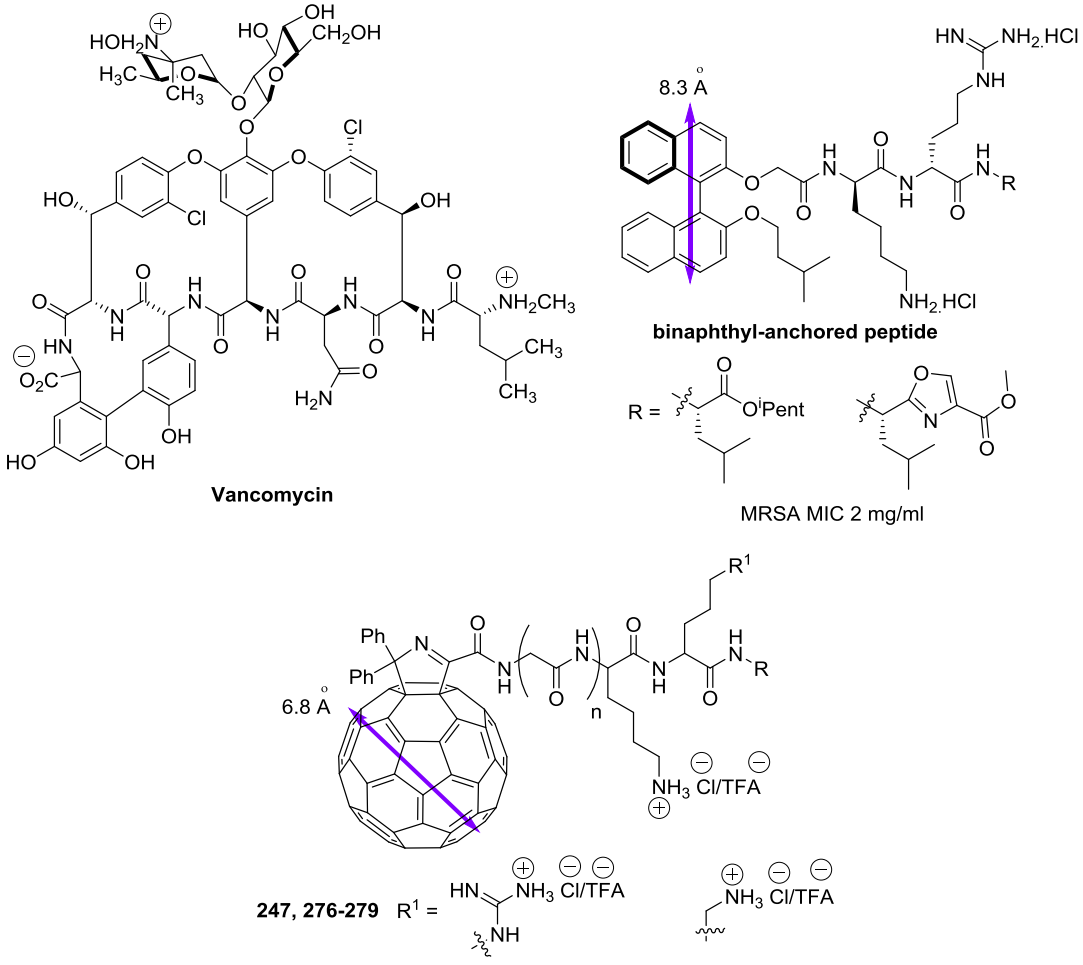
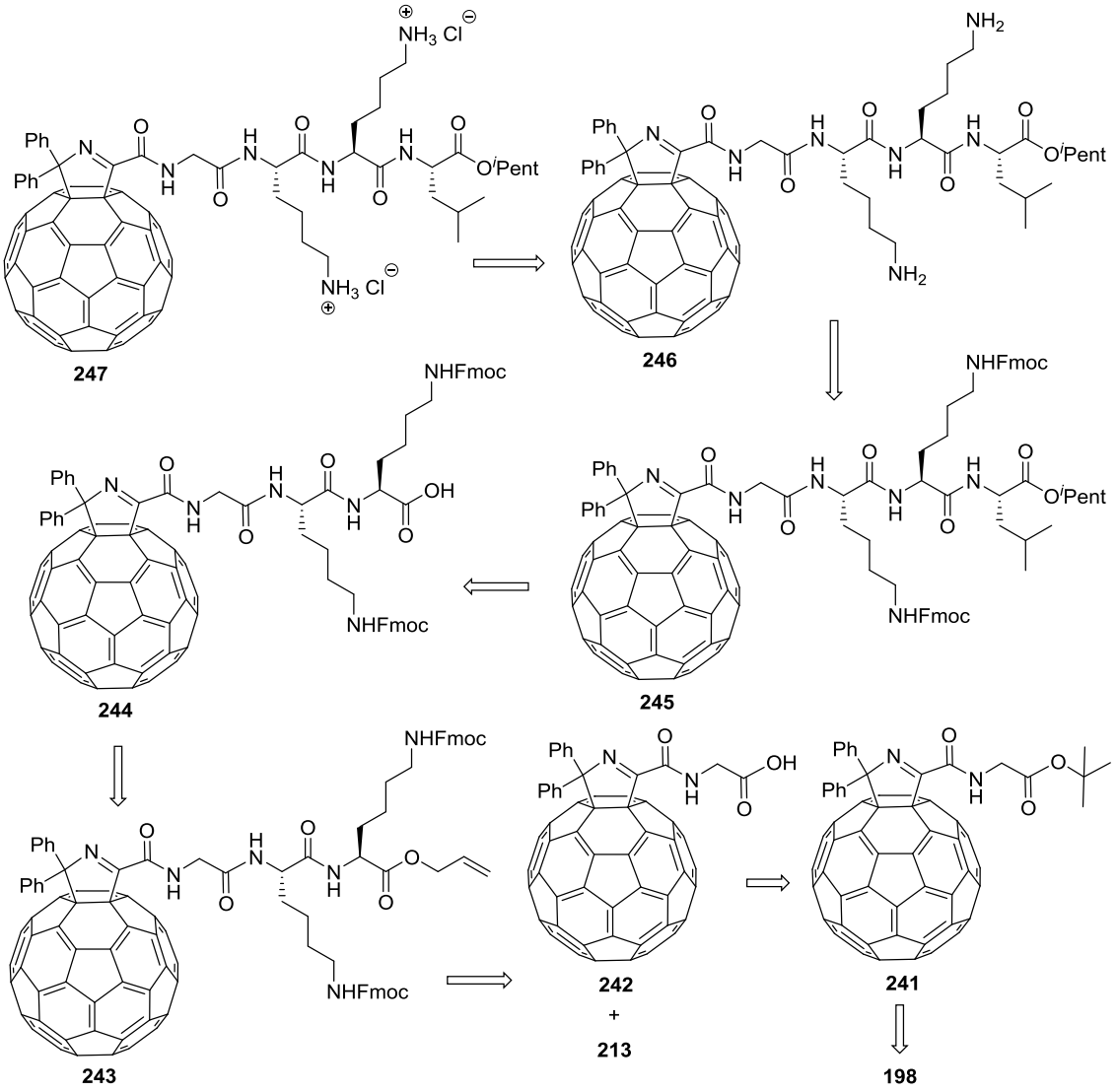


Figure 4.1. Binaphthyl-anchored peptide anti-biotic agents, Vancomycin and [*60*]Fullerene dicationic peptides.

4.2 Strategy 1: Coupling of a [*60*]fullerenoglycine derivative with a dipeptide followed by standard peptide couplings.

4.2.1 Proposed synthetic strategy for the initial peptoid 247.

The retro synthesis of the initial [*60*]fullerene based peptoid target **247**, starting from the previously reported 5,5-diphenylfullerenedihydropyrrole acid[33] **198**, is outlined in Scheme 4.1. A key step was the coupling of the fullerenyl acid **242** to the peptide **213** to give the tripeptide **243**.



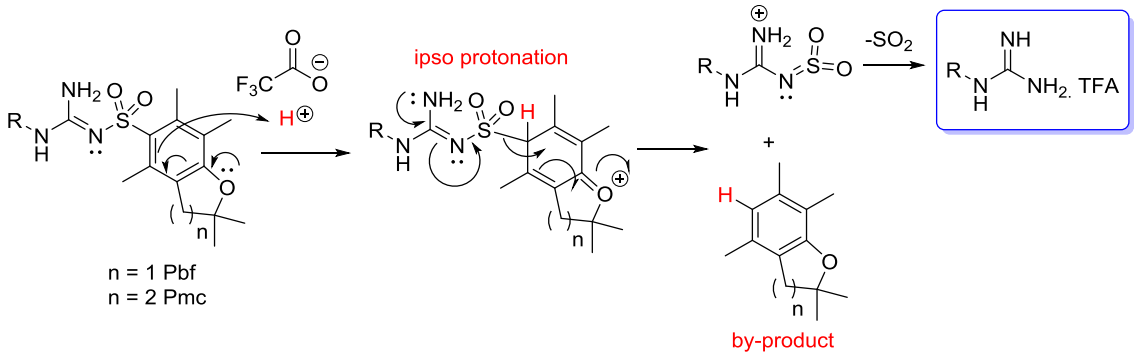
Scheme 4.1 Retrosynthetic analysis for the synthesis of [*60*]fullereno tetrapeptoid **247** from diphenylfullerenyldihydropyrrole acid **198** and amine **213**.

4.2.2 *N*-Boc, *N*-Pmc/Pbf and *tert*-butyl ester removal *via* acidolysis and HCl salt formation

A general procedure[97] mentioned in Section 2.2.3 (Scheme 2.7) was employed, with modifications, for the deprotection of *N*-Boc alone and/or *tert*-butyl ester group at rt for 4 h, and for all *N*-Boc and *N*-Pmc/Pbf containing intermediates at rt overnight. To form

the HCl salt, the residue was resuspended in a minimal volume of CH_2Cl_2 and the solution was then treated with an excess amount of 2 M HCl/ether (2 mL/0.01 mmol) solution and the solvent then evaporated. The product was purified by precipitation from CH_2Cl_2, with hexane and diethyl ether.

In this thesis the Boc, *tert*-butyl and Pbf/Pmc protecting groups were cleaved using TFA. The cleavage mechanism is similar for both substituents (Schemes 2.7 and 4.2) and proceeds *via* protonation followed by heterolytic cleavage and release of a stable carbocation. The acid-labile nature of the Boc and Pbf/Pmc groups lies in their ability to stabilize the resultant cationic species – for Boc it is a result of the *tert*-butyl carbocation and for Pbf/Pmc it is the 2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran and 2,2,5,7,8-pentamethylchromane respectively.[101,159] The tertiary nature of the *tert*-butyl cation stabilizes the positive charge by donating electron density onto the tertiary carbon (Scheme 2.7). Release of the cationic species from the *N*-protected substrate results in a carbamic acid for the Boc mechanism and the deprotected guanidine product in the Pbf/Pmc mechanism (Schemes 2.7 and 4.2). In the Boc mechanism, proton transfer followed by decomposition of the deprotonated carbamic acid gives CO_2 and the product amine as the TFA salt. In the Pbf/Pmc cleavage mechanism, the generated (alkylamino)(sulfonylamino)methaniminium undergoes decomposition to generate SO_2 and a guanidium salt (Scheme 4.2 – blue circle). [101,159]



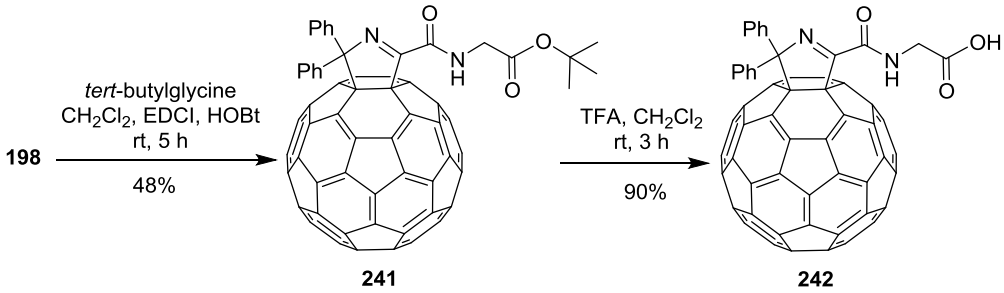
Scheme 4.2: Proposed mechanism for the acidolysis of an *N*-Pbf/Pmc group to give the product guanidine and by-products

The simple acidolytic conditions required for removal of both the *N*-Boc and *N*-Pbf groups allowed for the isolation of the crude TFA salts by simple solvent removal. Solvent-aided anion-exchange with anhydrous HCl allowed for the simple isolation of the final compounds as the mono- or di-hydrochloride salts by precipitation from diethyl ether. The general procedure that was developed allowed for at least a full 14 h reaction time, even though the Boc protecting groups have been observed to be fully deprotected in as little as 15 min. The longer reaction time was implemented to ensure complete deprotection of the Pbf/Pmc group, as shorter reaction times have been observed to give incomplete acidolysis. Due to the volatility of the Boc deprotection by-products, no resultant impurities were observed. The precipitation and repeated trituration of the final di-hydrochloride salt removes the majority of the benzofuran/chromene by-product. In the event of inefficient trituration, three small but distinctive peaks were noted in the ^{1}H NMR spectrum between δ 2.00 – 2.20 that were assigned to the benzofuran/chromene by-product (3 x CH_3). Overall, it was the relative ease, speed, and resultant product purity of the acidolytic deprotection method that allowed for the efficient turn-over of multiple derivatives.

4.2.3 From fullerenyldihydropyrrole derivatives towards fullerenyl peptides

4.2.4 Amide coupling of carboxylic acid 198, followed by ester deprotection yielding carboxylic acid 242

[*60*]Fullerenyl peptide **242** was prepared as shown in Scheme 4.3, from the fullerenyl carboxylic acid **198**.



Scheme 4.3: Synthesis of the fullerenyl amino acid **242**.

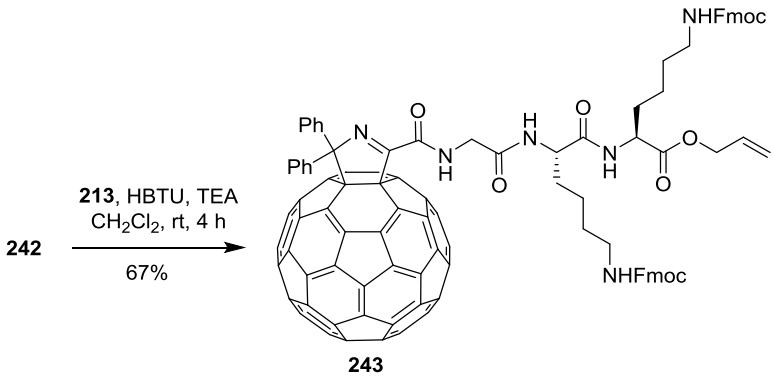
Under EDCI/HOBt amide coupling reaction conditions the fullerenyl carboxylic acid **198** was found to readily couple to *tert*-butyl glycinate in CH_2Cl_2 at rt in 5 h. The solution was washed with water then the solvent was reduced in volume to approximately half before being applied directly to the top of a silica gel column. Elution with CH_2Cl_2 provided the fullerenyl peptide **241** in 48% yield, which was converted to the acid **242** by treatment of a solution of **241** in CH_2Cl_2 with TFA at rt for 3 h (Scheme 4.3). Hexanes were then added and the precipitate was collected providing the acid **242** in 90% yield.

Evidence for the successful coupling was provided by analysis of the ^{1}H NMR spectrum of **241** which indicated loss of the resonance attributable to the carboxylic acid proton and the presence of a singlet, with a relative integration of 9H at δ 1.42, assigned to the *tert*-butyl protons. The presence of another singlet with an integration of 2H at δ 3.85

also provided strong evidence of the α-methylene protons of the *tert*-butyl glycine moiety. Analysis of the ESI-MS revealed an ion at *m/z* 1093 assigned as the sodiated molecular ion [$(M+Na)^+$ $C_{81}H_{22}N_2O_3Na$]. Successful deprotection of the *tert*-butyl group was provided by analysis of the ^1H NMR spectrum of **242**, which indicated loss of the resonance attributable to the *tert*-butyl group (δ 1.42 (9H, s)) along with the appearance of a broad singlet, with a relative integration of 1H, at δ 8.56 attributed to the carboxylic acid proton. Analysis of the ESI-MS, which showed a base peak at *m/z* 1015 assigned as the protonated molecular ion [$(M+H)^+$ $C_{77}H_{15}N_2O_3$].

4.2.5 Synthesis of the [*60*]fullereno tripeptide 243

The crucial [*60*]fullerenyl tripeptide **243** was prepared as shown in Scheme 4.4, starting with the coupling reaction of the fullerenyl carboxylic acid compound **242** and the dipeptide **213**.



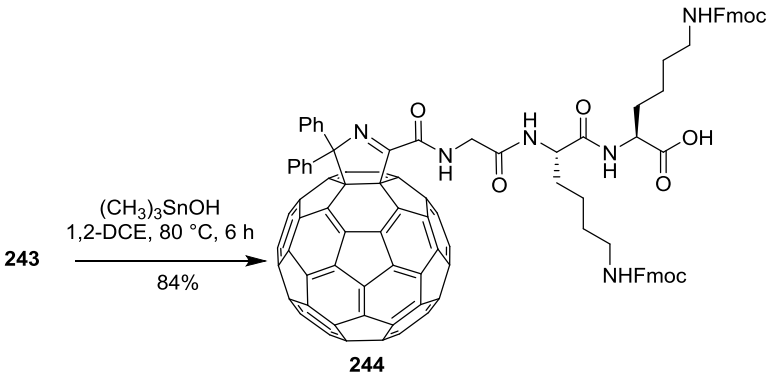
Scheme 4.4: Synthesis of [*60*]fullereno tripeptide.

Under EDCI/HOBt amide coupling reaction conditions the fullerenyl acid **242** was found to readily couple to a TFA salt of dipeptide **213** in CH_2Cl_2 at rt in 5 h in 45% yield. However, optimisation of the yield of this peptide coupling reaction under HBTU

rather than EDCI/HOBt, resulted in better yield of 67% for compound **243**, after purification by silica gel column chromatography. Analysis of the ^{1}H NMR spectrum of **243** showed a loss of the resonance attributable to the carboxylic acid proton and the presence of resonances for the characteristic protons of both the carboxylic acid **242** and amine **213**. A multiplet at δ 3.43-3.44 (-CH<u>N</u>H$_2$) in the ^{1}H NMR spectrum of **213** was no longer observed in the spectrum of **243** but a broad singlet at δ 4.51 with an integration of 2H was assigned to the two lysine α-methine protons. This evidence indicated that the α-proton, adjacent to the primary amine in precursor **213**, experienced a downfield shift as a result of the newly installed amide bond. Additionally, the ^{13}C NMR spectrum showed a resonance at δ 171.7, assigned to the new amide carbonyl group. The molecular structure of the product was verified by the appearance of the peak at *m*/*z* 1778.4565 in the HRMS, that was assigned to the sodiated molecular formula ($[M + Na]^+$ $C_{122}H_{62}N_6O_9Na$).

4.2.6 Selective deallylation of tripeptide 243 using $(CH_3)_3SnOH$ to provide carboxylic acid 244

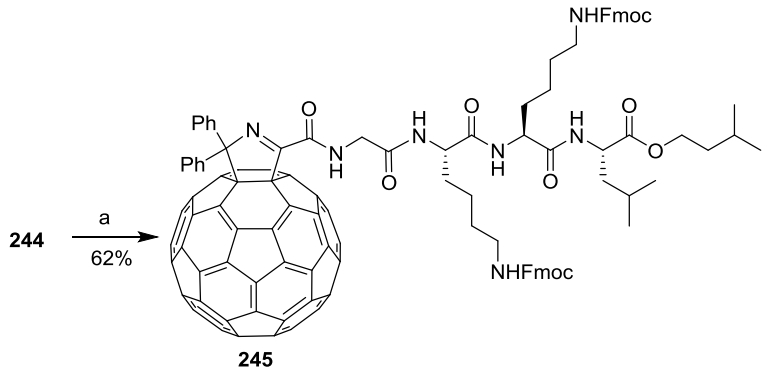
The [*60*]fullereno tripeptide **243** was treated with $(CH_3)_3SnOH$ under the above mentioned reaction conditions to selectively hydrolyse the allyl ester to give the acid **244** in 84% yield (Scheme 4.5) without any noticeable cleavage of the Fmoc carbamate protecting group. The acid **244** was then carried in to the subsequent reaction without any further characterisation, because of its poor solubility.



Scheme 4.5: Synthesis of the [*60*]fullereno tripeptide acid **244**.

4.2.7 Synthesis of novel tetrapeptide 245 from acid 244 under amide coupling conditions

The synthesis of tetrapeptide **245** (Scheme 4.6) was realized in 62% yield after stirring a CH_2Cl_2 solution of the commercially available amine, (*S*)-isopentyl 2-amino-4-methylpentanoate, and the acid derivative **244** with the coupling reagents EDCI and HOBt at rt for 4 h. Flash column chromatography followed by simple trituration from diethyl ether yielded the desired novel tetrapeptide product **245**.



Scheme 4.6: Synthesis of [*60*]fullereno tetrapeptide **245**. **Reagents and conditions**: (a) isopentyl *L*-leucinate hydrochloride, EDCI, HOBt, TEA, CH_2Cl_2, rt, 4 h.

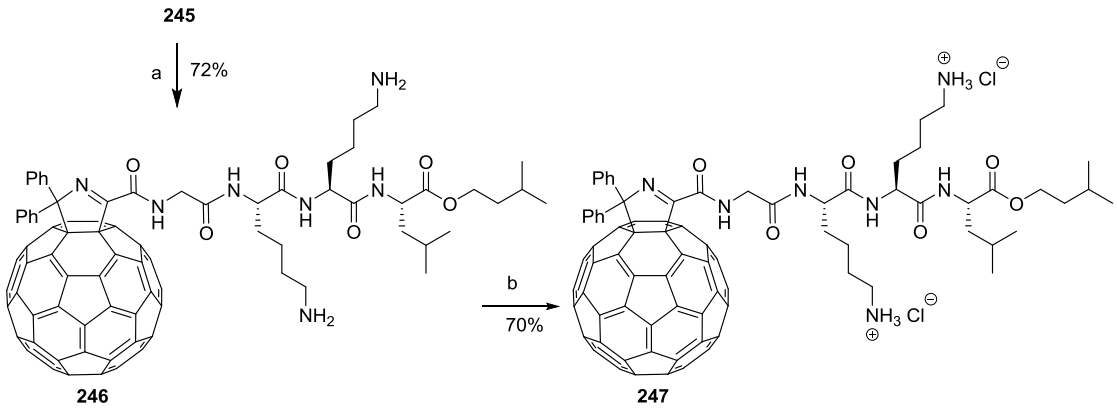
A broad singlet at δ 8.76 (-CHN<u>H</u>$_2$) in the ^{1}H NMR spectrum of (*S*)-isopentyl 2-amino-4-methylpentanoate was no longer observed in the spectrum of **245** but a triplet

resonance for the isopentyl ester –OC<u>H</u>$_2$ (δ 4.04 (2H, t, *J* = 6.5 Hz)) was observed. A multiplet at δ 4.11-4.17 for 4H was assigned to the leucine-α-methine proton including other protons, this evidence indicated that the α-proton, adjacent to the primary amine in precursor (*S*)-isopentyl 2-amino-4-methylpentanoate, experienced a downfield shift as a result of the newly installed amide bond. Additionally, the molecular structure of the product was verified by the appearance of a peak at *m*/*z* 1898.6011 in the HRMS, which was assigned to the protonated molecular formula ($[M+H]^+$, $C_{130}H_{79}N_7O_{10}$). This compound however, was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

4.2.8 Base-promoted *N*-Fmoc removal for the tetrapeptide 246, followed by HCl salt formation to give peptoid 247

A solution of tetrapeptide **245** in piperidine/acetonitrile (1:9 v/v) was stirred overnight at rt to produce the free amine **246**, which was carried forward to form the HCl salt by treatment with an excess amount of 1 M HCl/diethyl ether solution. The crude product was purified by precipitation from CH_2Cl_2, with hexane and diethyl ether (Scheme 4.7) to achieve a 70% yield of the hydrochloride salt **247**.

The molecular structure of this salt was verified by the appearance of a peak *m/z* 1454.4750 in the HRMS, which was assigned to the protonated molecular formula ($[M+H]^+$, $C_{100}H_{59}N_7O_6$). Unfortunately, this compound was not sufficiently soluble to generate an adequate ^1H or ^{13}C NMR spectrum.

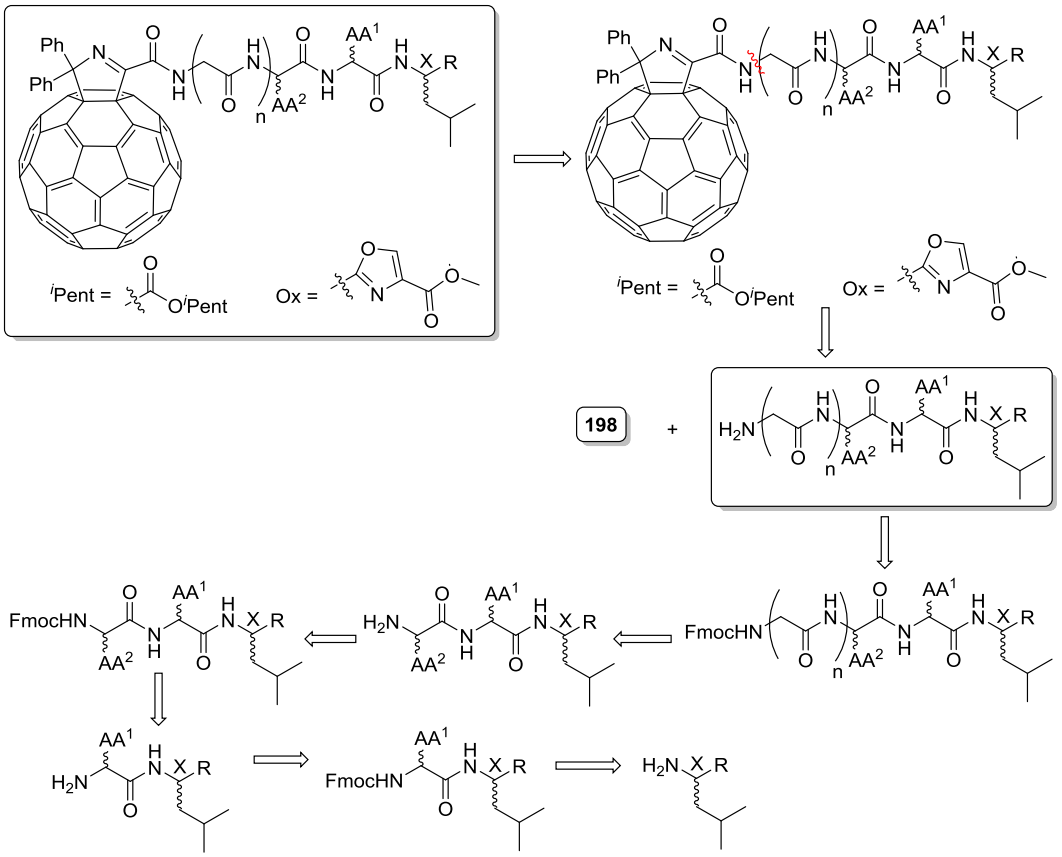


Scheme 4.7: Synthesis of [*60*]fullereno peptoid salt **247**. **Reagents and conditions**: (a) piperidine, CH_3CN, rt, 4 h; (g) 1 M $HCl.Et_2O$, 0 °C-rt, 0.5 h.

4.3 Strategy 2

4.3.1 Proposed synthetic strategy for the synthesis of [*60*]fullereno peptoids 272-279.

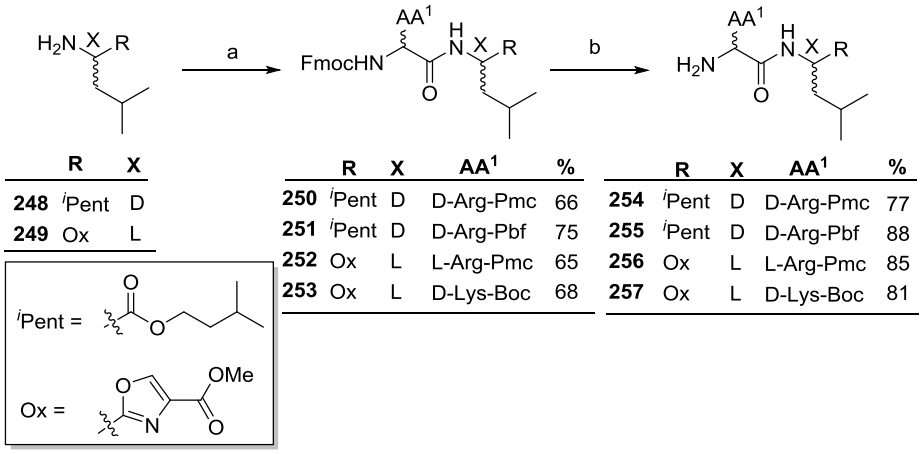
The synthesis of the second series of novel [*60*]fullereno peptoids **272-279** started from the previously mentioned 5,5-diphenylfullerenedihydropyrrole acid **198** and a variety of tri- and tetra-peptides was proposed in seven synthetic steps for the [*60*]fullereno pentapeptides, and six steps for the [*60*]fullereno tetrapeptides (Scheme 4.8). This strategy was adopted because of the relative ease of handling peptides in terms of solubility and purification without the fullerene moiety present, and additionally to minimise the exposure of the [*60*]fullerene containing peptide to acidic conditions, because of the possible cleavage of the [*60*]fullerene group especially under TFA reaction conditions.



Scheme 4.8 Retrosynthetic analysis for the synthesis of [*60*]fullereno tetra and pentapeptoids from the known diphenylfullerenyldihydropyrrole acid **198**, and amino acids **262**, **269**-**271**.

4.3.2 Synthesis of the known dipeptides 250-253 and the Fmoc deprotected dipeptides 254-257

The synthesis of the previously reported[94,96,97] free amine containing dipeptides **254**-**257** (Scheme 4.9) were achieved from commercially available starting materials **248** and **249**. Under EDCI/HOBt amide coupling reaction conditions the D/L-Arg-(Fmoc)-OH or D/L-Lys-(Boc)-OH acids were found to readily couple to free amines **248** and **249** in CH_2Cl_2 at rt in 5 h to produce dipeptides **250**-**253** in 65-75% yield. Fmoc deprotection of these compounds, then gave compounds **254**-**257**, respectively. These compounds exhibited NMR spectroscopic data that closely matched with those previously reported.[94,96,97] The yields for these individual reactions are summarised in Scheme 4.9.



	R	X
248	^{i}Pent	D
249	Ox	L

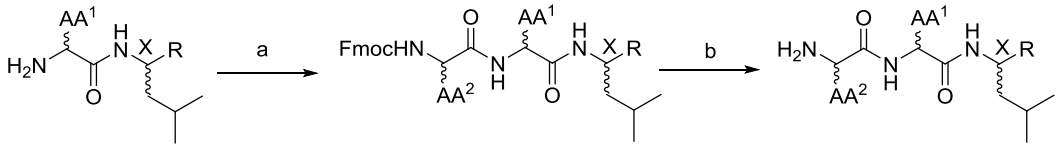
	R	X	AA^1	%
250	^{i}Pent	D	D-Arg-Pmc	66
251	^{i}Pent	D	D-Arg-Pbf	75
252	Ox	L	L-Arg-Pmc	65
253	Ox	L	D-Lys-Boc	68

	R	X	AA^1	%
254	^{i}Pent	D	D-Arg-Pmc	77
255	^{i}Pent	D	D-Arg-Pbf	88
256	Ox	L	L-Arg-Pmc	85
257	Ox	L	D-Lys-Boc	81

Scheme 4.9: Reagents and conditions: (a) Fmoc-*D*-arg-(Pmc/Pbf)-OH, Fmoc-*L*-arg-(Pmc)-OH or Fmoc-D-lys(Boc)-OH, EDCI, HOBt, CH_2Cl_2, rt, 4 h; (b) piperidine, CH_3CN, rt, 4 h.

4.3.3 Synthesis of the tripeptides 258-261 and the Fmoc deprotected tripeptides 262-265

Tripeptides **258**-**261** were prepared in 72-86% yields, by reacting **254**-**257** with Fmoc-*D* or *L*-Lys-(Boc)-OH under our standard amide coupling procedures (Section 2.2.2). Subsequent deprotection of the Fmoc group resulted in the compounds **262**-**265** in yields of 77-88%.



	R	X	AA^1
254	^{i}Pent	D	D-Arg-Pmc
255	^{i}Pent	D	D-Arg-Pbf
256	Ox	L	L-Arg-Pmc
257	Ox	L	D-Lys-Boc

	R	X	AA^1	AA^2
258	^{i}Pent	D	D-Arg-Pmc	D-Lys-Boc
259	^{i}Pent	D	D-Arg-Pbf	L-Lys-Boc
260	Ox	L	L-Arg-Pmc	D-Lys-Boc
261	Ox	L	D-Lys-Boc	D-Lys-Boc

	R	X	AA^1	AA^2
262	^{i}Pent	D	D-Arg-Pmc	D-Lys-Boc
263	^{i}Pent	D	D-Arg-Pbf	L-Lys-Boc
264	Ox	L	L-Arg-Pmc	D-Lys-Boc
265	Ox	L	D-Lys-Boc	D-Lys-Boc

Scheme 4.10: Reagents and conditions: (a) Fmoc-*D* or *L*-Lys-(Boc)-OH, EDCI, HOBt, CH_2Cl_2, rt, 4 h, 79% for **258**, 80% for **259**, 86% for **260** and 72% for **261**; (b) piperidine, CH_3CN, rt, 4 h, 88% for **262**, 77% for **263**, 84% for **264**, and 85% for **265**.

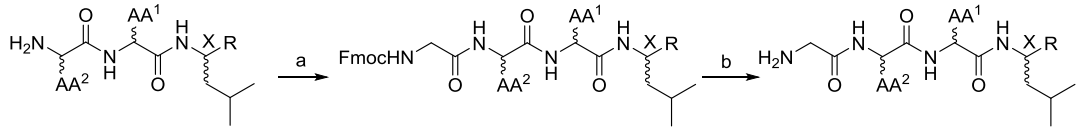
A broad singlet at δ 8.62 (-CHN$\underline{H}_2$) in the ^{1}H NMR spectrum of **254** was no longer observed in the spectrum of **258** but a signal appeared at δ 4.43-4.45 (m, 1H), that was

assigned to the arginine α-methine proton. This evidence indicated that the α-proton, adjacent to the primary amine in precursor **254**, experienced a downfield shift as a result of the newly installed amide bond. Additionally, the ^{1}H NMR singlet at δ 12.10 for the acid –OH in Fmoc-*D*-Lys-(Boc)-OH was absent in the ^{1}H NMR spectrum of compound **258**. Further analysis of the ^{13}C NMR spectra showed a signal at δ 172.5, assigned to the new amide carbonyl. The molecular structure of the product was verified by the appearance of the peak at *m*/*z* 1074.0 in the ESI MS, that was assigned to the protonated molecular ion, $[M + H]^{+}$.

Analysis of the ^{1}H NMR spectrum of amine **263** provided clear evidence for Fmoc deprotection as there was a notable absence of signals at approximately δ 4.10 (2H, m) and 4.32 (1H, t, *J* = 6.5 Hz) that were present in the spectrum of compound **259** and had been assigned to the three non-aromatic Fmoc protons (i.e. -OC$\underline{H}_2$-C$\underline{H}$-Ar). Furthermore, two distinct multiplet resonances at δ 7.26-7.29 (2H, m) and δ 7.35-7.38 (2H, m) in the spectrum of **259**, assigned as aromatic Fmoc protons, were no longer observed. This indicated the successful removal of the fluorenyl moiety and its eight aromatic protons. Analysis of the ^{13}C NMR data confirmed the absence of a deshielded methylene carbon signal at δ 67.4 and a methine carbon at δ 47.3 (assigned as the Fmoc CH_2 and CH carbons, respectively), as well as four aromatic signals between δ 120.2-144.1. The protonated molecular ion ($[M + H]^{+}$) of **263** was assigned to the peak at *m*/*z* 838.0 in the ESIMS.

4.3.4 Synthesis of the tetrapeptides 266-268 and the Fmoc deprotected tetrapeptides 269-271

The tetrapeptides **266**-**268** were prepared in 71-82% yields, by the coupling reactions of **263**-**265** with Fmoc-Gly-OH. Subsequent deprotection of their Fmoc groups resulted in the compounds **269**-**271**, respectively in yields of 80-88% yield.



	R	X	AA^1	AA^2
263	^{i}Pent	D	D-Arg-Pbf	L-Lys-Boc
264	Ox	L	L-Arg-Pmc	D-Lys-Boc
265	Ox	L	D-Lys-Boc	D-Lys-Boc

	R	X	AA^1	AA^2
266	^{i}Pent	D	D-Arg-Pbf	L-Lys-Boc
267	Ox	L	L-Arg-Pmc	D-Lys-Boc
268	Ox	L	D-Lys-Boc	D-Lys-Boc

	R	X	AA^1	AA^2
269	^{i}Pent	D	D-Arg-Pbf	L-Lys-Boc
270	Ox	L	L-Arg-Pmc	D-Lys-Boc
271	Ox	L	D-Lys-Boc	D-Lys-Boc

Scheme 4.11: Reagents and conditions: (a) Fmoc-Gly-OH, EDCI, HOBt, CH_2Cl_2, rt, 4 h, 75% for **266**, 71% for **267** and 82% for **268**; (b) piperidine, CH_3CN, rt, 4 h, 80% for **269**, 88% for **270**, and 86% for **271**.

A broad singlet at δ 6.29 (-CHN$\underline{H}_2$) in the ^{1}H NMR spectrum of **263** was no longer observed in the spectrum of **266** but a signal appeared at δ 4.58 (1H, m), which was assigned to the lysine α-methine proton. This evidence indicated that the α-proton, adjacent to the primary amine in precursor **263**, experienced a downfield shift as a result of the newly installed amide bond. Additionally, the ^{1}H NMR signal at δ 12.50 for the acid –OH of Fmoc-Gly-OH had disappeared in the spectrum of compound **266**. Further to this the ^{13}C NMR signal at δ 172.9 was assigned to the new amide carbonyl group. The molecular structure of the product was also supported by the appearance of the peak at *m*/*z* 1117.6 in the ESIMS, that was assigned to the protonated molecular ion, $[M + H]^+$.

Analysis of the ^{1}H NMR spectrum of amine **269** provided clear evidence for Fmoc deprotection as there was a notable absence of signals at δ 4.17 (t, J = 6.5 Hz, 1H) and

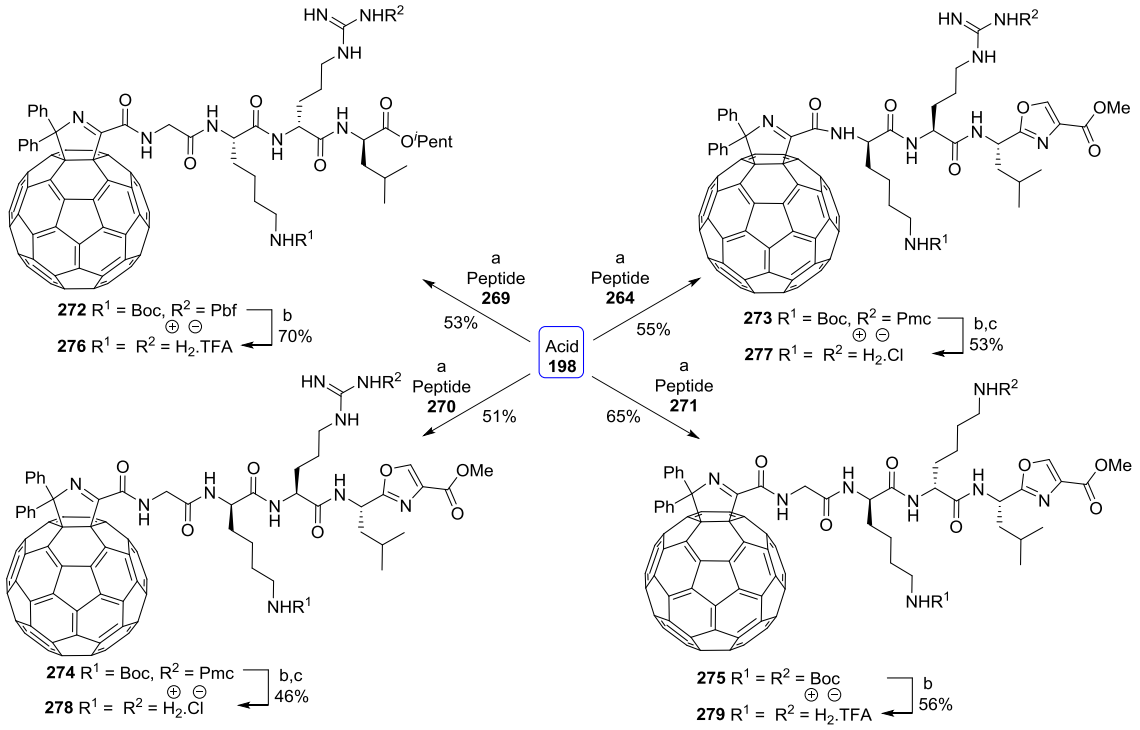
4.33 (d, *J* = 7.0 Hz, 2H) that were present in the spectrum of compound **266** and had been assigned to the three non-aromatic Fmoc protons (i.e. -OC$\underline{H_2}$-C$\underline{H}$-Ar). Furthermore, a clear absence of two distinct doublet resonances at δ 7.57 (d, *J* = 6.5 Hz, 2H) and δ 7.73 (d, *J* = 8.0 Hz, 2H) in the spectrum of **269**, which were present in the spectrum of **266**, and assigned as aromatic Fmoc protons. This indicated the successful removal of the fluorenyl moiety and its eight aromatic protons. Analysis of the ^{13}C NMR data of compound **269** was also conclusive by the absence of characteristic signals of deshielded methylene carbon at δ 67.4 and a methine carbon at δ 47.3 (assigned as the Fmoc CH_2 and CH carbons, respectively), as well as four aromatic signals between δ 121.9-144.3. The protonated molecular ion ([M + H]+) was assigned to the peak at *m*/*z* 895.6 in the ESIMS of **269** (Scheme 4.11).

4.3.5 Synthesis of the [*60*]fullereno tetra and pentapeptides 272-275 and their salts 276-279

The protected fullerenyl peptoids **272-275** (Scheme 4.12) were successfully synthesised by coupling [*60*]fullerenyldihydropyrrole acid **198** with the presynthesised peptides **269**, **264**, **270** and **271** under EDCI/HOBt peptide coupling conditions. In a typical example, the tetrapeptide **269** was added dropwise to a solution of acid **198** and HOBt in CH_2Cl_2, which was sonicated for 15 min. to aid solubility. EDCI was added at the same temperature and the reaction mixture was stirred for 4 h at room temperature. As the reaction progressed, the turbid solution transformed to a clear brown solution and upon workup, and the first-in-class [*60*]fullerenyl anchored tetrapeptide **272** was isolated. Synthesis of compound **273** was possible by coupling of the oxazole terminated tripeptide **264**, and the tetrapeptide version of **270**, with the extra glycine

amino acid in to the peptide chain producing **274**. Coupling the acid **198** with peptide **271**, which contained two Boc protected lysines with an oxazole terminus, yielded **275**. This reaction used $CHCl_3$:CH_2Cl_2 (1:1) as a solvent due to better solubility of the diBoc protected lysine containing peptide whereas the synthesis of compounds **273** and **274** only required CH_2Cl_2 as the single solvent (Scheme 4.12).

To improve the solubility of the [*60*]fullerene based peptides for antibacterial testing, it was necessary to use them as dicationic salts, which were prepared using either TFA and/or 1 M HCl in diethyl ether. Peptides **273** and **274** were made as the HCl salts **277** and **278**, respectively, by treating them with TFA/CH_2Cl_2 (1:1) to deprotect the Pmc/Pbf and *N*-Boc functional groups. Then, after removing the solvents under reduced pressure, the residue was resuspended in a minimal volume of CH_2Cl_2 and treated with an excess 1 M HCl in diethyl ether to obtain the targeted HCl salts. The peptides **272** and **275** were made as their TFA salts **276** and **279** by treating them with TFA/CH_2Cl_2 (1:1) to deprotect the Pmc/Pbf and *N*-Boc groups (Scheme 4.12).



Scheme 4.12: Synthesis of C_{60} fullerenyl anchored amino acid oxazoles and iso pentyl esters. **Reagents and conditions:** (a) EDCI, HOBt, CH_2Cl_2, rt, 4 h; (b) TFA/ CH_2Cl_2 (1:1), rt, 16 h; (c) 1 M HCl.Et_2O, 0-rt 0.5 h.

A broad singlet at δ 7.85 (-CHN$\underline{H}_2$) in the ^{1}H NMR spectrum of **269** was no longer observed in the spectrum of **272** but a signal appeared at δ 4.28 (bs, 2H), assigned to the glycine methylene protons. This evidence indicated that the methylene, adjacent to the primary amine in precursor **269**, experienced a downfield shift as a result of the newly installed amide bond. Additionally, a ^{1}H NMR signal at δ 8.63 assigned to the acid –OH proton in [*60*]fullerenyldihydropyrrole acid **198** was not observed in compound **272**. Further, the ^{13}C NMR signal at δ 172.9 assigned to the new amide carbonyl group in **272**. The molecular structure of the product was verified by the appearance of the peak at *m*/*z* 1856.5916 in HRMS, that was assigned to the sodiated molecular formula ([M + Na]$^+$, $C_{118}H_{83}N_9O_{11}SNa$) (Scheme 4.12).

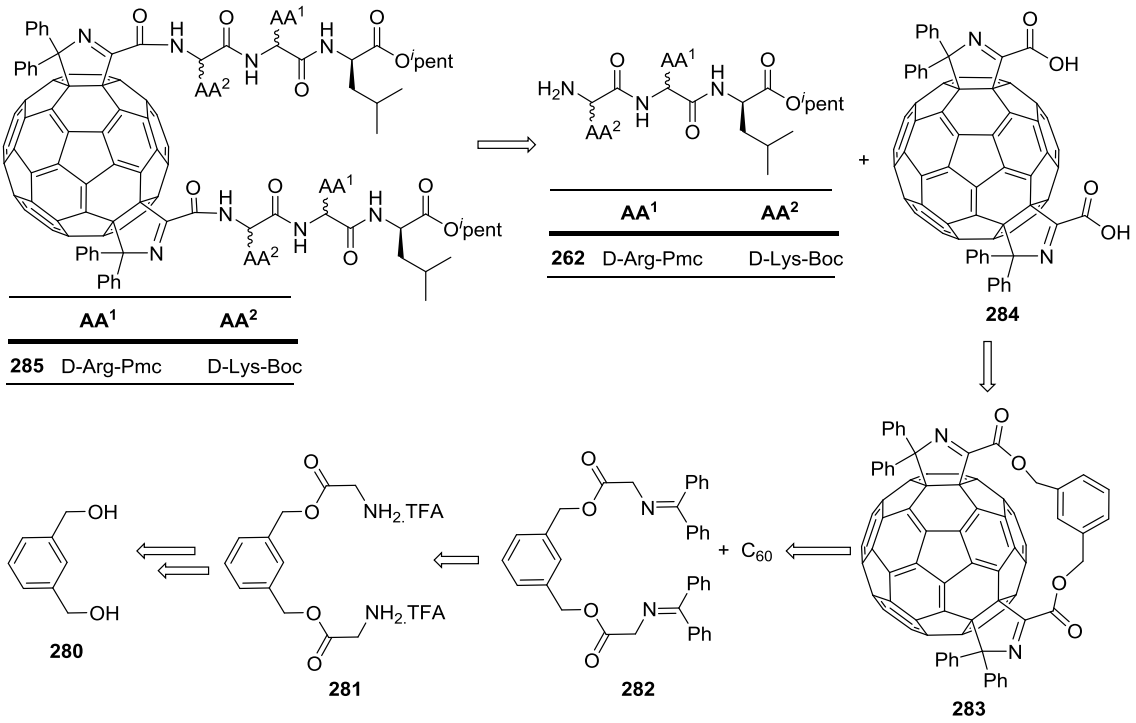
The salts **276**-**279** were characterised by HRMS, and additionally, compounds **276**, **277** and **278** returned ^{1}H NMR spectra of good quality, but not the compound **279**.

Unfortunately, due to the limited solubility of these salts, we were unable to obtain adequate ^{13}C NMR spectra.

The ^{1}H NMR spectrum of the compound **276** lacked the prominent 9H peak at δ 1.42 that was present in the spectrum of carbamate **272** and was assigned to the *tert*-butyl methyl protons. Furthermore, multiple diagnostic signals observed at δ 1.46, 2.08, 2.50, 2.57 and 2.95 assigned to the Pbf protecting group of carbamate **272**, were not observed in the ^{1}H NMR spectrum of the final compound **276**, indicating the successful removal of the Boc protecting group as well as Pbf group. HRMS analysis of compound **276** showed a peak at *m*/*z* 1482.4717 that was assigned to the protonated molecular ion ([M + H]+, $C_{100}H_{59}N_9O_6$).

4.3.6 [*60*]Fullerenyl bispeptides

Focus was then shifted to extending the carboxyl deprotection and coupling reactions, established on the mono-adducts (Section 4.3.1), to bisadducts (Scheme 4.13). Therefore, the synthesis of the tethered bisadduct **283** was performed using the previously reported procedures.[33,160] Hydrolysis of ester group on the tethered bisadduct **283** and subsequent amide coupling reaction with presynthesised peptide **262**, was anticipated to give the first target bispeptide **285**.



Scheme 4.13 Retrosynthetic analysis for the synthesis of [*60*]fullereno bispeptide of type **285** from the known diphenylfullerenyldihydropyrrole bisacid **284**, and peptide **262**.

4.3.7 Regioisomerism

In the formation of bis-methanofulleryl adducts, there are nine possible addition sites for functionalisation, assuming the reaction occurs across 6,6-fused bonds (Figure 4.2).[161,162,163,164,165,166,167,168,169,170,171,172,173,174] The basis for the positional algorithm is the contiguous numbering of carbon atoms of a [*60*]fullerene derivative in a spiral fashion.[171] If the second addition occurs in the same hemisphere as the first, it is assigned the *cis* regiochemistry with the numbering scheme (*cis*-1, *cis*-2, *cis*-3) increasing as the second addend *retreats* from the first site of addition. *Trans* regioisomers occur in the opposing hemisphere with the numbering scheme (*trans*-1, *trans*-2, *trans*-3, *trans*-4) increasing as the second addend *approaches* the first site of addition (Figure 4.2) (Table 4.1).

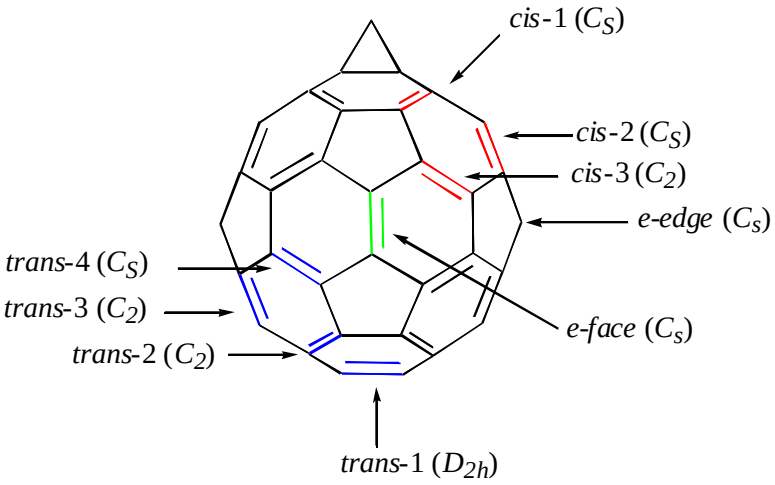


Figure 4.2: Positional notation and symmetry operations for regioisomeric methanofullerene bisadducts.[165]

Assignment	N° of Fulleryl ^{13}C NMR Resonances	N° of 1/2 Intensity ^{13}C NMR Resonances	Symmetry Operation
cis-1	32	4	C_S
cis-2	32	4	C_S
cis-3	30	0	C_2
e-face	60	0	C_S
e-edge	60	0	C_S
trans-1	8	0	D_{2h}
trans-2	30	0	C_2
trans-3	30	0	C_2
***trans*-4**	**32**	**4**	**C_S**

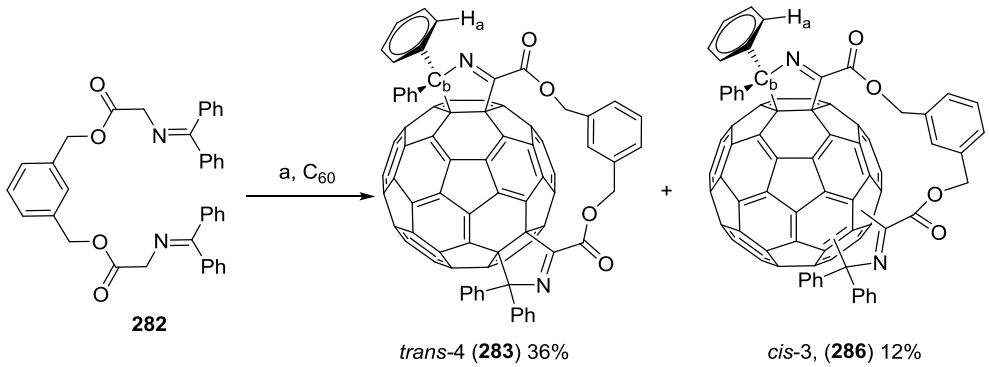
Table 4.1: Summary of regioisomeric assignments for methanofullerene bisadducts, the number of observed fullerenyl ^{13}C NMR resonances, and corresponding symmetry operations determined from ^{13}C NMR spectra.

4.3.8 Regioselective multifunctionalisation of [*60*]fullerene using tethered bis-*N*-(diphenylmethyleneglycinate) diesters

4.3.8.1 Double Bingel reactions with bis-*N*-(diphenylmethylene glycinate) diesters

The previously reported double Bingel reaction of **282**[33,160] with [*60*]fullerene was attempted using carbon tetrabromide (2.0 equiv.) and DBU (3.5 equiv.) (Scheme 4.14). The reaction mixture was stirred at rt, and typically took 6 h to reach completion.

Purification and separation of the resulting products **283** and **286** was achieved by elution of the crude reaction mixture through two silica gel columns with CH_2Cl_2:petroleum spirit (90:10) as the eluent. Final recrystallizations of these regiosiomers yielded pure samples of **283** and **286** in 36% and 12%, yields, respectively. The ESIMS spectrum of both **283** and **286** displayed a molecular ion at *m/z* 1298.



Scheme 4.14 (a) DBU, CBr_4, rt, toluene, C_{60}, 6 h, 45%.

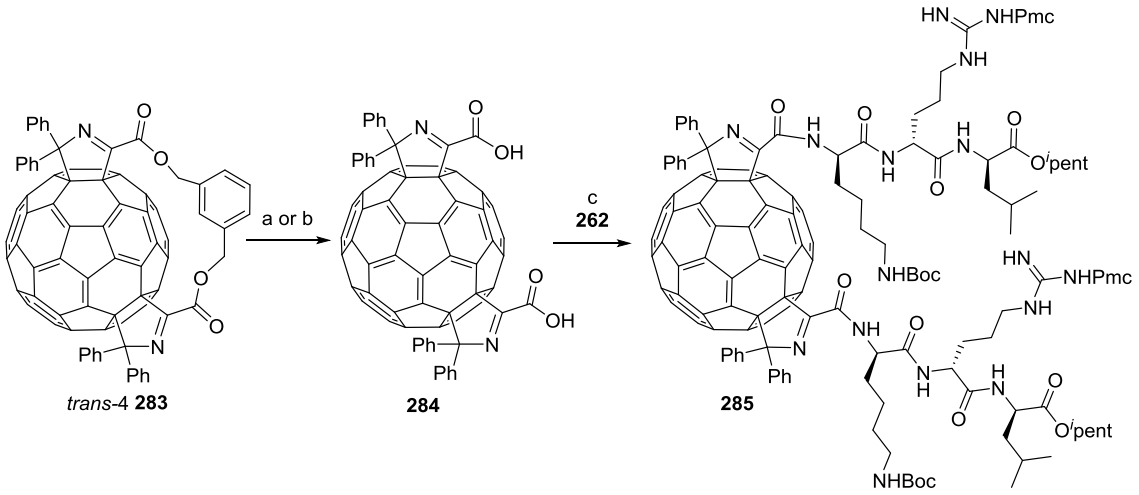
The ^{1}H NMR spectra of **283** and **286** revealed the loss of the methylene resonance (δ 3.17) associated with the *N*-(diphenylmethyleneglycinate) ester (**282**) as a result of Bingel reaction. Tether macrocyclisation induced the benzyl protons to become diastereotopic and these appeared as doublets [δ 5.06 and 5.71; *J* = 11.2 Hz for **283**; δ 5.32 and δ 5.41; *J* = 11.0 Hz for **286**] in contrast to the singlet resonance (δ 5.09) of the starting material (**282**).

The aromatic region of these bisfullerene derivatives exhibited a doubling up of resonances as a result of macrocyclisation. As with the monosubstituted derivatives, the *ortho* protons (H_a) exhibit a characteristic downfield shift [(δ 7.92, 2H, d, *J* = 8.4 Hz and δ 8.04, 2H, d, *J* = 8.4 Hz for **283**; δ 8.17, 2H, d, *J* = 7.6 Hz, δ 8.21, 2H, d, *J* = 7.6 Hz for **286**] compared to those corresponding protons in the starting material [**282**, (δ

7.64, 4H, dd, *J* = 8.4, 1.6 Hz)]. The ^{13}C NMR spectrum of **283** matched that previously reported.[33,90]

4.3.8.2 Diester deprotection of 283 followed by amide coupling to synthesise [*60*]fullerenyl bispeptide

We intended to synthesise fulleryl bispeptides by extension of the protocols of carboxyl deprotection and coupling reactions, established on the mono-adducts (Section 4.2.3), to bisadducts (Scheme 4.15). Therefore, a solution of **283** in 1,2-DCE was treated with $(CH_3)_3SnOH$ at 80 ^oC for 24 h before being quenched with 5% HCl solution. The product bisacid **284** was then isolated as a brown solid. The previously reported[90] BBr_3 mediated diester deprotection of **283** was also performed, but the former conditions ($(CH_3)_3SnOH$) resulted in a better yield of 65% compared to that of 58% using BBr_3.



Scheme 4.15 (a) $(CH_3)_3SnOH$, DCE, 80 ^oC, 24 h, 65%. (b) BBr_3, CH_2Cl_2, -10 ^oC - rt, 18 h, 58%. (c) pyridine/chloroform (1:2), EDCI, HOBt, 8 h, 50%.

Analysis of the ^{1}H NMR spectrum of bisacid **284** indicated the loss of the resonances attributed to the diastereotopic benzylic protons (δ 5.05 and 5.70). In addition, the aromatic region of the spectrum contained less peaks, notably the loss of the downfield

singlet (δ 7.18, 1H, s) assigned as the proton attached to C2 of the aromatic ring of the tether. In addition, the emergence of a broad singlet at δ 8.71, with a relative integration of 2H, indicated the presence of the carboxylic acid protons. ESIMS (+ve) analysis of compound **284** showed peaks at *m/*z 1195 (40%, $[M+H]^+$) and 1200 (100%, $[M+Li]^+$) which were assigned as the protonated molecular ion and the lithiated molecular ion, respectively.

Attempts to synthesize the [*60*]fullerene bispeptoid **285** using the standard coupling reaction conditions developed in Section 4.2.5, failed, due to poor solubility of the bisadduct **284**. No progress in the coupling reaction was observed even after longer reaction times and an increase in reaction temperature to 60 °C. Therefore, a change in solvent to a 1:2 ratio of pyridine/chloroform was used resulting in a 50% yield of the desired dipeptide **285** (Scheme 4.15). Unfortunately, attempted deprotection of **285** with TFA gave an insoluble product that could not be characterized.

Confirmation of the structure of the bispeptide **285** was provided by analysis of its ^{1}H NMR spectrum. The loss of the broad singlet at δ 8.71 (bs, 2H) corresponding to the carboxylic acid protons of **284** and the appearance of a broad 2H multiplet at δ 7.08, assigned to the amide protons of **285**, indicating that the dipeptide had been formed. Furthermore, compound **285** showed a singlet (18H) at δ 1.28 ppm, assigned to the two Boc groups attached to the lysine side chains. The presence of a single peak for these two groups indicates that both peptide side chains are in an equivalent environment and this is consistent with the expected C_s symmetry of the *trans-4* disubstituted [*60*]-fullerene (Table 4.1).[175] The presence of 30 sp^2 resonances and 2 sp^3 resonances (32 signals in total) further confirms this symmetry. Analysis of the HRMS of **285** indicated

a peak at *m/z* 2884.1675, which was assigned to the sodiated species of **285**, $C_{174}H_{164}N_{16}O_{20}S_2Na$.

4.3.9 Solubility and Antimicrobial Testing

The solubilities of the dicationic salts **247**, **276-279** in DMSO/H_2O were determined (Table 4.2); they had relatively poor solubilities ranging from 70-350 µg/mL

Table 4.2. Solubility of deprotected salts in DMSO/H_2O

Compound number	**247**	**276**	**277**	**278**	**279**
Solubility in DMSO/H_2O	5% DMSO in H_2O (270 µg/mL)	5% DMSO in H_2O (135 µg/mL)	5% DMSO in H_2O (350 µg/mL)	5% DMSO in H_2O (145 µg/mL)	5% DMSO in H_2O (70 µg/mL)

As an initial screen for biological activity, the [*60*]fullerenyl peptides **243**, **245**, **272-275** and **285** and the dicationic salts **247, 276-279** were subjected to *in vitro* antibacterial testing (Table 4.3) under the supervision of Prof. Thomas Riley at the University of Western Australia. These twelve [*60*]fullerene derivatives were tested using the broth microdilution method against *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* ATCC 10418 with positive controls for *E. coli* NCTC 10418 being Kanamycin (4 µg/mL) and chloramphenicol (2 µg/mL) while the positive controls used for *S. aureus* NCTC 6571 were Kanamycin (4 µg/mL) and Vancomycin (1 µg/mL). Unfortunately all fullerenyl-based compounds were inactive, even at higher concentrations, with minimum inhibitory concentrations (MICs) greater than 100 to 340 (µg/mL), which correlated well with their solubilities.

Table 4.3. Microdilution testing of fullerene compounds

Compound	Highest test Concentration (μg/mL) *	MIC	
		S. aureus NCTC 6571	*E. coli* ATCC 10418
272	210	> 210	> 210
273	340	> 340	> 340
274	135	> 135	> 135
275	135	> 135	> 135
285	100	> 100	> 100
243	315	> 315	> 315
245	190	> 190	> 190
276	165	> 165	> 165
277	155	> 155	> 155
278	100	> 100	> 100
279	210	> 210	> 210
247	210	> 210	> 210

* Concentration may be an overestimation as compounds were not fully solubilised in DMSO at the highest test concentration, with the exception of compounds **247** and **279**.

4.3.10 Future directions and conclusions

The synthesis of peptides anchored by [*60*]fullerenes was successful with a series of monosubstituted and disubstituted fullerene derivatives being realised. Although these derivatives did not show any substantial anti-bacterial activity, their synthesis has progressed the development of the important field of fullerenyl amino acids and peptide derivatives, in particular, their synthesis and isolation as peptide salts. This is highlighted by the modest solubility of the deprotected compounds in DMSO/water (Table 4.2) and the relative ease of purification of the protected fullerenyl peptides by column chromatography. Further, these fullerenyl amino acids have a more rigid, and thus more defined, tethered structure than most other fullerenyl peptides reported, which traditionally employ a flexible fullerene-peptide linkers. These more rigid properties could become increasingly important in the fields of medicinal chemistry or materials science.

This work can be applied to the potential synthesis of other bisadducts of the type **285**, and trisadducts as well as their corresponding salts for the better aqueous solubility.

CHAPTER 5

Conclusions and Future Directions

This thesis has demonstrated the successful synthesis of [*60*]fullerenyl based peptides and oligomers (Chapters 2 and 3) that may have potential solar cell applications. We also demonstrated the synthesis and biological screening of dicationic peptoids (Chapter 4) as possible antibacterial agents.

Chapter 2 has demonstrated the synthesis of the novel [*60*]fullerene monopeptide **201** and dipeptide **204** (Figure 5.1). [*60*]Fullerenoproline acid **198** was coupled to lysine amine **200** to yield the monopeptide **201**, which was deprotected to its amine salt **202** using TFA and to its acid derivative **203** with $Sn(CH_3)_3OH$. An amide coupling reaction of the amine **202** and the acid **203** resulted in the dipeptide **204**. Monopeptide **201** and the dipeptide **204** have been studied experimentally using spectroelectrochemical measurements with collaborators Prof. Timothy Clark and Prof. Dirk M. Guldi at the University of Erlangen-Nuremberg, Germany. The bis-fullerene-substituted peptide **204** provided experimental support for density-functional theory (DFT) calculations which indicate that van der Waals fullerene dimers can form between adjacent fullerenes in semiconductor layers resulting in interstitial electron traps.[142]

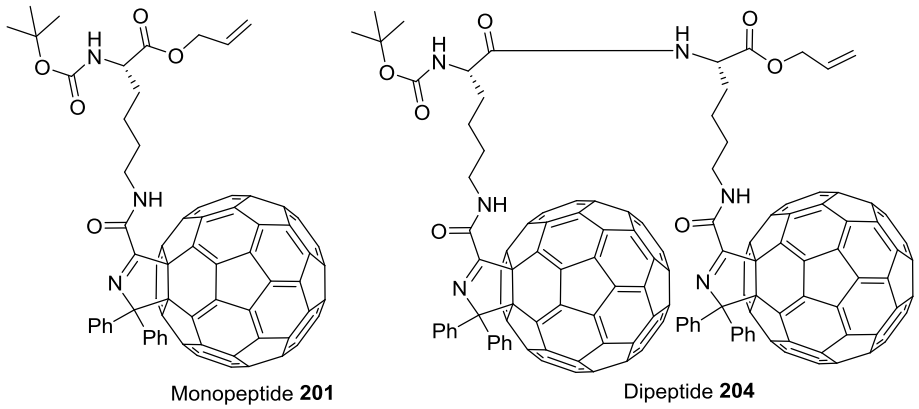


Figure 5.1: Structures of monopeptide **201** and dipeptide **204**

With the aim of preparing the tetrapeptide **207** (Figure 5.2), the dipeptide **204** was separately deprotected to the amine **205** and the acid **206**. A range of amide coupling reaction conditions were employed to couple these two components. These reactions were not successful in providing a pure sample of the tetrapeptide **207**. This material was difficult to purify and analyse because of its poor solubility properties.

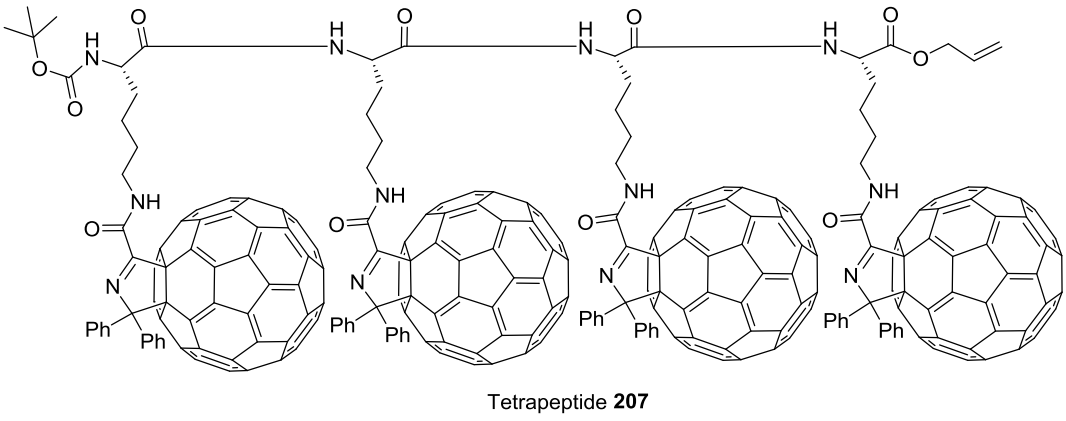


Figure 5.2: Structures of tetrapeptide **207**

An alternative synthesis of the tetrapeptide **207** was examined. The lysine tetrapeptide **216** and its tetramine **217** (Figure 5.3) were successfully synthesised. Attempts to couple **217** with [*60*]fullerenoproline acid **198** to produce tetrapeptide **207** were also unsuccessful, possibly due to steric hindrance problems because of the sterically demanding fullerene groups.

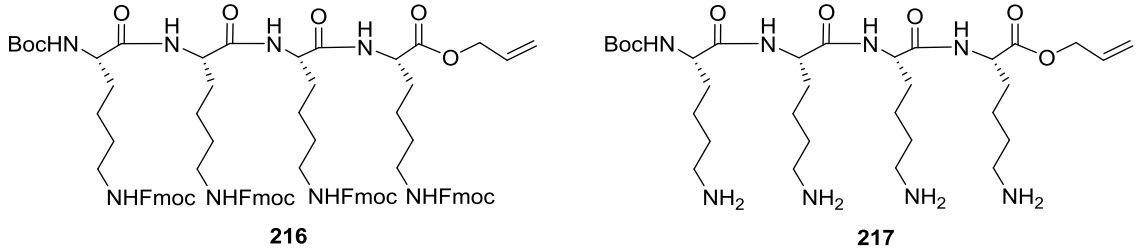


Figure 5.3: Structures of lysine tetrapeptide **216** and its tetramine **217**

Accordingly, it was postulated that the cyclic peptide tetrapeptide **220** (Figure 5.4) might help to solve these steric hindrance problems. Tetrapeptide **216** was successfully converted to the novel cyclic peptide **220** in three synthetic steps. However this route was not pursued because of the poor chemical yields and the difficulties with purifying **220**.

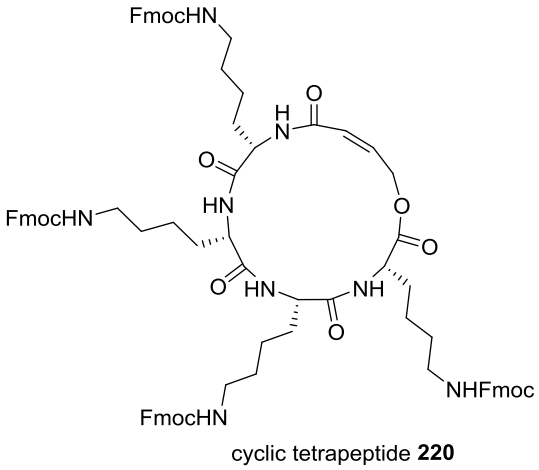
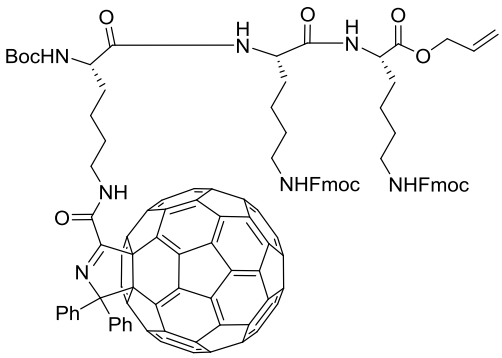


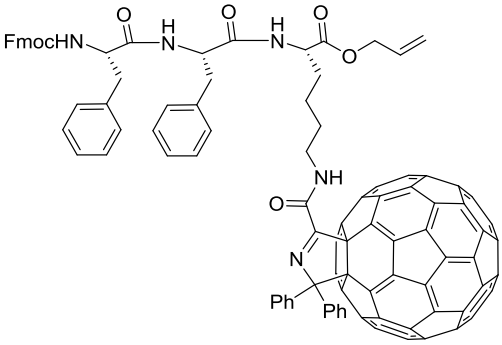
Figure 5.4: Structures of cyclictetrapeptide **220**

Chapter 3 reported an extension of [*60*]fullerenyl oligomers with the incorporation of spacers between the fullerenyl groups. Two types of amino acid spacers (L-lysine and L-phenylalanine) were introduced in to the oligomers (hexapeptide **227** and hexapeptide **238**, Figure 5.5). The synthesis of tripeptides with L-lysine spacers, **224**, and L-phenylalanine spacers **235** were achieved by coupling the acid **203** with the amine **213** and the amine **202** with the acid **234**, respectively. The previously used ester and *N*-Boc

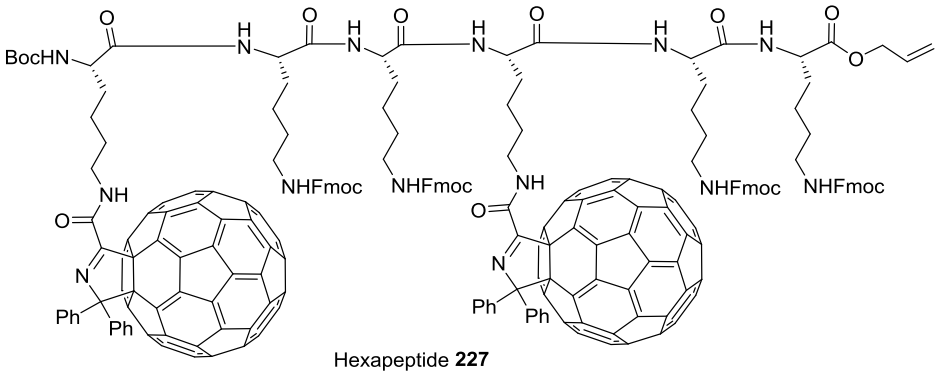
cleavage conditions were adopted to synthesise the corresponding acids (**225** and **236**) and amines (**226** and **237**) of **224** and **235** followed by amide couplings to provide the hexapeptides of **227** and **238**. Currently, these molecules are under study to form supramolecular complexes between [*60*]fullerene and porphyrin, and photoinduced charge separation experiments in Prof. Fukuzumi's laboratory at Osaka University, Japan.



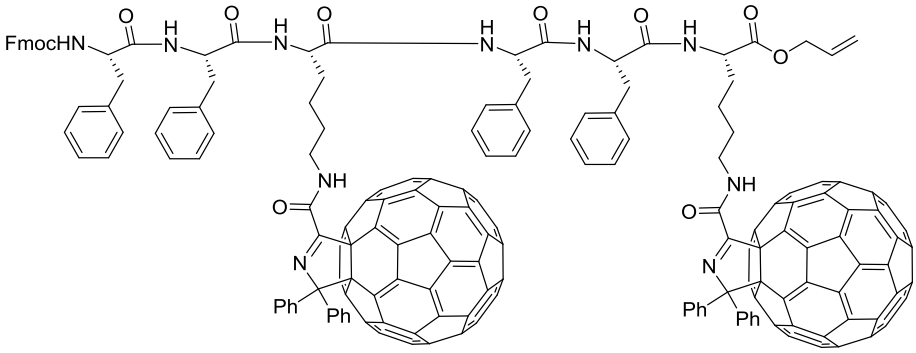
Tripeptide **224**



Tripeptide **235**



Hexapeptide **227**



Hexapeptide **238**

Figure 5.5: Structures of tripeptides **224** and **235**, and hexapeptides **227** and **238**

Chapter 4 reported the synthesis and antibacterial screening of mono substituted [*60*]fullerenyl peptides (**243**, **245**, **247**, **272-279**, Figure 5.6) and bis-[*60*]fullerenyl peptide (**285**). A range of tri, tetra and pentapeptides having an oxazole or isopentyl ester terminal group were synthesised. These peptides were then deprotected to form their HCl and TFA salts **247**, **276-279**. Antibacterial screening of these peptides (**243**, **245**, **247**, **272-279** and **285**) was performed at the University of Western Australia in Prof. Thomas Riley's laboratory, unfortunately, none of these compounds showed significant activity.

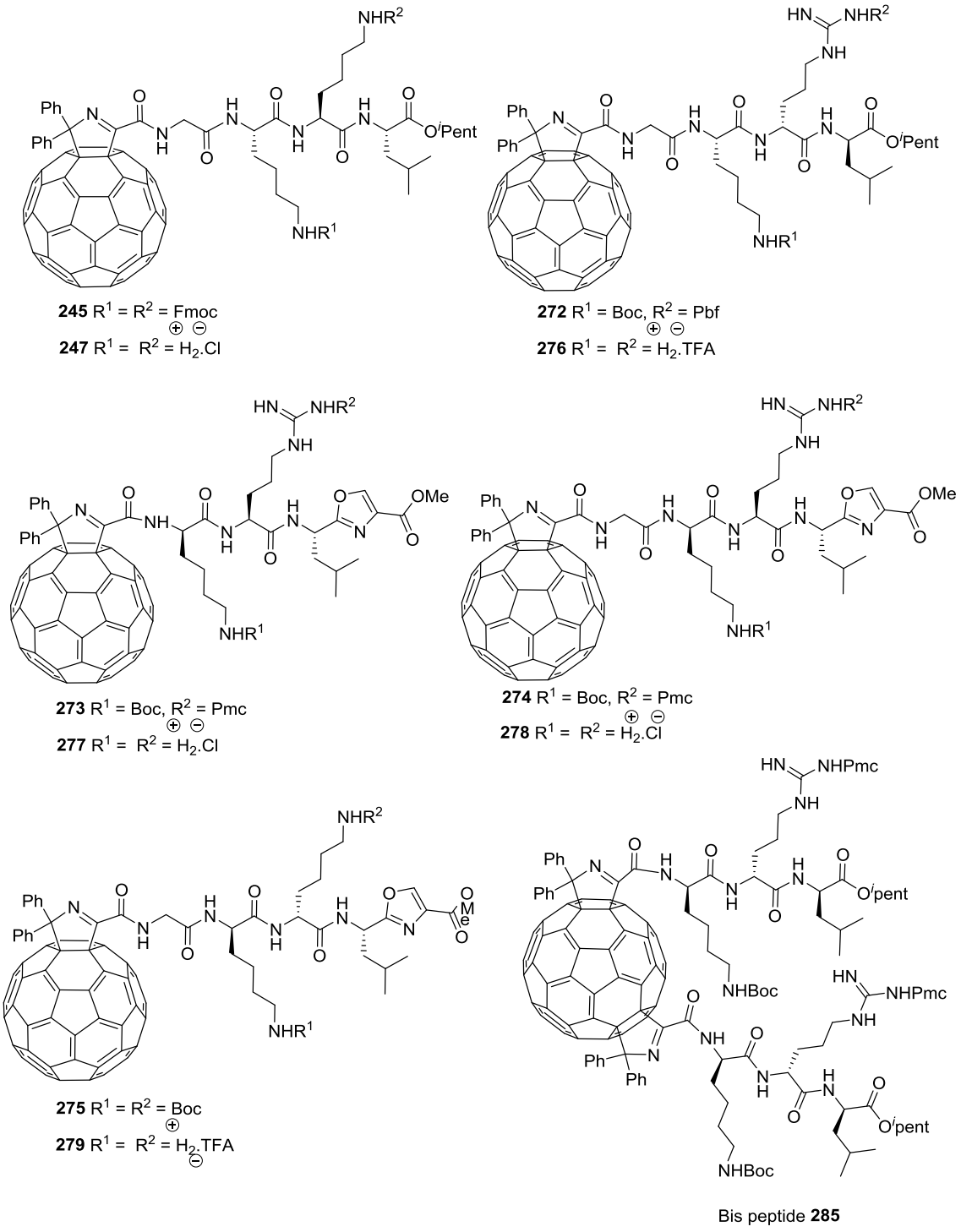
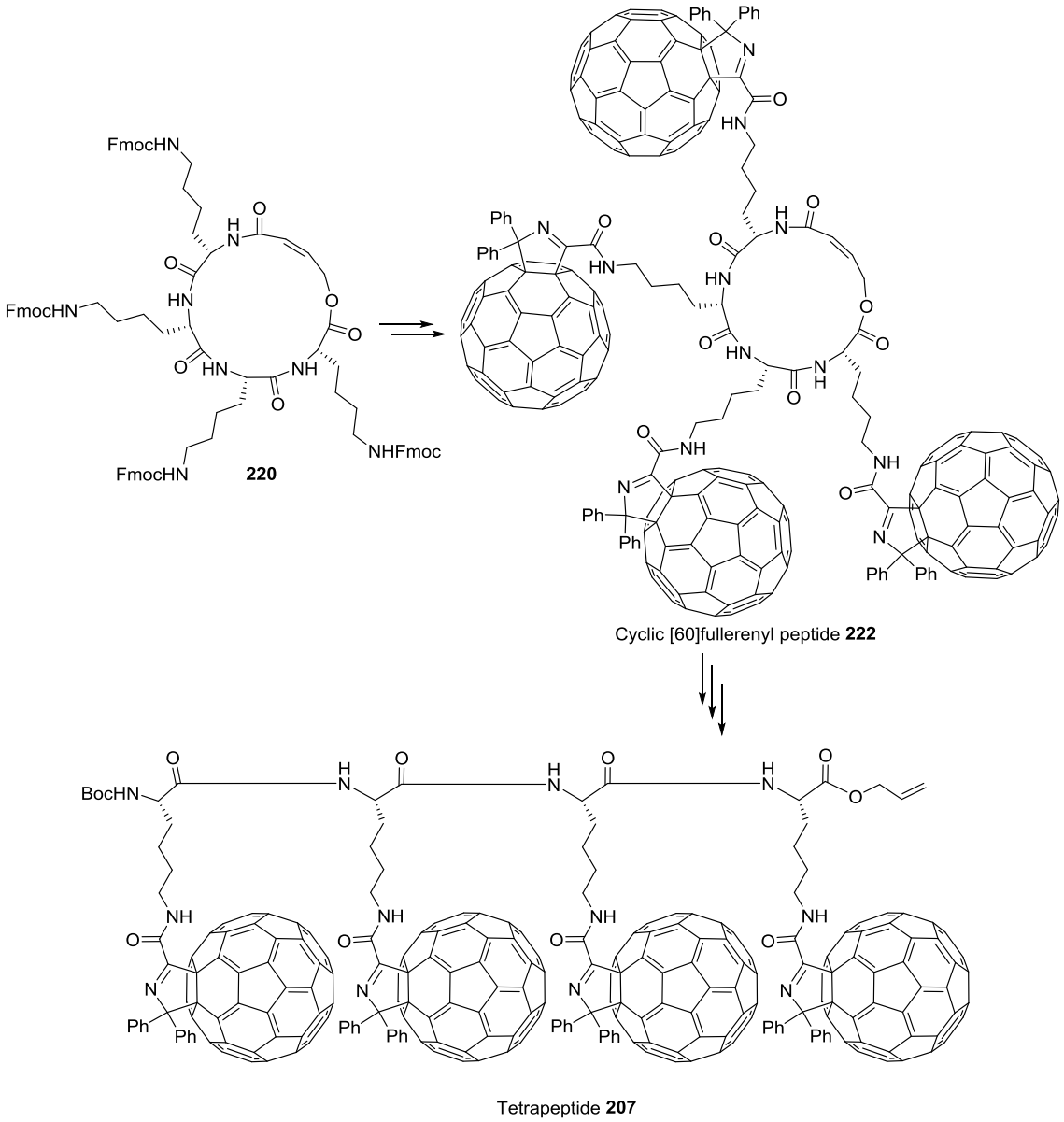


Figure 5.6: Structures of [*60*]fullerene-peptides **245**, **247**, **272-279** and **285**

In future work, a cyclic peptide synthetic route leading to the synthesis of compound **222** (Scheme 2.15, Chapter 2) followed by ring opening metathesis could be pursued depending on the availability of the starting material to achieve the tetrapeptide **207** in pure form.

Density functional theory calculations indicate that van der Waals fullerene dimers and larger oligomers can form interstitial electron traps in which the electrons are even more strongly bound than in isolated fullerene radical anions. Spectroelectrochemical measurements on the bis-fullerene-substituted peptide **204** provide experimental support. In the future, larger oligomers, including tetrapeptides **207**, **227** and **238**, could be studied for their spectroelectrochemical properties and these could possibly show better efficiencies as electron traps.

Currently, studies on the formation of supramolecular complexes between our [*60*]fullerene-peptides (**227** and **238**) and porphyrin are going on in Prof. Fukuzumi's laboratory at Osaka University, Japan. Based on the pending results, other spacers (Chapter 3) could be incorporated in to the [*60*]fullerene oligomer to which have less steric hindrance and are viable to synthesise in pure form to enhance the desired complexation and photoinduced charge separation properties.



Scheme 5.1: Proposed synthetic strategy to achieve tetrapeptide **207** from the cyclic tetrapeptide **222**.

There is wide scope for the synthesis of a variety of peptides or peptidomimetic replacements to incorporate in to the [*60*]fullerene to form more water soluble peptoids (Chapter 4) of the type **286** and **287** (Figure 5.7), hopefully leading to increased antibacterial activities. Expansion of substitution on to the [*60*]fullerene to form tris adducts **288** can also be considered for the better solubility and biological activity.

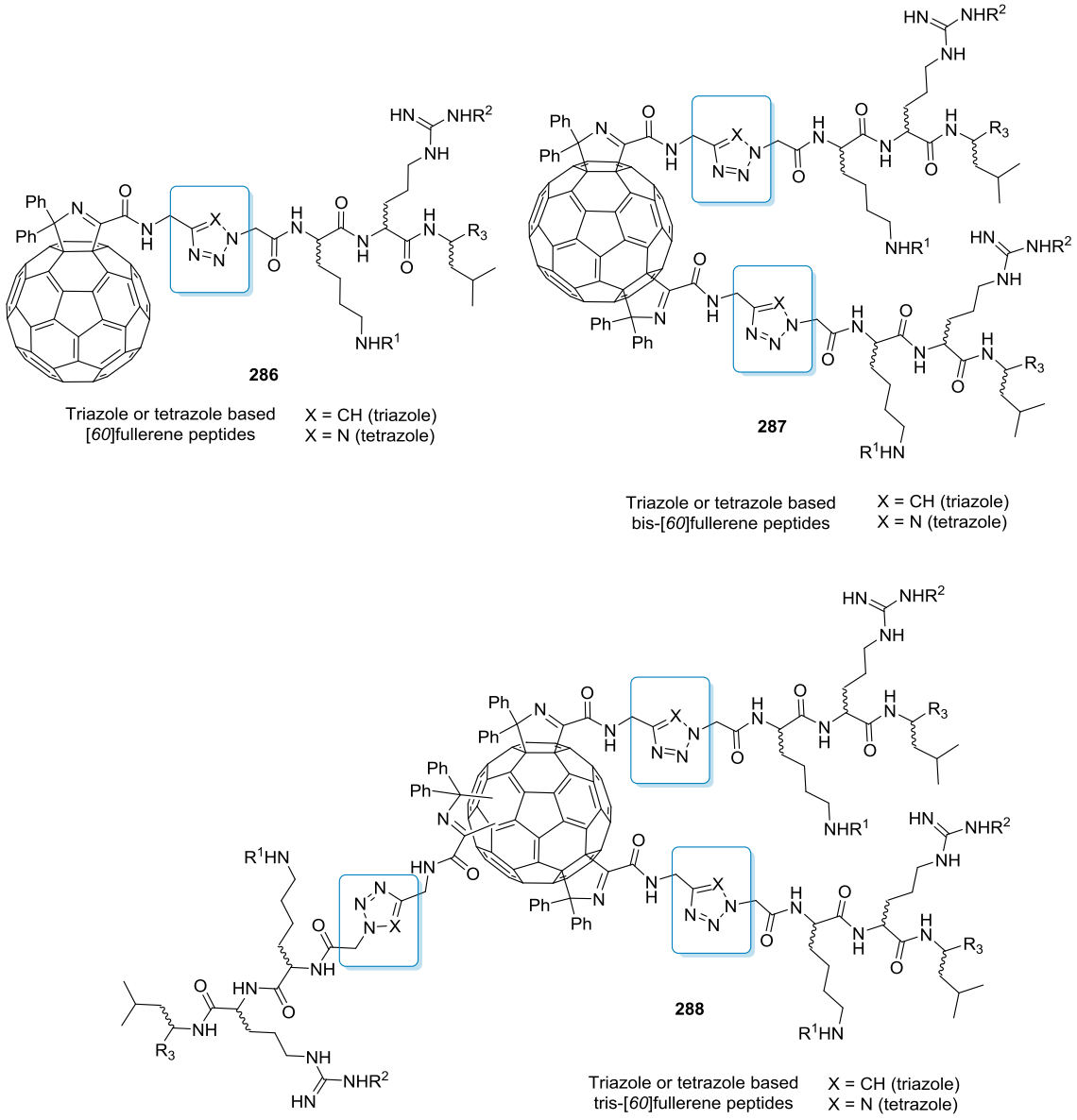
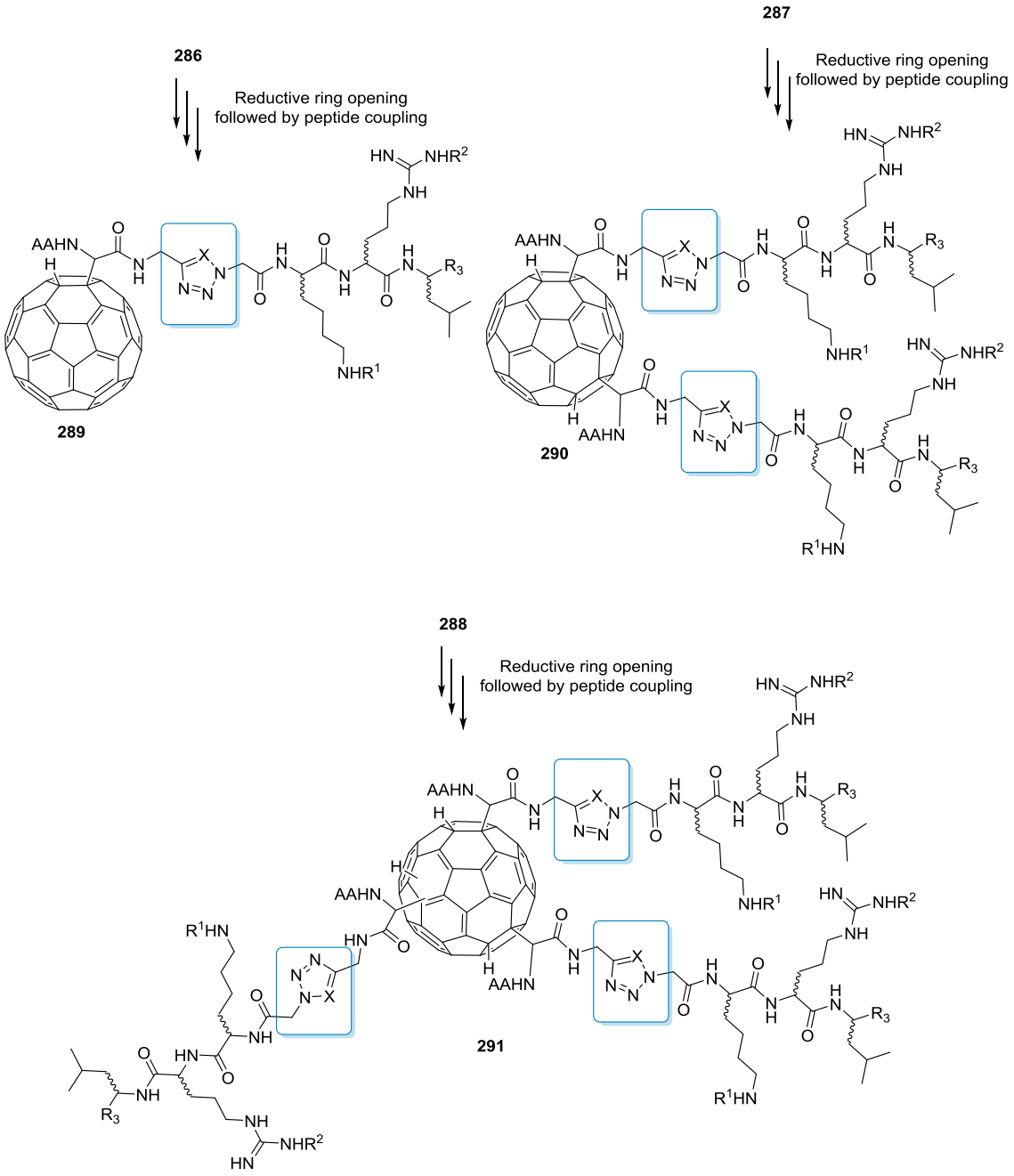


Figure 5.7: Structures of extended water soluble group incorporation in to the [*60*]fullerene mono, bis and tris peptides.

Reductive ring opening of compounds **286**, **287** and **288** followed by peptide coupling could provide ring-opened fullerenyl derivative **289**, **290** and **291** (Scheme 5.2), which are consider to be true fullerenyl "capped" amino acids and could potentially provide better water solubilities and biological activities.



Scheme 5.2: Proposed synthetic strategy of mono, bis and tris ring opened [*60*]fullerenyl "capped" peptides **289**, **290** and **291**.

CHAPTER 6

Experimental

6.1 General Experimental Procedures

Reagents and solvents were purchased reagent-grade and used without further purification. Toluene and THF were distilled from sodium benzophenone ketyl under a nitrogen atmosphere. CH_2Cl_2 was stored over $CaCl_2$ and distilled from CaH_2. MeCN was distilled from potassium carbonate. [60]Fullerene was purchased from MER Corporation Tucson, Arizona AZ 85706, USA. All reactions were performed in standard glassware under an atmosphere of nitrogen unless otherwise specified. Microwave reactions were performed using a Discover CEM Focused Microwave Synthesis System in 10 mL closed vessels. Flash column chromatography was performed using silica 60 (230-400 mesh, 0.040-0.063 mm) purchased from Merck. Petroleum spirit refers to a hydrocarbon fraction with a boiling point of 40-60 ^{o}C. The mass spectra of a fullerenyl derivatives were run on a Thermo Finnigan LTQ (Waltham, MA) fitted with a conventional IonMax electrospray ionization source. Spectra were obtained by infusion of a standard solution in $CHCl_3$/MeOH (2:1). Typical settings were spray voltages between 4 – 6 kV, capillary temperature 270 ^{o}C and sheath gas flow rates at 10 (arbitrary units). High resolution mass spectra were obtained using a QTOF mass spectrometer. ^{1}H NMR spectra were acquired on a Varian Unity 300 or 500 spectrometer at 300.1 and 499.9 MHz, respectively. ^{13}C NMR spectra were acquired on Varian Unity 300 or 500 spectrometer at 75.4 and 125.0 MHz, respectively. Deuterated solvents $CDCl_3$, CD_3OD, $(CD_3)_3SO$ were obtained commercially (Sigma-Aldrich or

Cambridge) and were greater than 99.5 atom % d. All chemical shifts are reported relative to TMS (δ 0.00).

^{1}H NMR spectral data are listed in the following order, chemical shift, multiplicity, integration, *J* value, and assignment. All fullerenyl carbon signals were reported as either a full resonance, (e.g. 148.0), or as a half-intensity peak denoted by a * or as an integer of a full resonance when there is peak overlap (e.g. 148.0, (2 x C)). When a reference is provided in the compound title it signifies that this reference procedure was used. Conversely, when a reference is provided with NMR spectroscopic data it signifies that our data is in agreement with those already reported using a different procedure.

Melting points (m.p.) were determined using a Gallenkamp (Griffin) melting point apparatus. Temperatures are expressed in degrees Celsius (°C) and are uncorrected. Optical rotations were measured using a Jasco polarimeter with a 10 mm path length and a wavelength of 589 nm. Concentrations are expressed as *c* (10 mg/mL). Infrared (IR) spectra were recorded using neat samples on a Nicolet Avatar 360 FT-IR spectrometer fitted with a Smart Omni-Sampler germanium crystal accessory.

Protocol 1: Peptide coupling

To a solution of the acid in CH_2Cl_2 (10 mL/0.10 mmol) at rt was added EDCI (1.2 equiv.), HOBt (1.2 equiv.), and the amine (1 equiv.). If the amine was a hydrochloride or TFA salt, Et_3N (1.2 equiv.) was also added. After stirring for 3-6 h, the solvent was removed under reduced pressure, and then the resulting residue was subjected to silica gel column chromatography MeOH/CH_2Cl_2 (0.5:99.5-1:99 for peptides and 1:49-1:4 for C_{60} attached peptides) as the eluent to afford the coupled product.

Protocol 2: *N*-Fmoc deprotection

The Fmoc-protected amine was stirred in 1 equiv. of piperidine/acetonitrile (5 mL/0.10 mmol) overnight at rt, unless otherwise stated. The solvent was removed under reduced pressure and the residue was subjected to column chromatography, using MeOH/CH_2Cl_2 (1:19) to yield the free amine.

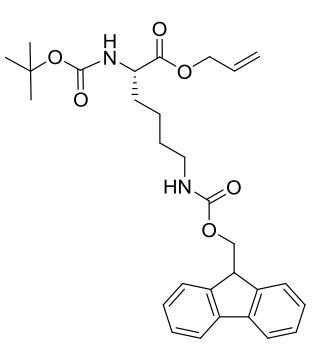
Protocol 3: *N*-Boc, Pbf and Pmc deprotection

The *N*-Boc, Pbf or Pmc protected amine was stirred for 4 h (for Boc) or overnight (for Pbf and Pmc) in 1:1 CH_2Cl_2/TFA (6 mL/0.10 mmol) solution at rt. The solvent was removed under reduced pressure, and the residue was resuspended in a minimal volume of CH_2Cl_2. The solution was then treated with an excess amount of 1 M HCl/diethyl ether (2 mL/0.01 mmol) solution and the solvent evaporated. The product was obtained by precipitation from CH_2Cl_2, with hexane and/or diethyl ether.

Protocol 4: Allyl and bis-1,3-benzyl ester hydrolysis

To a solution of the ester in 1,2-dichloroethane (100 mL/0.10 mmol) at rt was added $Sn(CH_3)_3OH$ (4 equiv.) and the solution was heated at 80 ^oC for 4 h, then further $Sn(CH_3)_3OH$ (4 equiv.) was added at the same temperature and the reaction was continued for 4 h. The reaction mixture was evaporated under reduced pressure and the resulting residue was taken up in CH_2Cl_2 (15-20 mL). The organic layer was washed with 5% HCl (3 x 10-15 mL), brine (3 x 10 mL) dried (Mg_2SO_4) and evaporated under reduced pressure to yield the corresponding acid.

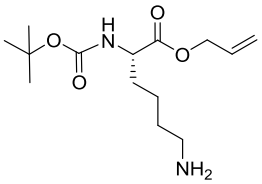
(*S*)-Allyl6-(9*H*-fluoren-9-yl)methoxycarbonylamino-2-(*tert*-butoxycarbonyl) aminohexanoate (199)



To a suspension of N^6-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^2-(*tert*-butoxycarbonyl)-*L*-lysine (1.20 g, 2.56 mmol) in CH_2Cl_2 (50 mL) was added HOBt (0.415 g, 3.07 mmol) and EDCI (0.587 g, 3.07 mmol) and the mixture was stirred for 15 min before a solution of allyl alcohol (1.74 mL, 25.6 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The resulting solution was stirred at rt for 14 h before the solvent was removed under reduced pressure. The crude residue was then subjected to flash silica gel chromatography, gradient elution with EtOAc/hexane (1:9 to 1:1) provided the title compound **199** as a colourless solid (0.954 g, 73%). m.p: 118-120 $^{\circ}$C; $[\alpha]_D^{25}$ -32.8 (*c* 4.16, $CHCl_3$); IR (neat, cm^{-1}): 3331 (w), 1722 (s), 1679 (s), 1530 (s); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.39-1.40 (m, 2H, $CHCH_2C\underline{H}_2$), 1.44 (s, 9H, Boc), 1.49-1.56 (m, 2H, $C\underline{H}_2CH_2NH$), 1.62-1.70 (m, 1H, $CHC\underline{H}HCH_2$), 1.80-1.84 (m, 1H, $CHCH\underline{H}CH_2$), 3.18-3.19 (m, 2H, $C\underline{H}_2N$ (Lys)), 4.21 (t, *J* = 6.3 Hz, 1H, CH (Fmoc)), 4.32 (bs, 1H, Lys-α-proton), 4.40 (d, *J* = 6.3 Hz, 2H, $OC\underline{H}_2$ (Fmoc)), 4.61-4.64 (m, 2H, $OC\underline{H}_2$), 4.81 (bs, 1H, $N\underline{H}$Boc), 5.04 (bs, 1H, $N\underline{H}$Fmoc), 5.25 (d, *J* = 10.5 Hz, 1H, $CH=C\underline{H}(Z)H$), 5.32 (d, *J* = 17.5 Hz, 1H, $CH=C\underline{H}(E)H$), 5.89-5.90 (m, 1H, $C\underline{H}=CH_2$), 7.31 (t, *J* = 7.0 Hz, 2H, $ArC\underline{H}$), 7.39 (t, *J* = 7.5 Hz, 2H, $ArC\underline{H}$), 7.59 (d, *J* = 7.5 Hz, 2H, $ArC\underline{H}$), 7.76 (d, *J* = 7.5 Hz, 2H, $ArC\underline{H}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 22.8 ($CHCH_2\underline{C}H_2$), 28.2 ($C(\underline{C}H_3)_3$), 29.0 ($\underline{C}H_2CH_2NH$), 32.4 ($CH\underline{C}H_2CH_2$), 39.8 ($\underline{C}H_2NH$), 47.2 ($\underline{C}H$ (Fmoc)), 53.2 ($CO\underline{C}HNH$), 65.8 ($\underline{C}H_2CH=CH_2$), 66.5 ($\underline{C}H_2$ (Fmoc)), 79.9 ($\underline{C}(CH_3)_3$), 118.9 ($CH=\underline{C}H_2$), 120.3 (2 x $Ar\underline{C}H$), 125.1 (2 x $Ar\underline{C}H$), 128.4 (2 x $Ar\underline{C}H$), 129.7 (2 x $Ar\underline{C}H$), 131.5 ($\underline{C}H=CH_2$),

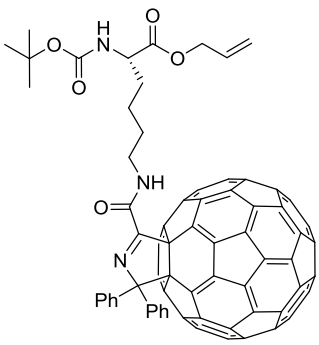
141.3 (2 x ArC), 142.5 (2 x ArC), 156.2 (CO (Boc)); 162.1 (CONH), 172.3 (CO, ester); HRMS (ESI +ve) calcd for $C_{29}H_{36}N_2O_6Na$, 531.2471, found 531.2446.

(*S*)-Allyl 6-amino-2-(*tert*-butoxycarbonyl)aminohexanoate (200)



To a suspension of allyl N^6-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^2-(*tert*-butoxycarbonyl)-*L*-lysinate **199** (0.52 g, 1.02 mmol) in acetonitrile (30 mL) was added piperidine (0.072 mL, 1.22 mmol) and the mixture was stirred at rt for 14 h before the solvent was removed under reduced pressure. The residue (0.292 g, 100%) was then taken through the next reaction without further purification. ESI-MS (+ve): *m/z* 287 (100%, $M+H^+$).

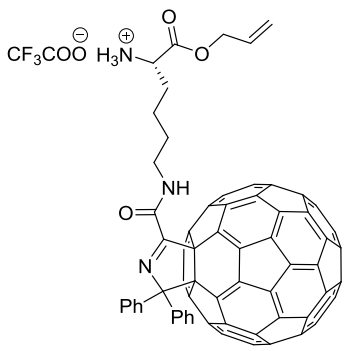
(*S*)-Allyl-6-(5,5-diphenylfullerenyldihydropyrrole-2-carbonylamino)-2-(*tert*-butoxycarbonyl)aminohexanoate (201)



This compound was prepared *via* protocol 1 using 5,5-diphenylfullerenyldihydropyrrole-2-carboxylic acid **198** (0.180 g, 0.187 mmol) and (*S*)-allyl 6-amino-2-(*tert*-butoxycarbonyl)aminohexanoate **200** (0.064 g, 0.224 mmol) to yield the title compound **201** as a brown solid (0.142 g, 62%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23-1.25 (m, 2H, $CHCH_2C\underline{H}_2$), 1.44 (s, 9H, Boc), 1.48-1.58 (m, 2H, $C\underline{H}_2CH_2NH$), 1.68-1.82 (m, 2H, $CHC\underline{H}_2CH_2$), 3.52-3.58 (m, 2H, $C\underline{H}_2N$ (Lys)), 4.36 (bs, 1H, Lys-α-proton), 4.58-4.64 (m, 2H, $OC\underline{H}_2$), 5.04 (bs, 1H, N<u>H</u>Boc), 5.28 (d, *J* = 10.0 Hz, 1H, CH=C<u>H</u>(*Z*)H), 5.30 (d, *J* = 17.0 Hz, 1H, CH=C<u>H</u>(*E*)H), 5.84-5.94 (m, 1H, C<u>H</u>=CH_2), 7.42 (t, *J* = 7.5 Hz, 2H, ArC<u>H</u>), 7.53 (t, *J* = 7.5 Hz, 4H, ArC<u>H</u>); 7.88 (bs, 1H, NH), 8.43 (d, *J* = 7.5 Hz, 4H, ArC<u>H</u>); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 22.8 ($CHCH_2\underline{C}H_2$), 28.2 ($C(\underline{C}H_3)_3$), 29.0 ($\underline{C}H_2CH_2NH$), 32.4

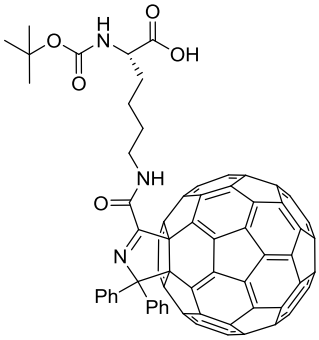
(CH<u>C</u>H$_2$CH$_2$), 39.8 (<u>C</u>H$_2$NH), 53.2 (NH<u>C</u>HCO), 65.8 (O<u>C</u>H$_2$), 79.9 (<u>C</u>(CH$_3$)$_3$), 82.2* ($C_{60}sp^3$), 83.3* ($C_{60}sp^3$), 95.3 (<u>C</u>Ph$_2$), 118.9 (CH=<u>C</u>H$_2$), 128.4 (2 x Ar<u>C</u>H), 129.7 (Ar<u>C</u>H), 131.5 (<u>C</u>H=CH$_2$), 134.7 (Ar<u>C</u>), [136.5, 139.0, 139.5, 140.8, 141.2, 141.7, 141.76, 141.8, 142.3, 142.5, 142.6, 142.7, 142.9, 144.0, 144.3, 144.8, 145.0, 145.1, 145.3 (2 x C), 145.4, 145.7, 145.9, 146.0, 146.3, 146.8*, 147.0*, 148.5, 149.1, 153.1 ($C_{60}sp^2$)]; 155.3 (<u>C</u>=N), 161.2 (<u>C</u>O, Boc); 162.1 (<u>C</u>ONH), 172.3 (<u>C</u>O, ester); HRMS (ESI +ve) calcd for $C_{89}H_{35}N_3O_5Na$, 1248.2474, found 1248.2494.

(*S*)-6-(5,5-Diphenylfullerenyldihydropyrrole-2-carbonylamino)-1-(allyloxy)-1-oxohexan-2-aminium 2,2,2-trifluoroacetate (202)



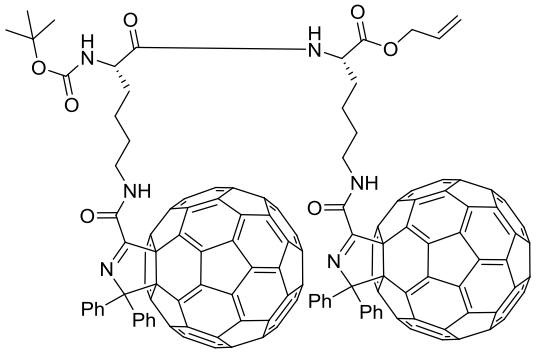
This compound was prepared *via* protocol 3, using **201** (0.10 g, 0.081 mmol) to afford the title compound **202** (0.082 g, 90%) as a fine brown solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.25-1.28 (m, 2H, CHCH$_2$C<u>H</u>$_2$), 1.46-1.62 (m, 2H, C<u>H</u>$_2$CH$_2$NH), 1.78-1.82 (m, 2H, CHC<u>H</u>$_2$CH$_2$), 3.45-3.56 (m, 2H, C<u>H</u>$_2$N (Lys)), 4.06 (bs, 1H, Lys-α-proton), 4.62 (bs, 2H, OC<u>H</u>$_2$), 5.25-5.38 (m, 2H, CH=C<u>H</u>$_2$), 5.80-5.92 (m, 1H, C<u>H</u>=CH$_2$), 7.40 (t, *J* = 7.5 Hz, 2H, ArC<u>H</u>), 7.51 (t, *J* = 7.5 Hz, 4H, ArC<u>H</u>), 7.84 (bs, 1H, NH), 8.02 (d, *J* = 7.5 Hz, 4H, ArC<u>H</u>); ESI-MS (+ve): *m/z* 1126 (100%, M+H$^+$). This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

(*S*)-6-(5,5-Diphenylfullerenyldihydropyrrole-2-carbonylamino)-2-(*tert*-butoxy carbonyl)aminohexanoic acid (203)



The titled compound was prepared *via* protocol 4 using **201** (0.080 g, 0.065 mmol), 1,2-dichloroethane (80 mL) and $(CH_3)_3SnOH$ (0.047 g, 0.260 mmol x 2) to yield the title **203** as a brown solid (0.068 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23-1.25 (m, 2H, $CHCH_2C\underline{H}_2$), 1.45 (s, 9H, Boc), 1.49-1.59 (m, 2H, $C\underline{H}_2CH_2NH$), 1.66-1.82 (m, 2H, $CHC\underline{H}_2CH_2$), 3.50-3.60 (m, 2H, $C\underline{H}_2N$ (Lys)), 4.38 (bs, 1H, Lys-α-proton), 5.02 (bs, 1H, N<u>H</u>Boc), 7.40 (t, *J* = 7.5 Hz, 2H, ArC<u>H</u>), 7.56 (t, *J* = 7.5 Hz, 4H, ArC<u>H</u>); 7.88 (bs, 1H, NH), 8.02 (d, *J* = 7.5 Hz, 4H, ArC<u>H</u>); HRMS (ESI +ve) calcd for $C_{86}H_{31}N_3O_5Na$, 1208.2161, found 1208.2145. This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

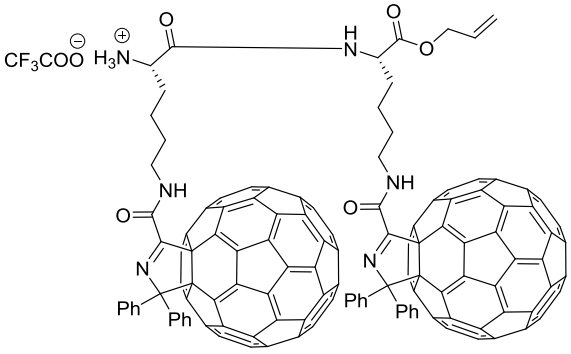
(*S*)-Allyl-6-((5,5-diphenylfullerenyldihydropyrrole-2-carbonyl)amino)-2-(*S*)-6-(5,5-diphenylfullerenyldihydropyrrole-2-carbonylamino)-2-(*tert*-butoxycarbonyl) aminohexanamido)hexanoate (204)



This compound was prepared *via* protocol 1, using **202** (0.056 g, 0.047 mmol) and **203** (0.053 g, 0.047 mmol) 0.056 mmol) to yield the title compound as a brown solid (0.062 g, 57%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08-1.18 (m, 2H, $CHCH_2C\underline{H}_2$), 1.20-1.30 (m, 2H, $CHCH_2C\underline{H}_2$), 1.45 (s, 9H, Boc), 1.48-1.58 (m, 4H, $C\underline{H}_2CH_2NH$), 1.60-1.98 (m, 4H, $CHC\underline{H}_2CH_2$), 3.40-3.58 (m, 4H, $C\underline{H}_2N$ (Lys)), 4.06 (bs, 1H, Lys-α-proton), 4.58-

4.68 (m, 3H, OC<u>H</u>$_2$ and Lys-α-proton), 5.04 (bs, 1H, N<u>H</u>Boc), 5.28 (d, *J* = 10.5 Hz, 1H, CH=C<u>H</u>(*Z*)H), 5.30 (d, *J* = 17.0 Hz, 1H, CH=C<u>H</u>(*E*)H), 5.82-5.94 (m, 1H, C<u>H</u>=CH$_2$), 6.68 (bs, 1H, NH), 7.40 (t, *J* = 7.5 Hz, 4H, ArC<u>H</u>), 7.48-7.58 (m, 8H, ArC<u>H</u>); 7.80-7.98 (m, 2H, 2 x NH), 8.08 (d, *J* = 7.5 Hz, 8H, ArC<u>H</u>); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 22.8 (CHCH$_2$<u>C</u>H$_2$), 23.0 (CHCH$_2$<u>C</u>H$_2$), 28.6 (C(<u>C</u>H$_3$)$_3$), 29.0 (<u>C</u>H$_2$CH$_2$NH), 29.2 (<u>C</u>H$_2$CH$_2$NH), 32.4 (CH<u>C</u>H$_2$CH$_2$), 40.0 (2 x <u>C</u>H$_2$NH), 52.1 (NH<u>C</u>HCO), 66.3 (O<u>C</u>H$_2$), 80.8 (<u>C</u>(CH$_3$)$_3$), 82.5* ($C_{60}sp^3$), 83.6* ($C_{60}sp^3$), 95.6 (<u>C</u>Ph$_2$), 119.3 (CH=<u>C</u>H$_2$), 128.7 (2 x Ar<u>C</u>H), 130.0 (Ar<u>C</u>H), 131.6 (<u>C</u>H=CH$_2$), 135.0 (Ar<u>C</u>), [136.6, 139.3, 139.8, 141.1, 141.5, 142.01, 142.04, 142.1, 142.6, 142.7, 142.97, 143.0, 143.1, 144.3, 144.5, 145.0, 145.3, 145.6, 145.7 (2 x C), 146.0, 146.2, 146.3*, 146.5*, 147.1, 147.3, 148.8, 149.4, 149.42, 153.4 ($C_{60}sp^2$)],; 155.3 (<u>C</u>=N), 161.6 (<u>C</u>O, Boc), 161.7 (<u>C</u>ONH), 162.7 (<u>C</u>ONH), 171.9 (<u>C</u>O, ester); HRMS (ESI +ve) calcd for $C_{170}H_{56}N_6O_7Na$, 2315.4108, found 2315.4114.

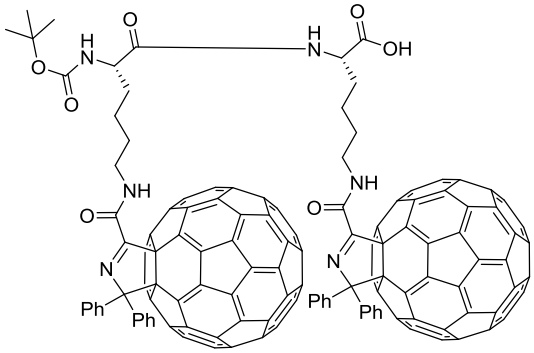
(*S*)-1-(*S*)-1-Allyloxy-6-(5,5-diphenylfullerenyldihydropyrrole-2-carboxamido)-1-oxohexan-2-ylamino)-6-(5,5-diphenylfullerenyldihydropyrrole-2-carboxamido)-1-oxohexan-2-ammonium trifluoroacetate (205)



This compound was prepared *via* protocol 3, using **204** (0.025 g, 0.010 mmol) to afford the title compound **205** (0.020 g, 86%) as a amorphous brown solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.21-1.35 (m, 4H, CHCH$_2$C<u>H</u>$_2$), 1.58-1.90 (m, 8H, C<u>H</u>$_2$CH$_2$NH and CHC<u>H</u>$_2$CH$_2$), 3.35-3.58 (m, 6H, 2x C<u>H</u>$_2$N (Lys), 2 x Lys-α-proton), 4.60-4.62 (m, 2H, OC<u>H</u>$_2$), 5.28 (d, *J* = 10.5 Hz, 1H, CH=C<u>H</u>(*Z*)H), 5.30 (d, *J*

= 17.0 Hz, 1H, CH=C<u>H</u>(*E*)H), 5.82-5.94 (m, 1H, C<u>H</u>=CH_2), 7.38-7.58 (m, 4H, ArC<u>H</u>), 7.60-7.68 (m, 8H, ArC<u>H</u>), 7.78-7.98 (m, 2H, N<u>H</u>), 8.02-8.06 (m, 8H, ArC<u>H</u>); HRMS (ESI +ve) calcd for $C_{165}H_{48}N_6O_5$, 2193.3794, found 2193.3764. This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

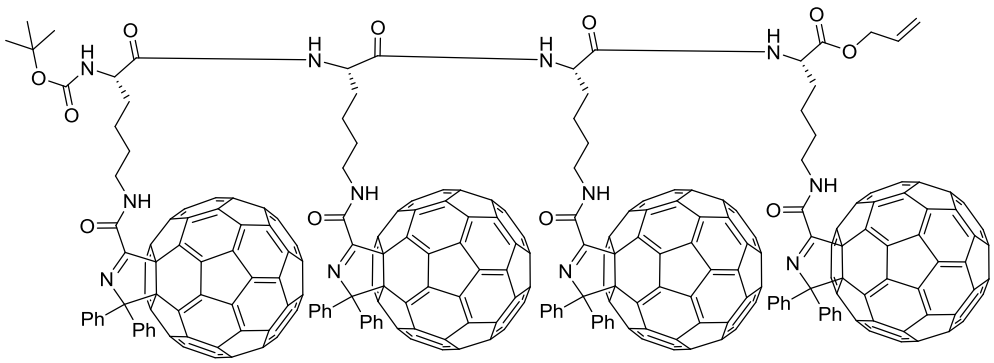
(*S*)-6-((5,5-Diphenylfullerenyldihydropyrrole-2-carbonyl)amino)-2-((*S*)-6-((5,5-diphenylfullerenyldihydropyrrole-2-carbonyl)amino)-2-((*tert*-butoxycarbonyl)amino)hexanamido)hexanoic acid (206)



This compound was prepared *via* protocol 4, using **204** (0.025 g, 0.010 mmol), 1,2-dichloroethane (30 mL) and $(CH_3)_3SnOH$ (0.008 g, 0.04 mmol x 2) to yield the title compound **206** (0.018 g, 75%) as a brown solid. ^1H NMR (500 MHz, $CDCl_3$): δ 1.21-1.35 (m, 4H, $CHCH_2C\underline{H}_2$), 1.44 (s, 9H, Boc), 1.58-1.90 (m, 8H, $C\underline{H}_2CH_2NH$ and $CHC\underline{H}_2CH_2$), 3.35-3.38 (m, 4H, $C\underline{H}_2N$ (Lys)), 4.08 (bs, 1H, NH), 4.38 (bs, 2H, Lys-α-proton), 5.04 (bs, 1H, N<u>H</u>Boc), 7.41-7.42 (m, 4H, ArC<u>H</u>), 7.50-7.53 (m, 8H, ArC<u>H</u>); 7.88 (bs, 1H, NH), 8.02-8.07 (m, 8H, ArC<u>H</u>); ESI-MS (+ve): *m/z* 2254 (50%, $M+H^+$), 2276 (100%, M+Na). This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

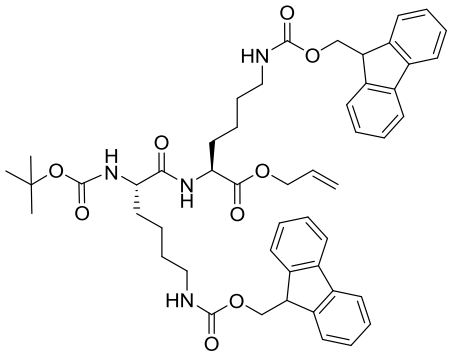
Allyl (6*S*,9*S*,12*S*,15*S*)-6,9,12,15-tetrakis(4-(5,5-diphenylfullerenyldihydropyrrole-2-carboxamido)butyl)-2,2-dimethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecane-16-oate (207)

This compound was prepared *via* protocol 1, using **205** (0.022 g, 0.010 mmol) and **206** (0.021 g, 0.010 mmol) to yield the title compound **207** as a brown solid (0.022 g, 51%).



^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08-1.18 (m, 4H, $CHCH_2C\underline{H}_2$), 1.22-1.38 (m, 4H, $CHCH_2C\underline{H}_2$), 1.44 (s, 9H, Boc), 1.48-1.98 (m, 16H, $C\underline{H}_2CH_2NH$ and $CHC\underline{H}_2CH_2$), 3.20-3.64 (m, 8H, 4 x $C\underline{H}_2N$ (Lys)), 4.02 (bs, 1H, Lys-α-proton), 4.12 (bs, 1H, Lys-α-proton), 4.26 (bs, 1H, Lys-α-proton), 4.56-4.58 (m, 3H, $OC\underline{H}_2$ and Lys-α-proton), 4.80 (bs, 1H, NH), 4.98 (bs, 2H, N<u>H</u>), 5.25 (d, *J* = 9.0 Hz, 1H, CH=C<u>H</u>(*Z*)H), 5.27 (d, *J* = 17.0 Hz, 1H, CH=C<u>H</u>(*E*)H), 5.82-5.92 (m, 1H, C<u>H</u>=CH_2), 7.32-7.37 (m, 8H, ArC<u>H</u>), 7.48-7.50 (m, 16H, ArC<u>H</u>); 7.80-7.84 (m, 4H, 4 x NH), 8.01 (d, *J* = 7.5 Hz, 16H, ArC<u>H</u>); This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum and not sufficiently soluble in MeOH to generate an adequate ESI-MS.

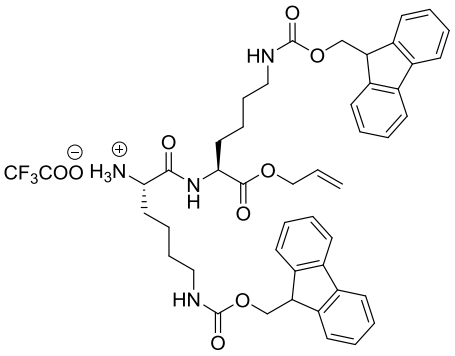
(*S*)-Allyl 6-(9*H*-fluoren-9-yl)methoxycarbonylamino-2-(*S*)-6-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-((*tert*-butoxycarbonyl)amino)hexanamido) hexanoate (212)



This compound was prepared *via* protocol 1 using allyl N^6-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-lysinate (500 mg, 1.22 mmol) and Fmoc-(*S*)-Lys(Boc)-OH (573 mg, 1.22 mmol) to yield the desired product **212** as a white foam (920 mg, 87%). IR (neat, cm^{-1}): δ 3302 (w), [1665, 1662, 1641, 1558 (s)], 1507, 1198, 1131 (m); ^{1}H NMR (500 MHz, $CDCl_3$): 1.29-1.39 (m, 4H, $CHCH_2C\underline{H}_2$), 1.42 (s, 9H, Boc), 1.50-1.51 (m, 4H, $C\underline{H}_2CH_2NH$), 1.62-1.68 (m, 2H, $C\underline{H}CH_2CH_2$), 1.85-1.86 (m, 2H, $C\underline{H}CH_2CH_2$), 3.16-3.17 (bs, 4H, $C\underline{H}_2N$ (Lys)), 4.12 (bs, 1H, Lys-α-proton), 4.18 (bs, 1H, Lys-α-proton), 4.19-4.20 (m, 2H, 2 x C<u>H</u> (Fmoc)), 4.37 (d, *J* = 6.5 Hz, 2H, $C\underline{H}_2CH$ (Fmoc)), 4.41 (d, *J* = 7.0 Hz, 2H, $C\underline{H}_2CH$ (Fmoc)), 4.57-4.61 (m, 3H, $C\underline{H}_2CH=CH_2$ and N<u>H</u>), 4.97 (bs, 1H, N<u>H</u>), 5.03 (bs, 1H, N<u>H</u>), 5.22 (d, *J* = 10.5 Hz, 1H, CH=C<u>H</u>(*Z*)H), 5.29 (d, *J* = 17.5 Hz, 1H, CH=C<u>H</u>(*E*)H), 5.82-5.90 (m, 1H, $C\underline{H}=CH_2$), 6.75 (bs, 1H, N<u>H</u>), 7.29 (t, *J* = 7.5 Hz, 4H, ArC<u>H</u>), 7.38 (t, *J* = 7.5 Hz, 4H, ArC<u>H</u>), 7.57-7.60 (m, 4H, ArC<u>H</u>), 7.75 (d, *J* = 7.5 Hz, 4H, ArC<u>H</u>). MS (ESI +ve) *m/z* 881.7 ($[M+Na]^+$, 60%).

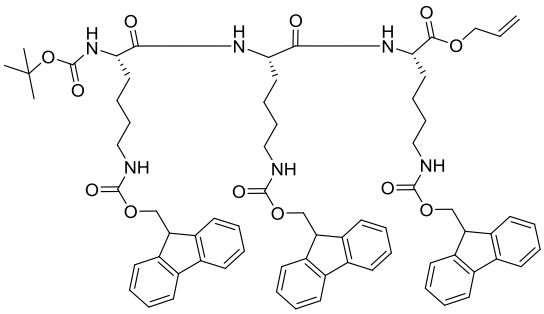
(9*S*,12*S*)-9-((Allyloxy)carbonyl)-1,20-di-(9*H*-fluoren-9-yl)-3,11,18-trioxo-2,19-dioxa-4,10,17-triazaicosan-12-ammonium trifluoroacetate (213)

This compound was prepared *via* protocol 3, using **212** (150 mg, 0.174 mmol) to yield **213** as a white foam (110 mg, 90%). IR (neat, cm^{-1}): 3309 (w), [1667, 1662, 1635 (s)], 1194 (m), 1135 (m); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34-1.46 (m, 4H, $CHCH_2C\underline{H}_2$),



1.53 (m, 4H, C<u>H</u>$_2$CH$_2$NH), 1.59 (bs, 2H, C<u>H</u>CH$_2$CH$_2$), 1.74-1.77 (m, 2H, C<u>H</u>CH$_2$CH$_2$), 3.12 (bs, 1H, Lys-α-proton), 3.18-3.20 (m, 4H, C<u>H</u>$_2$N (Lys)), 3.43-3.44 (m, 2H, N<u>H</u>$_2$), 4.12 (bs, 1H, Lys-α-proton), 4.20 (t, *J* = 6.5 Hz, 2H, CH$_2$C<u>H</u> (Fmoc)), 4.40 (d, *J* = 6.5 Hz, 4H, 2 x C<u>H</u>$_2$CH (Fmoc)), 4.57 (bs, 1H, N<u>H</u>), 4.61 (d, *J* = 5.5 Hz, 2H, C<u>H</u>$_2$CH=CH$_2$), 4.86 (bs, 2H, N<u>H</u>), 5.24 (d, *J* = 10.5 Hz, 1H, CH=C<u>H</u>(*Z*)H), 5.32 (d, *J* = 17.0 Hz, 1H, CH=C<u>H</u>(*E*)H), 5.88-5.94 (m, 1H, C<u>H</u>=CH$_2$), 7.30 (t, *J* = 7.5 Hz, 4H, ArC<u>H</u>), 7.39 (t, *J* = 7.5 Hz, 4H, ArC<u>H</u>), 7.58 (d, *J* = 7.5 Hz, 4H, ArC<u>H</u>); 7.76 (d, *J* = 7.5 Hz, 4H, ArC<u>H</u>). MS (ESI +ve) *m/z* 759 ([M+H]$^+$, 100%).

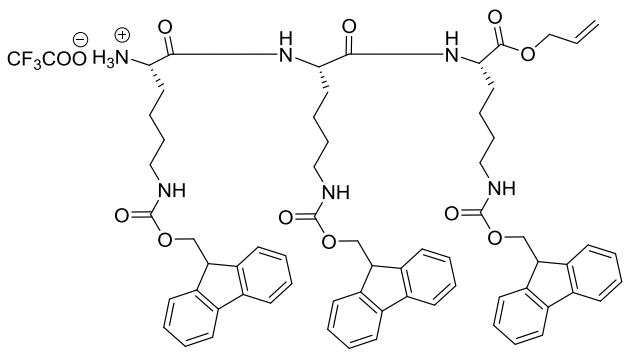
Allyl (9*S*,12*S*,15*S*)-12,15-bis(4-(9*H*-fluoren-9-yl)methoxycarbonylaminobutyl)-9-(*tert*-butoxycarbonylamino)-1-(9*H*-fluoren-9-yl)-3,10,13-trioxo-2-oxa-4,11,14-triazahexadecane-16-oate (214)



This compound was prepared *via* protocol 1, using (*S*)-6-((((9*H*-fluoren-9-yl)methoxy) carbonyl)amino)-2-(*tert*-butoxycarbonyl) amino hexanoic acid (0.150 g, 0.320 mmol) and (9*S*,12*S*)-9-((allyloxy)carbonyl)-1,20-di-(9*H*-fluoren-9-yl)-3,11,18-trioxo-2,19-dioxa-4,10,17-triazaicosan-12-aminium 2,2,2-trifluoroacetate **213** (0.242 g, 0.320 mmol) to afford the title compound **214** as a white solid (0.325 g, 83%). m.p: 147-149 ºC; $[\alpha]_D^{25}$ -44.6 (*c* 3.18, $CHCl_3$); IR (neat, cm^{-1}): 3329 (w), [1719, 1679, 1532 (s)]; ^{1}H NMR (500 MHz, DMSO-d6): δ 1.18-1.33 (m, 6H, CHCH$_2$C<u>H</u>$_2$), 1.30 (s, 9H, Boc), 1.40-1.48 (m, 6H,

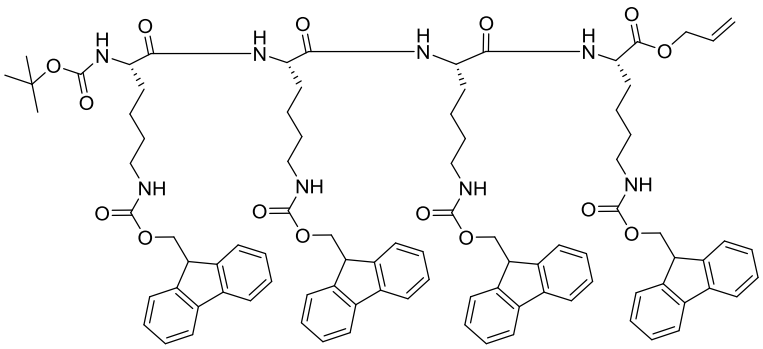
C<u>H</u>$_2$CH$_2$NH), 1.48-1.68 (m, 6H, CHC<u>H</u>$_2$CH$_2$), 2.88-2.89 (m, 6H, C<u>H</u>$_2$N(Lys)), 3.92 (bs, 1H, NH), 4.14-4.15 (m, 6H, 3 x CH$_2$C<u>H</u> (Fmoc) and 3 x Lys-α-protons), 4.22 (bs, 6H, 3 x CHC<u>H</u>$_2$ (Fmoc)), 4.48 (bs, 2H OC<u>H</u>$_2$), 5.13 (d, *J* = 10.5 Hz, 1H, CH=C<u>H</u>(*Z*)H), 5.22 (d, *J* = 17.0 Hz, 1H, CH=C<u>H</u>(*E*)H), 5.78-5.82 (m, 1H, C<u>H</u>=CH$_2$), 6.72 (bs, 1H, N<u>H</u>), 6.81 (bs, 1H, N<u>H</u>), 7.18 (t, *J* = 7.0 Hz, 2H, ArC<u>H</u>), 7.26 (t, *J* = 7.0 Hz, 6H, ArC<u>H</u>); 7.35 (t, *J* = 7.0 Hz, 6H, ArC<u>H</u>), 7.58 (bs, 1H, N<u>H</u>), 7.60-7.62 (m, 4H, ArC<u>H</u>), 7.67 (d, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 7.0 Hz, 6H, ArC<u>H</u>), 8.22 (bs, 2H, N<u>H</u>); ESI-MS (+ve): *m/z* 1231 (100%, M+Na$^+$). This sample turned into a gum over a period of time, so without measuring the ^{13}C NMR spectrum, the material was carried forward to the subsequent reaction.

(9*S*,12*S*,15*S*)-12-(4-(9*H*-Fluoren-9-yl)methoxycarbonylaminobutyl)-9-((allyloxy)carbonyl)-1,23-di(9*H*-fluoren-9-yl)-3,11,14,21-tetraoxo-2,22-dioxa-4,10,13,20-tetraazatricosan-15-ammonium trifluoroacetate (215)



This compound was prepared *via* protocol 3, using **214** (0.300 g, 0.248 mmol) to afford the title compound **215** (0.270 g, 98%) as a colourless solid initially which became a gummy mass. This material was taken for the next reaction by characterising only by mass spectrometry. ESI-MS (+ve): *m/z* 1109 (100%, M+H$^+$).

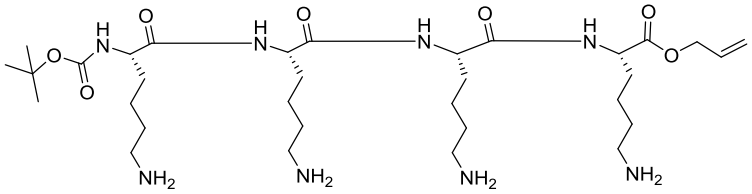
Allyl-(9*S*,12*S*,15*S*,18*S*)-12,15,18-tris(4-(9*H*-fluoren-9-yl)methoxycarbonyl amino butyl)-9-((*tert*-butoxycarbonyl)amino)-1-(9*H*-fluoren-9-yl)-3,10,13,16-tetraoxo-2-oxa-4,11,14,17-tetraazanonadecane-19-oate (216)



This compound was prepared *via* protocol 1, using (*S*)-6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(*tert*-butoxycarbonyl)aminohexanoic acid **210** (0.105 g, 0.225 mmol) and **215** (0.250 g, 0.225 mmol) to afford the title compound **216** as an off white solid (0.249 g, 70%). m.p: 136-138 ºC; $[\alpha]_D^{25}$ -37.2 (*c* 2.48, $CHCl_3$); IR (neat, cm^{-1}): 3330, 1718, 1680, 1537; ^{1}H NMR (500 MHz, DMSO-d6): δ 1.12-1.30 (m, 8H, $CHCH_2C\underline{H}_2$), 1.29 (s, 9H, Boc), 1.32-1.48 (m, 8H, $C\underline{H}_2CH_2NH$), 1.50-1.68 (m, 8H, $CHC\underline{H}_2CH_2$), 2.88 (bs, 8H, $C\underline{H}_2N$(Lys)), 3.82 (bs, 1H, N<u>H</u>), 4.12-4.14 (m, 8H, 4 x $CH_2C\underline{H}$ (Fmoc), and 4 x Lys-α- protons), 4.16-4.22 (m, 8H, 4 x $CHC\underline{H}_2$ (Fmoc)), 4.46 (bs, 2H $C\underline{H}_2CH{=}CH_2$), 5.11 (d, *J* = 10.5 Hz, 1H, CH=C<u>H</u>(*Z*)H), 5.21 (d, 1H, *J* = 17.5 Hz, CH=C<u>H</u>(*E*)H), 5.78-5.82 (m, 1H, $C\underline{H}{=}CH_2$), 6.82 (d, 1H, *J* = 7.5 Hz, N<u>H</u>), 6.81 (bs, 1H, N<u>H</u>), 7.17 (m, 4H, ArC<u>H</u>), 7.25 (t, *J* = 7.5 Hz, 8H, ArC<u>H</u>); 7.33 (t, *J* = 7.5 Hz, 8H, ArC<u>H</u>), 7.55 (bs, 1H, N<u>H</u>), 7.60 (d, *J* = 7.0 Hz, 6H, ArC<u>H</u>), 7.70 (bs, 2H, N<u>H</u>), 7.81 (d, *J* = 7.5 Hz, 6H, ArC<u>H</u>), 8.18 (bs, 2H, NH); ^{13}C NMR (125 MHz, DMSO-d6): δ 22.5 ($CHCH_2\underline{C}H_2$), 28.3 ($C(\underline{C}H_3)_3$), 29.0 ($CH\underline{C}H_2CH_2$), 29.3 ($\underline{C}H_2CH_2NH$), 31.6 ($CH\underline{C}H_2CH_2$), 38.4 (4 x $\underline{C}H_2NH$ (Lys)), 47.2 ($CH_2\underline{C}H$ (Fmoc)), 55.0 (NH<u>C</u>HCO, Lys α carbon), 64.9 ($\underline{C}H_2CH{=}CH_2$), 65.1 ($\underline{C}H_2CH$ (Fmoc)), 78.2 ($\underline{C}(CH_3)_3$), 109.8, 117.9 ($CH{=}\underline{C}H_2$), 120.2 (8 x Ar<u>C</u>H), 121.5 (8 x Ar<u>C</u>H), 123.3

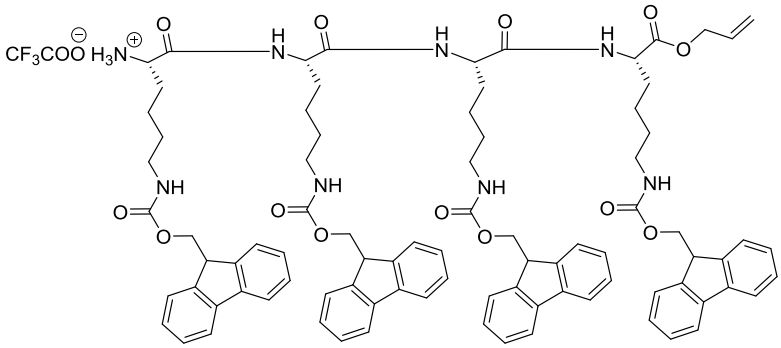
(ArCH), 127.4 (8 x ArCH), 129.1 (8 x ArCH), 132.5 (CH=CH_2), 137.5 (8 x ArC), 139.5 (4 x ArC), 142.7 (4 x ArC), 152.8 (2 x CONH), 155.5 (CO, Boc), [158.2 (3 x CO), 169.1, 171.8 (CONH)], 172.3 (CO, ester); HRMS (ESI +ve) calcd for $C_{92}H_{102}N_8O_{15}Na$, 1581.7362, found 1581.7362.

Allyl (6*S*,9*S*,12*S*,15*S*)-6,9,12,15-tetrakis(4-aminobutyl)-2,2-dimethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecane-16-oate (217)



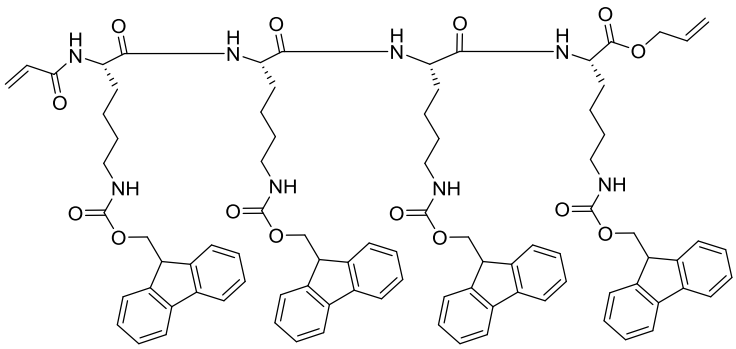
This compound was prepared *via* protocol 2, using **216** (0.050 g, 0.032 mmol) to afford a crude product (0.021 g, 100%), which was then taken through the next reaction without further purification. ESI-MS (+ve): *m/z* 671 (100%, $M+H^+$).

(9*S*,12*S*,15*S*,18*S*)-12,15-Bis(4-(9*H*-fluoren-9-yl)methoxycarbonylaminobutyl)-9-((allyloxy)carbonyl)-1,26-di(9*H*-fluoren-9-yl)-3,11,14,17,24-pentaoxo-2,25-dioxa-4,10,13,16,23-pentaazahexacosan-18-ammonium trifluoroacetate (218)



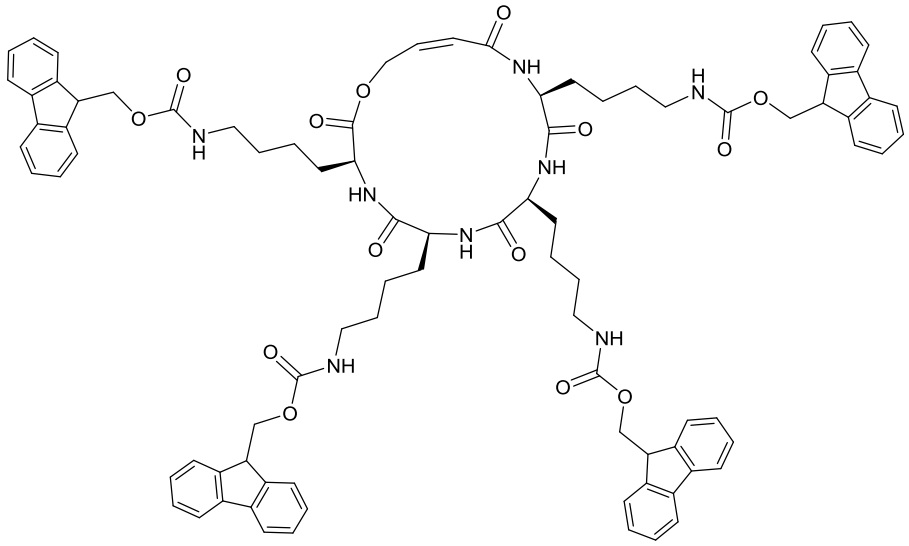
This compound was prepared *via* protocol 3, using **216** (0.300 g, 0.248 mmol) to afford the title compound **218** (0.270 g, 98%) as a gummy mass. This material was carried forward to the subsequent reaction. ESI-MS (+ve): *m/z* 1459 (100%, $M+H^+$).

Allyl (9*S*,12*S*,15*S*,18*S*)-12,15,18-tris(4-(9*H*-fluoren-9-yl)methoxycarbonyl aminobutyl)-9-acrylamido-1-(9*H*-fluoren-9-yl)-3,10,13,16-tetraoxo-2-oxa-4,11,14,17-tetraazanonadecane-19-oate (219)



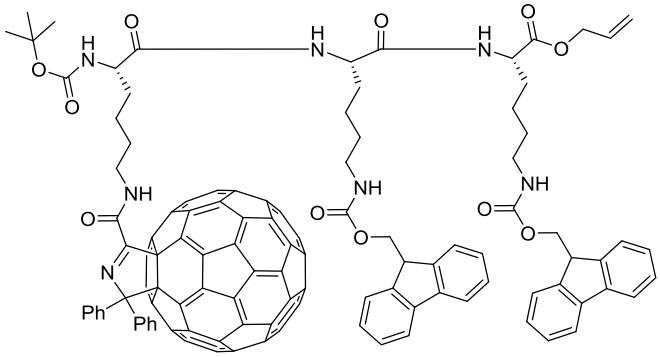
This compound was prepared *via* protocol 1, using acrylic acid (0.051 g, 0.684 mmol) and **218** (0.250 g, 0.171 mmol) to afford the title compound **219** as an off white solid (0.194 g, 75%). ^{1}H NMR (500 MHz, ($CDCl_3$/CD_3OD (1:1)): δ 1.26-1.30 (m, 8H, $CHCH_2C\underline{H}_2$), 1.32-1.48 (m, 8H, $C\underline{H}_2CH_2NH$), 1.55-1.82 (m, 8H, $CHC\underline{H}_2CH_2$), 3.11-3.18 (bs, 8H, $C\underline{H}_2N$(Lys)), 3.60 (bs, 1H, N<u>H</u>), 4.17-4.23 (m, 8H, 4 x $CH_2C\underline{H}$ (Fmoc) and 4 x Lys-α protons), 4.35 (bs, 2H, $CHC\underline{H}_2$ (Fmoc)), 4.47 (bs, 6H, $CHC\underline{H}_2$ (Fmoc)), 4.56 (bs, 2H $OC\underline{H}_2$), 5.20-5.33 (m, 2H, $CH=C\underline{H}_2$), 5.63-5.65 (m, 1H, $C\underline{H}=CH_2$), 5.81-5.83 (m, 1H, CH=CH<u>H</u>), 6.06-6.20 (m, 1H, CH=C<u>H</u>H), 6.25-6.28 (m, 1H, C<u>H</u>=CHH), 7.28-7.29 (m, 6H, ArC<u>H</u>), 7.36-7.37 (m, 8H, ArC<u>H</u>), 7.39 (d, *J* = 7.0 Hz, 6H, ArC<u>H</u>), 7.58 (d, *J* = 7.5 Hz, 6H, ArC<u>H</u>), 7.74 (d, *J* = 7.5 Hz, 6H, ArC<u>H</u>); ^{13}C NMR (126 MHz,$CDCl_3$/CD_3OD (1:1)): This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum. ESI-MS (+ve): *m/z* 1513 (70% $M+H^+$), 1535 (100%, $M+Na^+$).

Bis((9*H*-fluoren-9-yl)methyl)-(3*R*,6*R*,9*R*,12*R*,*Z*)-6,9-bis(4-(9*H*-fluoren-9-ylmethoxycarbonylaminobutyl)-2,5,8,11,14-pentaoxo-1-oxa-4,7,10,13-tetraazacycloheptadec-15-ene-3,12-diyl)bis(butane-4,1-diyl))dicarbamate (220)



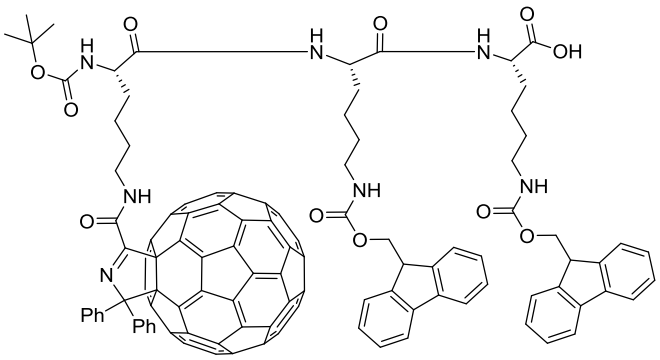
A solution of **219** (0.110 g, 0.072 mmol) in CH_2Cl_2 (100 mL) was heated at reflux then Grubbs' II catalyst (0.006 g, 0.0072 mmol) was added in one portion. The colour became pink then purple and then darkened to orange. The reaction was heated at reflux for 4 h, after which TLC analysis showed complete consumption of **219**. The crude residue was then subjected to flash silica chromatography, elution with MeOH/CH_2Cl_2 (1:25) provided the title compound **220** (0.021 g, 19%) as an off white solid. This compound was not sufficiently soluble to generate an adequate ^{1}H or ^{13}C NMR spectrum. ESI-MS (+ve): *m/z* 1486 (80%, $M+H^+$).

Allyl (6*S*,9*S*,12*S*)-9,12-bis(4-(9*H*-fluoren-9-yl)methoxycarbonylaminobutyl)-6-(4-(5,5-diphenylfullerenyldihydropyrrole-2-carboxamido)butyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecane-13-oate (224)



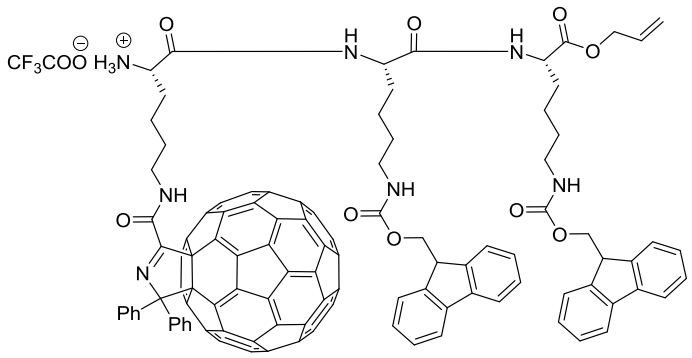
This compound was prepared *via* protocol 1, using **203** (0.120 g, 0.101 mmol) and **213** (0.076 g, 0.101 mmol) to yield the title compound **224** as a brown solid (0.122 g, 62%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34-1.47 (m, 6H, $CHCH_2C\underline{H}_2$), 1.40 (s, 9H, Boc), 1.60-1.75 (m, 6H, $C\underline{H}_2CH_2NH$), 1.80-1.86 (m, 6H, $CHC\underline{H}_2CH_2$), 3.11-3.18 (m, 5H, 2 x $C\underline{H}_2NHFmoc$ and Lys-α-proton), 3.47-3.48 (m, 2H, $C\underline{H}_2N$ (Lys)), 4.08-4.20 (m, 3H, $CHC\underline{H}_2$ and $CH_2C\underline{H}$ (Fmoc)), 4.37-4.43 (m, 5H, $OC\underline{H}_2CH=CH_2$, $CHC\underline{H}_2$ (Fmoc), and Lys-α-proton), 4.54-4.60 (m, 3H, $CHC\underline{H}_2$ and $CH_2C\underline{H}$ (Fmoc), 5.07 (bs, 1H, $N\underline{H}$Boc), 5.22-5.26 (m, 2H, $CH=C\underline{H}_2$), 5.82-5.88 (m, 1H, $C\underline{H}=CH_2$), 6.74-6.82 (m, 2H, $N\underline{H}$), 7.28-7.30 (m, 4H, $Ar\underline{C}H$), 7.35-7.40 (m, 6H, $Ar\underline{C}H$); 7.50 (t, *J* = 7.5 Hz, 4H, $Ar\underline{C}H$ (Fmoc)), 7.57 (t, *J* = 7.5 Hz, 4H, $Ar\underline{C}H$ (Fmoc)), 7.73 (d, *J* = 7.0 Hz, 4H, $Ar\underline{C}H$ (Fmoc)), 8.05 (d, *J* = 7.5 Hz, 4H, $Ar\underline{C}H$ (Fmoc)); HRMS (ESI +ve) calcd for $C_{131}H_{79}N_7O_{11}Na$, 1948.5735, found 1948.5831. This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

(6*S*,9*S*,12*S*)-9,12-Bis(4-(9*H*-fluoren-9-yl)methoxycarbonylaminobutyl)-6-(4-(5,5-diphenylfullerenyldihydropyrrole-2-carboxamido)butyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecane-13-oic acid (225)



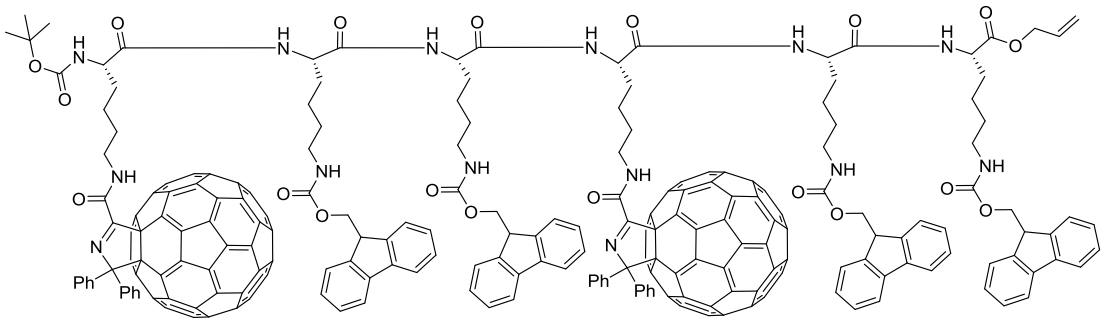
This compound was prepared *via* protocol 4, using **224** (0.060 g, 0.031 mmol), 1,2-dichloroethane (80 mL) and $(CH_3)_3SnOH$ (0.024 g, 0.124 mmol x 2) to yield **225** (0.045 g, 77%) as a brown solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25-1.48 (m, 6H, $CHCH_2C\underline{H}_2$), 1.39 (s, 9H, Boc), 1.70-1.74 (m, 6H, $C\underline{H}_2CH_2NH$), 1.80-1.88 (m, 6H, $CHC\underline{H}_2CH_2$), 3.12-3.16 (m, 5H, 2 x $C\underline{H}_2NH$Fmoc and Lys-α-proton), 3.47 (bs, 2H, $C\underline{H}_2N$ (Lys)), 4.06-4.22 (m, 3H, $CHC\underline{H}_2$ and $CH_2C\underline{H}$ (Fmoc)), 4.36-4.58 (m, 4H, $CHC\underline{H}_2$, Lys-α-proton and $CH_2C\underline{H}$ (Fmoc)), 4.96-5.18 (m, 2H, N$\underline{H}$Boc and Lys-α-proton), 6.60-6.78 (m, 2H, N$\underline{H}$), 7.28-7.29 (m, 4H, Ar$\underline{C}$H), 7.37-7.38 (m, 6H, Ar$\underline{C}$H); 7.50 (t, *J* = 7.0 Hz, 4H, Ar$\underline{C}$H (Fmoc)), 7.58 (bs, 4H, Ar$\underline{C}$H (Fmoc)), 7.73 (d, *J* = 6.0 Hz, 4H, Ar$\underline{C}$H (Fmoc)), 8.05 (d, *J* = 8.0 Hz, 4H, Ar$\underline{C}$H (Fmoc)); HRMS (ESI +ve) calcd for $C_{128}H_{75}N_7O_{11}Na$, 1909.5455, found 1909.5359. This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

(9*S*,12*S*,15*S*)-12-(4-(9*H*-Fluoren-9-yl)methoxycarbonylaminobutyl)-9-((allyloxy) carbonyl)-21-(5,5-diphenylfullerenyldihydropyrrolyl)-1-(9*H*-fluoren-9-yl)-3,11,14,21-tetraoxo-2-oxa-4,10,13,20-tetraazahenicosan-15-ammonium trifluoroacetate (226)



This compound was prepared *via* protocol 3, using **225** (0.060 g, 0.031 mmol) to afford the title compound **226** (0.050 g, 89%) as a fine brown solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.22-1.38 (m, 6H, $CHCH_2C\underline{H}_2$), 1.72-1.88 (m, 12H, $C\underline{H}_2CH_2NH$ and $CHC\underline{H}_2CH_2$), 3.10-3.20 (m, 5H, 2 x $C\underline{H}_2$NFmoc (Lys) and Lys-α-proton), 3.40 (bs, 2H, $C\underline{H}_2$N (Lys)), 4.08-4.22 (m, 3H, $CHC\underline{H}_2$ (Fmoc) and $CH_2C\underline{H}$ (Fmoc)), 4.34-4.60 (m, 4H, $CHC\underline{H}_2$, Lys-α-proton and $CH_2C\underline{H}$ (Fmoc)), 4.96-5.18 (m, 2H, N$\underline{H}$ and Lys-α-proton), 5.70-5.90 (bs, 2H, N$\underline{H}$), 7.29-7.38 (m, 10H, ArC$\underline{H}$), 7.45-7.49 (m, 4H, ArC$\underline{H}$), 7.68-7.75 (m, 8H, ArC$\underline{H}$), 8.01-8.04 (m, 4H, ArC$\underline{H}$); HRMS (ESI +ve) calcd for $C_{126}H_{71}N_7O_9$, 1826.5392, found 1826.5789. This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

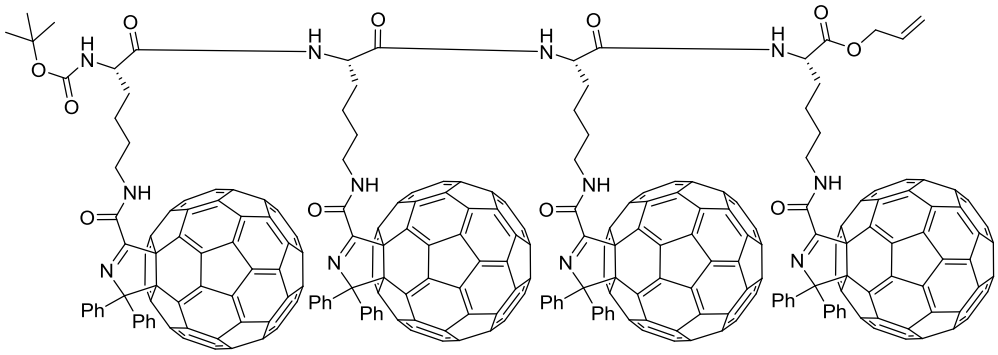
Attempted synthesis of Allyl (6*S*,9*S*,12*S*,15*S*,18*S*,21*S*)-9,12,18,21-tetrakis(4-(9*H*-fluorene-9-yl)methoxycarbonylaminobutyl)-6,15-bis(4-(5,5-diphenylfullerenyl dihydropyrrole-2-carboxamido)butyl)-2,2-dimethyl-4,7,10,13,16,19-hexaoxo-3-oxa-5,8,11,14,17,20-hexaazadocosane-22-oate (227)



This compound was prepared *via* protocol 1, using **225** (0.040 g, 0.021mmol) and **226** (0.038 g, 0.021 mmol) to yield the title compound **227** as a brown solid (0.035 g, 44%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.20-1.55 (m, 12H, $CHCH_2C\underline{H}_2$), 1.39 (s, 9H, Boc), 1.64-1.76 (m, 12H, $C\underline{H}_2CH_2NH$), 1.80-1.90 (m, 12H, $CHC\underline{H}_2CH_2$), 3.06-3.20 (m, 8H, 4 x $C\underline{H}_2NHFmoc$), 3.40-3.48 (m, 4H, 2 x $C\underline{H}_2N$ (Lys)), 3.67-3.69 (m, 1H, N<u>H</u>), 4.00 (bs, 1H, NH), 4.17-4.20 (m, 4H, 4 x $CH_2C\underline{H}$ (Fmoc)), 4.37-4.46 (m, 8H, 4 x $CHC\underline{H}_2$ (Fmoc)), 4.50-4.62 (m, 8H, $OC\underline{H}_2CH=CH_2$ and 6 x Lys-α-protons), 4.90 (bs, 1H, N<u>H</u>), 5.03-5.06 (m, 3H, 3 x N<u>H</u>), 5.20-5.22 (m, 2H, $CH=C\underline{H}_2$), 5.81-5.86 (m, 1H, $C\underline{H}=CH_2$), 6.83-6.84 (m, 2H, 2 x N<u>H</u>), 6.94-6.96 (m, 2H, 2 x N<u>H</u>), 7.29 (d, *J* = 6.5 Hz, 8H), 7.37 (m, 12H); 7.49 (d, *J* = 7.0 Hz, 8H), 7.56 (d, *J* = 7.5 Hz, 8H), 7.74 (t, *J* = 7.0 Hz, 8H), 8.05 (d, *J* = 8.0 Hz, 8H), 8.11-8.12 (bs, 2H, NH); This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum and not sufficiently soluble in MeOH to generate an adequate ESI-MS.

Attempted preparation of Allyl (6*S*,9*S*,12*S*,15*S*)-6,9,12,15-tetrakis(4-(5,5-diphenylfullerenyldihydropyrrole-2-carboxamido)butyl)-2,2-dimethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecane-16-oate (207)

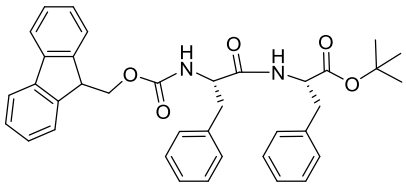
To a suspension of **198** (0.118 g, 0.124 mmol) in pyridine/$CHCl_3$ (90 mL) was added HOBt (0.020 g, 0.148 mmol) and EDCI (0.028 g, 0.148 mmol) and the mixture was stirred for 15 min before a solution of **217** (0.021 g, 0.031 mmol) in pyridine/$CHCl_3$ (25 mL) was added dropwise. The resulting solution was stirred at rt for 8 h before the solvent was removed



under reduced pressure. The crude residue was then subjected to flash silica gel chromatography with gradient elution with MeOH/$CHCl_3$ (1:49 to 1:19). This resulted in the isolation of three different products (10 mg, 14 mg and 56 mg), none of them were the desired product and these could not be characterised by ^{1}H NMR spectroscopy or mass spectrometry.

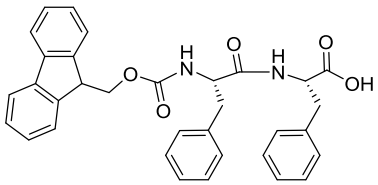
(*S*)-*tert*-Butyl-3-(phenyl)-2-((*S*)-3-(phenyl)-2-(9*H*-fluoren-9-yl)methoxycarbonyl aminopropanamido)propanoate [143] (233)

This compound was prepared *via* protocol 1, using (((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-phenylalanine **231** (0.220 g, 0.568 mmol) and *tert*-butyl *L*-phenylalaninate **232** (0.125 g, 0.568 mmol) to afford the title compound **233** as a



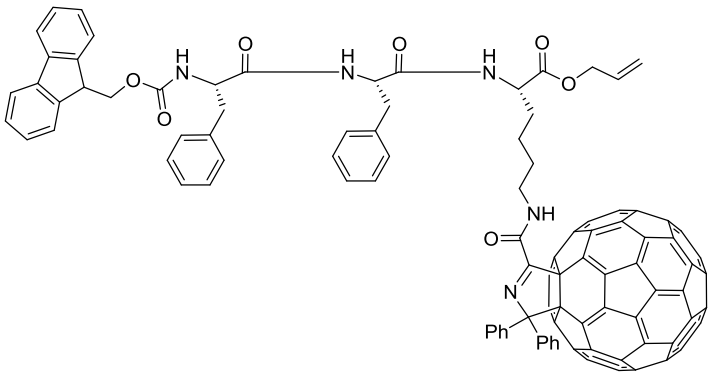
colourless solid (0.310 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36 (s, 9H, C(C<u>H</u>$_3$)$_3$), 3.01 (d, *J* = 6.0 Hz, 2H, C<u>H</u>$_2$Ph), 3.05 (bs, 2H, C<u>H</u>$_2$Ph), 4.17 (t, *J* = 7.0 Hz, 1H, α-Phe proton), 4.28 (bs, 1H, (CH$_2$C<u>H</u> (Fmoc)), 4.40-4.43 (m, 2H, C<u>H</u>$_2$CH (Fmoc)), 4.63 (m, 1H, α-Phe proton), 5.34 (bs, 1H, NH), 6.31 (bs, 1H, N<u>H</u>), 7.02 (d, *J* = 6.0 Hz, 2H, ArC<u>H</u>), 7.17-7.31 (m, 10H, ArC<u>H</u>), 7.39 (t, *J* = 7.5 Hz, 2H, ArC<u>H</u>), 7.52 (t, *J* = 8.5 Hz, 2H, ArC<u>H</u>), 7.75 (d, *J* = 7.0 Hz, 2H, ArC<u>H</u>); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.1 (C(<u>C</u>H$_3$)$_3$), 38.0 (<u>C</u>H$_2$Ph), 38.2 (<u>C</u>H$_2$Ph), 47.3 (CH$_2$<u>C</u>H (Fmoc)), 53.9 (<u>C</u>HCH$_2$Ph), 56.1 (<u>C</u>HCH$_2$Ph), 67.2 (<u>C</u>H$_2$ (Fmoc)), 82.5 (<u>C</u>(CH$_3$)$_3$)), [120.2, 125.0, 127.3, 127.6, 128.6, 128.9, 129.5, 129.6 (ArC<u>H</u>)], [136.0, 136.3, 141.4, 143.8 (Ar<u>C</u>)], 155.9 (C=O (Fmoc)), 170.0 (C=O), 170.2 (C=O). HRMS (ESI +ve) calcd for $C_{37}H_{38}N_2O_5$ 591.2859, found 591.2865.

(*S*)-3-(Phenyl)-2-((*S*)-3-(phenyl)-2-(9*H*-fluoren-9-yl)methoxycarbonylamino propanamido)propanoic acid 234



This compound was prepared *via* protocol 3, using **233** (0.250 g, 0.423 mmol) to afford the title compound **234** (0.205 g, 90%) as a fine colourless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.00-3.02 (m, 2H, C<u>H</u>$_2$Ph), 3.07 (bs, 2H, C<u>H</u>$_2$Ph), 4.16 (t, *J* = 7.0 Hz, 1H, α-Phe proton), 4.25 (bs, 1H, (CH (Fmoc)), 4.41-4.43 (m, 2H, CH$_2$ (Fmoc)), 4.74 (m, 1H, α-Phe proton), 5.37 (bs, 1H, NH), 6.35 (bs, 1H, N<u>H</u>), 7.02-7.05 (m, 2H, ArC<u>H</u>), 7.20-7.32 (m, 10H, ArC<u>H</u>), 7.38 (t, *J* = 7.5 Hz, 2H, ArC<u>H</u>), 7.52 (t, *J* = 8.5 Hz, 2H, ArC<u>H</u>), 7.75 (d, *J* = 7.0 Hz, 2H, ArC<u>H</u>); HRMS (ESI +ve) calcd for $C_{33}H_{30}N_2O_5$ 535.2233, found 535.2238.

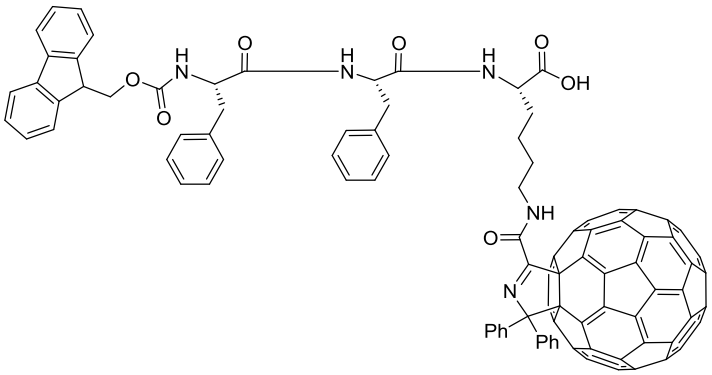
Allyl-(5*S*,8*S*,11*S*)-5,8-dibenzyl-11-(4-(5,5-diphenylfullerenyldihydropyrrole-2-carboxamido)butyl)-1-(9*H*-fluoren-9-yl)-3,6,9-trioxo-2-oxa-4,7,10-triazadodecane-12-oate (235)



This compound was prepared *via* protocol 1, using (*S*)-3-(phenyl)-2-((*S*)-3-(phenyl)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)propanoic acid **234** (0.052 g, 0.097 mmol) and (*S*)-6-((5,5-diphenylfullerenyldihydropyrrole-2-carbonyl)amino)-1-(allyloxy)-1-oxohexan-2-aminium 2,2,2-trifluoroacetate **202** (0.109 g, 0.097 mmol) to afford the title compound **235** as a colourless solid (0.095 g, 60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25-1.36 (m, 2H, $CHCH_2C\underline{H}_2$), 150-1.52 (m, 2H, $C\underline{H}_2CH_2NH$), 1.80-1.86 (m, 2H, $CHC\underline{H}_2CH_2$), 2.95-3.01 (m, 4H, $C\underline{H}_2N$ (Lys) and $C\underline{H}_2Ph$), 3.42-3.45 (m, 2H, $C\underline{H}_2Ph$), 4.06-4.11 (m, 1H, $CH_2C\underline{H}$ (Fmoc)), 4.22-4.30 (m, 2H, α-Phe proton), 4.39-4.59 (m, 5H, $OC\underline{H}_2$, $CHC\underline{H}_2$ (Fmoc), Lys-α-proton), 5.19-5.25 (m, 2H, $CH=C\underline{H}_2$), 5.84-5.90 (m, 1H, $C\underline{H}=CH_2$), 6.57 (bs, 2H, N$\underline{H}$), 7.05-7.28 (m, 20H, Ar$C\underline{H}$), 7.36-7.41 (m, 2H, Ar$C\underline{H}$); 7.45-7.48 (m, 2H, Ar$C\underline{H}$), 7.69-7.74 (m, 2H, Ar$C\underline{H}$), 8.01-8.06 (m, 4H, Ar$C\underline{H}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 22.8 ($CHCH_2\underline{C}H_2$), 29.0 ($\underline{C}H_2CH_2NH$), 31.9 ($CH\underline{C}H_2CH_2$), 37.8 ($\underline{C}H_2Ph$), 39.8 ($\underline{C}H_2NH$), 47.1 ($CH_2\underline{C}H$ (Fmoc)), 52.3 (NH$\underline{C}$HCO), 53.5 ($\underline{C}HCH_2Ph$), 54.6 ($\underline{C}HCH_2Ph$), 66.1 ($\underline{C}H_2CH=CH_2$), 67.2 (OCH_2 (Fmoc)), 83.4* ($C_{60}sp^3$), 83.6* ($C_{60}sp^3$), 95.5 ($\underline{C}Ph_2$), 119.2 ($CH=\underline{C}H_2$), [120.1, 125.1, 125.5, 127.2, 127.3, 127.9, 128.5, 128.7, 128.9, 129.3, 129.9, 131.5 (Ar$\underline{C}$H)], [134.8, 134.85 (Ar$\underline{C}$)], [136.2, 136.6, 139.2, 139.6, 141.0, 141.4, 141.9, 142.4,

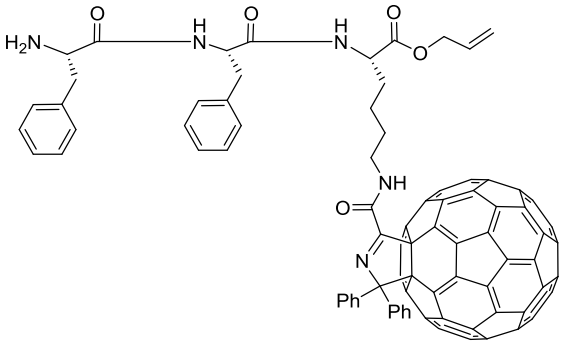
142.6 ($C_{60}sp^2$)], [142.6, 142.8(Ar<u>C</u>)], [143.0, 144.2, 144.4, 144.9, 145.2, 145.3 (2 x C), 145.33, 145.5, 145.6, 145.9, 146.1, 146.4* (1/2 x C), 146.9* (1/2 x C), 147.2, 148.6, 149.2, 153.2 (2 x C) ($C_{60}sp^2$)], 153.3 (<u>C</u>=N), 161.4 (2 x <u>C</u>O), 162.4 (2 x <u>C</u>O), 171.3 (<u>C</u>O, ester); HRMS (ESI +ve) calcd for $C_{117}H_{55}N_5O_7$,1641.4101, found 1641.4235.

(5*S*,8*S*,11*S*)-5,8-Dibenzyl-11-(4-(5,5-diphenylfullerenyldihydropyrrole-2-carboxamido) butyl)-1-(9H-fluoren-9-yl)-3,6,9-trioxo-2-oxa-4,7,10-triazadodecane-12-oic acid (236)



This compound was prepared *via* protocol 4, using **235** (0.042 g, 0.025mmol), 1,2-dichloroethane (40 mL) and $(CH_3)_3SnOH$ (0.020 g, 0.100 mmol) mL) to yield **236** (0.030 g, 75%) as a brown solid. This compound was carried forward without any spectroscopic characterisation.

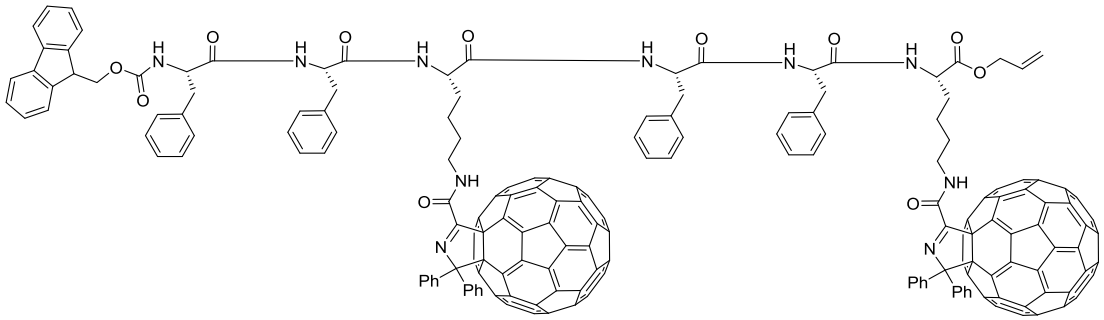
Allyl (*S*)-2-((*S*)-2-((*S*)-2-amino-3-phenylpropanamido)-3-phenylpropanamido)-6-(5,5-diphenylfullerenyldihydripyrrole-2-carboxamido)hexanoate (237)



This compound was prepared *via* protocol 2, using **235** (0.045 g, 0.027 mmol) to afford the title compound **237** (0.38 g, 99%) as a fine colourless solid. HRMS (ESI +ve) calcd for $C_{102}H_{45}N_5O_5$ 1419.3421, found 1419.3529. This

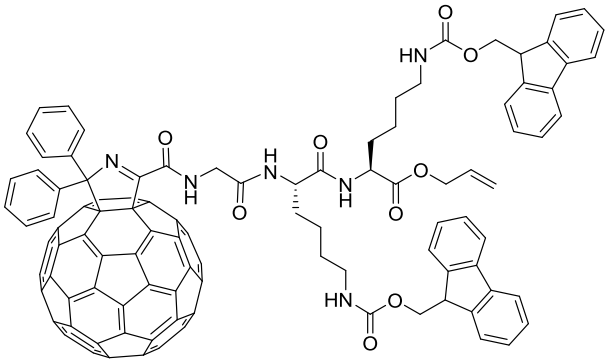
compound was carried forward to the subsequent reaction without any further characterisation.

Allyl-(5*S*,8*S*,11*S*,14*S*,17*S*,20*S*)-5,8,14,17-tetrabenzyl-11,20-bis(4-(5,5-diphenyl fullerenyldihydropyrrole-2-carboxamido)butyl)-1-(9*H*-fluoren-9-yl)-3,6,9,12,15,18-hexaoxo-2-oxa-4,7,10,13,16,19-hexaazahenicosane-21-oate (238)



This compound was prepared *via* protocol 1, using **236** (0.025 g, 0.015 mmol) and **237** (0.021 g, 0.015 mmol) to yield the title compound **238** as a colourless solid (0.018 g, 40 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21-1.39 (m, 4H, $CHCH_2C\underline{H}_2$), 146-1.57 (m, 4H, $C\underline{H}_2CH_2NH$), 1.80-1.88 (m, 4H, $CHC\underline{H}_2CH_2$), 2.96-3.06 (m, 8H, 2 x $C\underline{H}_2N$ (Lys) and 2 x $C\underline{H}_2Ph$), 3.41-3.45 (m, 4H, 2 x $C\underline{H}_2Ph$), 4.04-4.15 (m, 1H, $CH_2C\underline{H}$ (Fmoc)), 4.20-4.30 (m, 4H, 4 x α-Phe proton), 4.37-4.61 (m, 6H, $C\underline{H}_2CH=CH_2$, $C\underline{H}_2$ (Fmoc) and 2 x Lys-α-proton), 5.17-5.25 (m, 2H, $CH=C\underline{H}_2$), 5.86-5.92 (m, 1H, $C\underline{H}=CH_2$), 6.60 (bs, 2H, N<u>H</u>), 6.65 (bs, 2H, N<u>H</u>), 7.18-7.36 (m, 32H, ArC<u>H</u>), 7.36-7.41 (m, 4H, ArC<u>H</u>), 7.45-7.48 (m, 8H, ArC<u>H</u>), 7.69-7.74 (m, 2H, ArC<u>H</u>), 8.01-8.06 (m, 2H, ArC<u>H</u>). This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum and not sufficiently soluble in MeOH to generate an adequate ESI-MS.

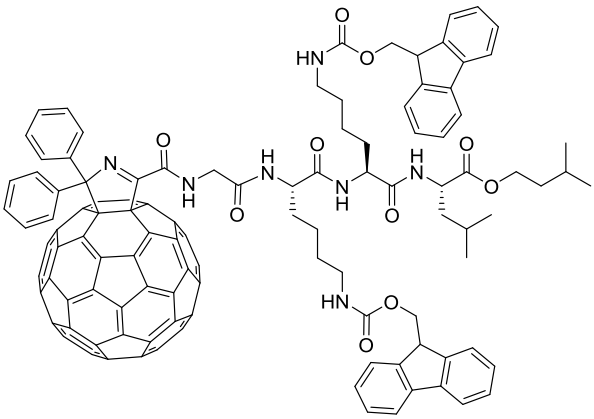
Allyl (*S*)-6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-((*S*)-6-(9*H*-fluoren-9-yl)methoxycarbonylamino-2-(2-(5,5-diphenylfullerenyldihydropyrrole-2-carbonyl)aminoacetamido)hexanamido)hexanoate (243)



To a suspension of fullerenoprolineglycine-OH **242** (133 mg, 0.131 mmol) in CH_2Cl_2 (100 mL) was added HBTU (59 mg, 0.157 mmol) and Et_3N (22 µL, 0.157 mmol) and the mixture was stirred for 15 min before a solution of **213** (100 mg, 0.131 mmol) in CH_2Cl_2 (15 mL) was added dropwise. The resulting solution was stirred at rt for 4 h, before the solvent was removed under reduced pressure. The crude residue was then subjected to flash silica gel chromatography, elution with MeOH/CH_2Cl_2 (1:99) provided the title compound **243** as a brown solid (155 mg, 67%). IR (neat, cm^{-1}): 3303 (w), [1695, 1683, 1653, 1647 (s)], 1246 (m), 1139; ^{1}H NMR (500 MHz, $CDCl_3$:CS_2 (4:1)): δ 1.23-1.25 (m, 4H, $CHCH_2C\underline{H}_2$), 142-1.49 (m, 4H, $C\underline{H}_2CH_2NH$), 1.68-1.78 (m, 2H, $CHC\underline{H}_2CH_2$), 1.85 (bs, 2H, $CHC\underline{H}_2CH_2$), 3.10-3.18 (m, 4H, 2 x $C\underline{H}_2N$ (Lys)), 4.16-4.22 (m, 4H, 2 x C<u>H</u> (Fmoc) and 2 x Lys-α-proton), 4.36-4.40 (m, 5H, 2 x $C\underline{H}_2CH$ (Fmoc) and N<u>H</u>), 4.51 (bs, 2H, $NHC\underline{H}_2CO$ (Gly)), 4.60 (bs, 2 x N<u>H</u>), 4.90-5.11 (m, 2H, $C\underline{H}_2CH{=}CH_2$), 5.21 (d, *J* = 10.5 Hz, 1H, CH=C<u>H</u>(*Z*)H), 5.28 (d, *J* = 17.0 Hz, 1H, CH=C<u>H</u>(*E*)H), 5.82-5.94 (m, 1H, $C\underline{H}{=}CH_2$), 6.86 (bs, 1H, N<u>H</u>), 7.27-7.40 (m, 10H, ArC<u>H</u>), 7.47 (t, *J* = 7.5 Hz, 4H, ArC<u>H</u>), 7.56 (bs, 4H, ArC<u>H</u>); 7.72 (d, *J* = 7.0 Hz, 4H, ArC<u>H</u>), 8.05 (d, *J* = 7.5 Hz, 4H, ArC<u>H</u>), 8.57 (bs, 1H, N<u>H</u>); ^{13}C NMR (125 MHz, $CDCl_3$:CS_2 (4:1)): δ 22.0 ($CHCH_2\underline{C}H_2$), 22.3 ($CHCH_2\underline{C}H_2$), 29.3 ($\underline{C}H_2CH_2NH$), 31.1 ($CH\underline{C}H_2CH_2$), 31.5

(CH<u>C</u>H$_2$CH$_2$), 38.6 (<u>C</u>H$_2$NH (Lys)), 40.2 (<u>C</u>H$_2$NH (Lys)), 43.0 (NH<u>C</u>H$_2$CO), 47.2 (CH$_2$<u>C</u>H (Fmoc)), 52.3 (NH<u>C</u>HCO, Lys α carbon), 53.1 (NH<u>C</u>HCO, Lys α carbon), 66.0 (<u>C</u>H$_2$CH=CH$_2$), 66.5 (<u>C</u>H$_2$CH (Fmoc)), 66.9 (<u>C</u>H$_2$CH (Fmoc)), 95.5 (N<u>C</u>Ph$_2$), 118.9 (CH=<u>C</u>H$_2$), [119.9, 125.0, 127.0, 127.6, 128.4, 129.7 (Ar<u>C</u>H)], 131.4 (<u>C</u>H=CH$_2$), 134.7 (2 x Ar<u>C</u>), [136.6, 139.1, 139.4, 140.8, 141.2, 141.3, 141.7, 142.3, 142.4 ($C_{60}sp^2$)], 142.7 (2 x Ar<u>C</u>), 142.9 (2 x Ar<u>C</u>), 143.9 (4 x Ar<u>C</u>), [144.0, 144.2 (2 x C), 144.8, 145.0, 145.3 (2 x C), 145.4, 145.7, 145.9, 146.3* (1/2 x C), 146.8* (1/2 x C), 147.0, 148.3, 148.9, 153.1 (2 x C) ($C_{60}sp^2$)], 156.7 (C=N), [156.8, 161.7, 165.7, 168.2, 171.3, 171.7 (CO)]; Two $C_{60}sp^3$ signals were not observed due to poor signal to noise. MS (ESI +ve) *m/z* 1778.0 ($[M+Na]^+$, 100%), HRMS (ESI +ve) calcd for $C_{122}H_{62}N_6O_9Na$ 1778.4509, found 1778.4565.

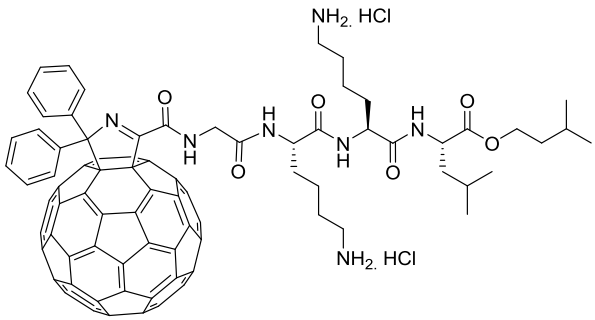
Isopentyl (9*S*,12*S*,15*S*)-12-(4-(9*H*-fluoren-9-yl)methoxycarbonylaminobutyl)-9-(2-(5,5-diphenylfullerenyldihydropyrrole-2-carbonyl)aminoacetamido)-1-(9*H*-fluoren-9-yl)-15-isobutyl-3,10,13-trioxo-2-oxa-4,11,14-triazahexadecane-16-oate (245)



This compound was prepared *via* protocol 4 using **243** (110 mg, 0.062 mmol) to yield the desired carboxylic acid precursor of **244** as a brown solid (90 mg, 84%). This product was carried into the next reaction without any further purification, *via* protocol 1 using the acid **244** (75 mg, 0.043 mmol) and (*S*)-isopentyl 2-amino-4-methylpentanoate (17 mg, 0.086 mmol) to yield the desired product **245** as a brown solid (52 mg, 62%). IR (neat, cm^{-1}): 3305 (w), [1669, 1663,

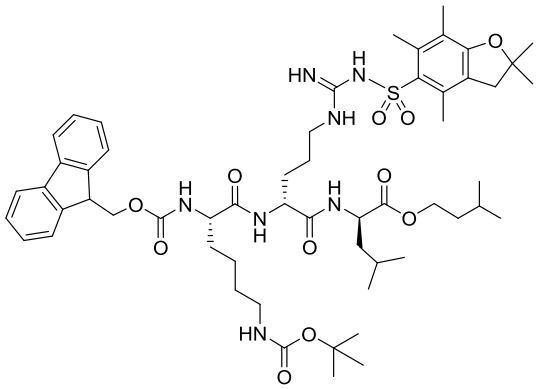
1647, 1632, 1558 (s)], 1515 (m), 1196, 1132 (m); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.80-0.94 (m, 12H, 4 x C<u>H</u>$_3$ (Leu and isopentyl)), 1.38-1.44 (m, 4H, CHCH$_2$C<u>H</u>$_2$), 1.51-1.57 (m, 7H, 2 x C<u>H</u>$_2$CH$_2$NH, OCH$_2$C<u>H</u>$_2$ and C<u>H</u>(CH$_3$)$_2$ (Leu)), 1.66-1.71 (m, 4H, CHC<u>H</u>$_2$CH$_2$ (Lys)), 1.74-1.80 (m, 1H, C<u>H</u>(CH$_3$)$_2$ (isopentyl)), 1.87 (m, 2H, C<u>H</u>$_2$CH(CH$_3$)$_2$ (Leu)), 3.44 (t, *J* = 6.0 Hz, 4H, 2 x C<u>H</u>$_2$N (Lys)), 4.04 (t, *J* = 6.5 Hz, 2H, CO$_2$C<u>H</u>$_2$ (ester)), 4.11-4.17 (m, 4H, NHC<u>H</u>$_2$CO and 2 x C<u>H</u>CH$_2$ (Fmoc)), 4.19 (d, *J* = 6.0 Hz, 2H, CHC<u>H</u>$_2$ (Fmoc)), 4.35-4.40 (m, 4H, CHC<u>H</u>$_2$ (Fmoc) and 2 x Lys-α-proton), 4.49 (bs, 1H, Leu-α-proton), 5.08 (bs, 1H, N<u>H</u>), 5.19 (bs, 1H, N<u>H</u>), 6.50 (bs, 1H, N<u>H</u>), 6.97 (bs, 1H, N<u>H</u>), 6.40 (bs, 1H, N<u>H</u>), 7.27-7.41 (m, 14H, ArC<u>H</u>), 7.57 (bs, 4H, ArC<u>H</u>), 7.61 (d, *J* = 7.5 Hz, 4H, ArC<u>H</u>), 7.73-7.78 (m, 4H, ArC<u>H</u>), 8.05 (bs, 1H, N<u>H</u>); HRMS (ESI +ve) calcd for $C_{130}H_{79}N_7O_{10}$ 1898.5967, found 1898.6011. This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum.

(*S*)-5-(2-(5,5-Diphenylfullerenyldihydropyrrole-2-carbonyl)aminoacetamido)-6-(*S*)-6-ammonio-1-(*S*)-1-(isopentyloxy)-4-methyl-1-oxopentan-2-yl)amino-1-oxohexan-2-ylamino-6-oxohexan-1-ammonium chloride (247)



This compound was prepared *via* protocol 2, followed by protocol 3, using **245** (32 mg, 0.016 mmol) to yield **247** as a brown solid (17 mg, 70%). IR (neat, cm^{-1}): 3237 (w), 3064 (w), [1647, 1635, 1558, 1539 (s)], 1507, 1185, 1155 (m). This compound was not sufficiently soluble to generate an adequate ^{1}H NMR and ^{13}C NMR spectrum. HRMS (ESI +ve) calcd for $C_{100}H_{59}N_7O_6$ 1454.4604, found 1454.4750.

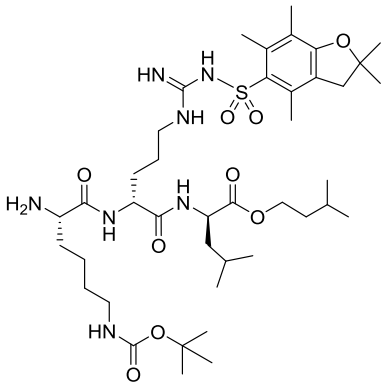
(10*S*,13*R*,16*R*)-Isopentyl 10-(9*H*-fluoren-9-yl)methoxycarbonylamino-16-isobutyl-2,2-dimethyl-4,11,14-trioxo-13-(3-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-3-oxa-5,12,15-triazaheptadecan-17-oate (259)



This compound was prepared *via* protocol 1 using isopentyl N^{ω}-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-*D*-arginyl-*D*-leucinate **255** (250 mg, 0.410 mmol), Fmoc-(*L*)-Lys(Boc)-OH (192 mg, 0.410 mmol) to yield the desired product **259** as a white foam (350 mg, 80%). IR (neat, cm^{-1}): 3306 (w), [1668, 1662, 1647, 1635, 1558 (s)], 1190, 1132 (m); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84-0.88 (m, 12H, 4 x C<u>H</u>$_3$ (Leu and isopentyl)), 1.40 (s, 9H, Boc), 1.43 (s, 6H, C(C<u>H</u>$_3$)$_2$, Pbf), 1.46-1.49 (m, 4H, CHCH$_2$C<u>H</u>$_2$ (Lys) and CHCH$_2$C<u>H</u>$_2$ (Arg)), 1.57-1.72 (m, 6H, CHC<u>H</u>$_2$CH$_2$ (Lys), OCH$_2$C<u>H</u>$_2$ and C<u>H</u>$_2$CH$_2$NH (Lys)), 1.76 (bs, 1H, C<u>H</u>(CH$_3$)$_2$ (Leu)), 1.92 (bs, 2H, CHC<u>H</u>$_2$ (Leu)), 1.99 (bs, 1H, C<u>H</u>(CH$_3$)$_2$ (isopentyl)), 2.07 (s, 3H, C<u>H</u>$_3$ (Pbf)), 2.50 (s, 3H, C<u>H</u>$_3$ (Pbf)), 2.57 (s, 3H, C<u>H</u>$_3$ (Pbf)), 2.92 (s, 2H, C<u>H</u>$_2$ (Pbf)), 3.06 (bs, 2H, C<u>H</u>$_2$N (Arg)), 3.23 (bs, 2H, C<u>H</u>$_2$N (Lys)), 4.05-4.10 (m, 2H, N<u>H</u>), 4.18 (t, *J* = 6.5 Hz, 2H, CO$_2$C<u>H</u>$_2$), 4.32-4.34 (m, 2H, C<u>H</u>CH$_2$ (Fmoc) and Lys-α-proton), 4.43-4.45 (m, 2H, CHC<u>H</u>$_2$ (Fmoc)), 4.56-4.57 (m, 2H, Leu-α-proton and N<u>H</u>), 4.82 (bs, 1H, Arg-α-proton), 5.29 (bs, 1H, N<u>H</u>), 5.82 (bs, 1H, N<u>H</u>), 6.29 (bs, 2H, N<u>H</u>), 7.26-7.29 (m, 2H, ArC<u>H</u>), 7.35-7.38 (m, 2H, ArC<u>H</u>), 7.57 (t, *J* = 6.5 Hz, 2H, ArC<u>H</u>), 7.73 (d, *J* = 7.5 Hz, 2H, ArC<u>H</u>); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.8 (<u>C</u>H$_3$, Pbf), 18.3 (<u>C</u>H$_3$, Pbf), 19.6 (<u>C</u>H$_3$, Pbf), 21.9 (CHCH$_2$<u>C</u>H$_2$ (Lys)), 22.6 (2 x <u>C</u>H$_3$ (Leu)), 22.7 (2 x <u>C</u>H$_3$ (isopentyl)),

22.8 ($CHCH_2\underline{C}H_2$ (Arg)), 23.0 ($\underline{C}H(CH_3)_2$ (isopentyl)), 25.0 ($\underline{C}H(CH_3)_2$ (Leu)), 25.2 ($CH\underline{C}H_2CH_2$ (Lys)), 28.7 ($C(\underline{C}H_3)_3$), 28.8 ($C(\underline{C}H_3)_2$ (Pbf)), 29.6 ($CH\underline{C}H_2CH_2$ (Arg)), 29.8 ($\underline{C}H_2CH_2NH$ (Lys)), 37.5 ($\underline{C}H_2CH(CH_3)_2$ (isopentyl) and $CH_2\underline{C}H_2NH$ (Lys)), 40.3 ($\underline{C}H_2CH(CH_3)_2$ (Leu)), 40.6 ($NH\underline{C}H_2$ (Lys)), 43.9 ($NHC\underline{H}_2$ (Arg)), 47.3 ($\underline{C}H_2$ (Pbf)), 51.6 ($CH_2\underline{C}H$ (Fmoc)), 52.7 ($NH\underline{C}HCO$ (Leu)), 53.7 ($NH\underline{C}HCO$ (Lys)), 55.5 ($NH\underline{C}HCO$ (Arg)), 64.3 ($O\underline{C}H_2$ (isopentyl)), 67.4 ($\underline{C}H_2CH$ (Fmoc)), 79.6 ($O\underline{C}(CH_3)_3$), 86.6 ($O\underline{C}$ (Pbf)), 117.7 (Ar$\underline{C}$), 120.2 (Ar$\underline{C}$H), 124.8 (Ar$\underline{C}$), [125.4, 125.5, 126.3 (Ar$\underline{C}$H)], [128.0, 132.5, 133.1, 138.6, 141.4, 141.5, 143.9, 144.1 (Ar$\underline{C}$)], 156.6 (CO, Boc), 156.7 ($\underline{C}$=N), 159.0 (Ar$\underline{C}$ of Pbf), 171.9 (CO), 172.5 (CO), 173.4 (CO, ester); MS (ESI +ve) *m/z* 1060.0 ($[M+H]^+$, 100%), 1082.6 ($[M+Na]^+$, 90%).

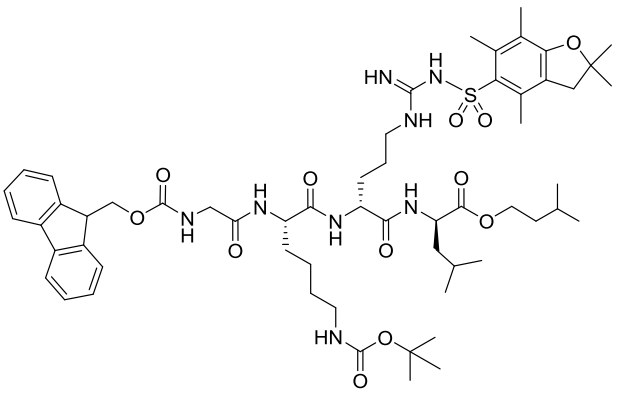
(10*S*,13*R*,16*R*)-Isopentyl 10-amino-16-isobutyl-2,2-dimethyl-4,11,14-trioxo-13-(3-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-3-oxa-5,12,15-triazaheptadecan-17-oate (263)



This compound was prepared *via* protocol 2 using **259** (320 mg, 0.301 mmol) to yield the desired product **263** as a white foam (195 mg, 77%). IR (neat, cm^{-1}): 3300 (w), [1668, 1662, 1647, 1558, 1507 (s)], 1194, 1131 (m); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90-0.91 (m, 12H, 4 x CH_3), 1.42 (s, 9H, Boc), 1.46 (s, 6H, $C(C\underline{H}_3)_2$ Pbf), 1.49-1.52 (m, 4H, $CHCH_2C\underline{H}_2$ (Lys) and $CHCH_2C\underline{H}_2$ (Arg)), 1.56-1.62 (m, 4H, $CHC\underline{H}_2CH_2$ (Lys), and $C\underline{H}_2CH_2NH$ (Lys)), 1.64-1.75 (m, 2H, $OCH_2C\underline{H}_2$), 1.80 (bs, 1H, $C\underline{H}(CH_3)_2$ (Leu)), 1.96 (bs, 5H, $CHC\underline{H}_2$ (Leu), $CHC\underline{H}_2$ (Arg) and $C\underline{H}(CH_3)_2$ (isopentyl)), 2.08 (s, 3H, $C\underline{H}_3$ (Pbf)), 2.51 (s, 3H, $C\underline{H}_3$ (Pbf)), 2.58 (s, 3H, $C\underline{H}_3$ (Pbf)), 2.95 (s, 2H, $C\underline{H}_2$ (Pbf)), 3.06-3.09 (m, 2H, $C\underline{H}_2N$ (Arg)), 3.24 (bs, 2H, $C\underline{H}_2N$ (Lys)),

3.40 (bs, 1H, NH), 4.10-4.14 (m, 2H, NH), 4.44-4.49 (m, 1H, Lys-α-proton), 4.52-4.53 (m, 1H, Leu-α-proton), 4.78 (bs, 1H, NH), 6.17 (bs, 1H, NH), 6.29 (bs, 2H, NH_2), 7.30 (bs, 1H, NH), 7.90 (bs, 2H, NH), ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.6 (CH_3, Pbf), 18.0 (CH_3, Pbf), 19.4 (CH_3, Pbf), 21.9 ($CHCH_2CH_2$ (Lys)), 22.6 (2 x CH_3 (Leu)), 22.8 (2 x CH_3 (isopentyl)), 23.0 ($CHCH_2CH_2$ (Arg)), 24.9 ($CH(CH_3)_2$ (isopentyl)), 25.1 ($CH(CH_3)_2$ (Leu)), 25.2 ($CHCH_2CH_2$ (Lys)), 28.6 ($C(CH_3)_3$), 28.7 ($C(CH_3)_2$ (Pbf)), 29.9 ($CHCH_2CH_2$ (Arg)), 30.1 (CH_2CH_2NH (Lys)), 34.6 ($CH_2CH(CH_3)_2$ (isopentyl)), 37.3 (CH_2CH_2NH (Lys)), 40.4 ($CH_2CH(CH_3)_2$ (Leu)), 40.7 ($NHCH_2$ (Lys)), 43.4 ($NHCH_2$ (Arg)), 51.3 (CH_2 (Pbf)), 52.3 (NHCHCO (Leu)), 55.1 (NHCHCO (Arg)), 64.2 (OCH_2 (isopentyl)), 79.3 ($OC(CH_3)_3$), 86.5 (OC(Pbf), [117.6, 124.7, 132.4, 133.1, 138.5 (ArC)]; 156.4 (CO, Boc), 156.5 (C=N), 158.9 (ArC, Pbf), 171.9 (CO), 173.3 (CO), 175.7 (CO, ester). MS (ESI +ve) *m/z* 738 ([M-Boc]+, 100%), 838 ([M+H]+, 95%), 860 ([M+Na]+, 22%).

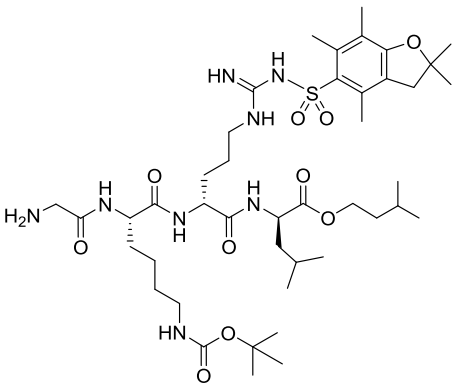
(8*S*,11*R*,14*R*)-Isopentyl 8-(4-(*tert*-butoxycarbonylamino)butyl)-1-(9*H*-fluoren-9-yl)-14-isobutyl-3,6,9,12-tetraoxo-11-(3-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxa-4,7,10,13-tetraazapentadecan-15-oate (266)



This compound was prepared *via* protocol 1 using **263** (170 mg, 0.203 mmol), Fmoc-Gly-OH (60 mg, 0.203 mmol) to yield the desired product **266** as a white foam (169 mg, 75%). IR (neat, cm^{-1}): 3302 (w), [1671, 1660, 1647, 1633, 1558, 1505 (s)], 1193, 1135 (m); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.85 (bs,

12H, 4 x C<u>H</u>$_3$ (Leu and isopentyl)), 1.38 (s, 9H, Boc), 1.42 (s, 6H, C(C<u>H</u>$_3$)$_2$ Pbf)), 1.46-1.50 (m, 4H, CHCH$_2$C<u>H</u>$_2$ (Lys) and CHCH$_2$C<u>H</u>$_2$ (Arg)), 1.56-1.66 (m, 4H, CHC<u>H</u>$_2$CH$_2$ (Lys), OCH$_2$C<u>H</u>$_2$)), 1.67-1.79 (m, 2H, C<u>H</u>$_2$CH$_2$NH (Lys)), 1.86 (bs, 1H, C<u>H</u>(CH$_3$)$_2$ (Leu)), 1.91 (bs, 1H, 1H, C<u>H</u>(CH$_3$)$_2$ (isopentyl)), 1.95 (s, 3H, C<u>H</u>$_3$ (Pbf)), 2.06 (s, 3H, C<u>H</u>$_3$ (Pbf)), 2.57 (s, 3H, C<u>H</u>$_3$ (Pbf)), 2.90 (s, 2H, C<u>H</u>$_2$ (Pbf)), 3.06-3.08 (m, 2H, C<u>H</u>$_2$N (Arg)), 3.19 (bs, 2H, C<u>H</u>$_2$N (Lys)), 3.94-4.00 (m, 2H, N<u>H</u>), 4.01-4.09 (m, 2H, CHC<u>H</u>$_2$ (Leu)), 4.17 (t, *J* = 6.5 Hz, 1H, C<u>H</u>CH$_2$ (Fmoc)), 4.33 (d, *J* = 7.0 Hz, 2H, CHC<u>H</u>$_2$ (Fmoc)), 4.46-4.47 (m, 2H, C<u>H</u>$_2$ (Gly)), 4.58 (bs, 1H, Leu-α-proton), 5.01 (bs, 1H, Lys-α-proton), 6.31 (bs, 4H, Arg-α-proton and 3 x NH), 7.23-7.25 (m, 3H, 2 x ArC<u>H</u> and NH), 7.34-7.37 (m, 2H, ArC<u>H</u>), 7.44 (bs, 2H, ArC<u>H</u>), 7.57 (d, *J* = 6.5 Hz, 2H, ArC<u>H</u>), 7.73 (d, *J* = 8.0 Hz, 2H, ArC<u>H</u>). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.6 (<u>C</u>H$_3$, Pbf), 18.3 (<u>C</u>H$_3$, Pbf), 19.4 (<u>C</u>H$_3$, Pbf), 21.8 (CHCH$_2$<u>C</u>H$_2$ (Lys)), 22.4 (2 x <u>C</u>H$_3$ (Leu)), 22.9 (2 x <u>C</u>H$_3$ (isopentyl)), 22.95 (CHCH$_2$<u>C</u>H$_2$ (Arg)), 23.0 (<u>C</u>H(CH$_3$)$_2$ (isopentyl)), 25.2 (<u>C</u>H(CH$_3$)$_2$ (Leu)), 25.25 (CH<u>C</u>H$_2$CH$_2$ (Lys)), 28.8 (C(<u>C</u>H$_3$)$_3$), 29.0 (C(<u>C</u>H$_3$)$_2$ Pbf), 29.5 (CH<u>C</u>H$_2$CH$_2$ (Arg)), 29.8 (<u>C</u>H$_2$CH$_2$NH (Lys)), 31.7 (CH<u>C</u>H$_2$ (Leu)), 37.6 (2 x <u>C</u>H$_2$ (<u>C</u>H$_2$CH(CH$_3$)$_2$ (isopentyl) and CH$_2$<u>C</u>H$_2$NH (Lys)), 40.3 (<u>C</u>H$_2$CH(CH$_3$)$_2$ (Leu)), 40.6 (NH<u>C</u>H$_2$ (Lys)), 43.9 (NHC<u>H</u>$_2$ (Arg)), 44.4 (<u>C</u>H (Gly)), 47.3 (<u>C</u>H$_2$ (Pbf)), 51.3 (CH$_2$<u>C</u>H (Fmoc)), 52.7 (NH<u>C</u>HCO (Leu)), 53.7 (NH<u>C</u>HCO (Lys)), 55.9 (NH<u>C</u>HCO (Arg)), 64.3 (O<u>C</u>H$_2$ (isopentyl)), 67.4 (<u>C</u>H$_2$CH (Fmoc)), 79.6 (O<u>C</u>(CH$_3$)$_3$), 86.6 (O<u>C</u>(CH$_3$)$_2$), 117.6 (Ar<u>C</u>), 121.9 (2 x Ar<u>C</u>H), 124.9 (2 x Ar<u>C</u>H), [125.4, 125.5, 128.0 (Ar<u>C</u>H)], [132.5, 133.1, 138.6, 141.4, 141.5, 143.9, 144.3 (Ar<u>C</u>)], 156.6 (C=N), 156.7 (CO, Boc), 158.9 (Ar<u>C</u>), 172.0 (2 x CO), 172.9 (2 x CO), 173.7 (CO, ester); MS (ESI +ve) *m/z* 1017.6 ([M-Boc]$^+$, 100%), 1117.6 ([M+H]$^+$, 95%), 1139.6 ([M+Na]$^+$, 22%).

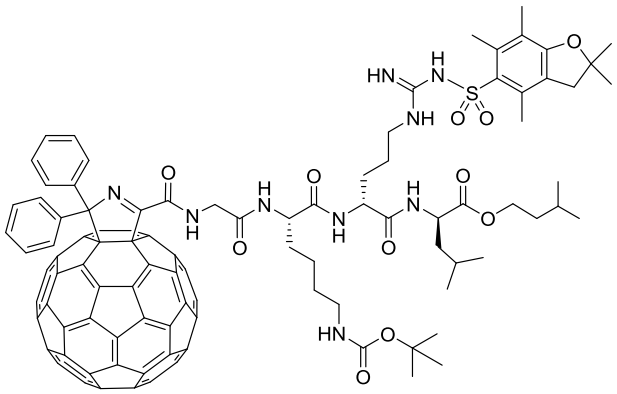
(10*S*,13*R*,16*R*)-Isopentyl 10-(2-aminoacetamido)-16-isobutyl-2,2-dimethyl-4,11,14-trioxo-13-(3-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-3-oxa-5,12,15-triazaheptadecan-17-oate (269)



This compound was prepared *via* protocol 2 using **266** (140 mg, 0.125 mmol) to yield the desired product **269** as a white foam (90 mg, 80%). IR (neat, cm^{-1}): 3300 (w), [1666, 1662, 1647, 1635, 1557, 1502 (s)], [1194, 1137 (m)]; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87-0.91 (m, 12H, 4 x C$\underline{H}_3$ (Leu and isopentyl)), 1.41 (s, 9H, Boc), 1.45 (s, 6H, C(C$\underline{H}_3$)$_2$ Pbf), 1.48-1.52 (m, 4H, CHCH$_2$C$\underline{H}_2$ (Lys) and CHCH$_2$C$\underline{H}_2$ (Arg)), 1.56-1.61 (m, 4H, CHC$\underline{H}_2$CH$_2$ (Lys), and C$\underline{H}_2$CH$_2$NH (Lys)), 1.63-1.74 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.86-1.94 (m, 3H, C$\underline{H}$(CH$_3$)$_2$ (Leu) and CHC$\underline{H}_2$ (Arg)), 1.96 (bs, 1H, C$\underline{H}$(CH$_3$)$_2$ (isopentyl)), 2.02 (bs, 2H, CHC$\underline{H}_2$ (Leu) and), 2.08 (s, 3H, C$\underline{H}_3$ (Pbf)), 2.51 (s, 3H, C$\underline{H}_3$ (Pbf)), 2.58 (s, 3H, C$\underline{H}_3$ (Pbf)), 2.95 (s, 2H, C$\underline{H}_2$ (Pbf)), 3.06-3.08 (m, 2H, C$\underline{H}_2$N (Arg)), 3.22 (bs, 2H, C$\underline{H}_2$N (Lys)), 3.41 (m, 2H, N$\underline{H}$), 4.07-4.14 (m, 2H, C$\underline{H}_2$ (Gly)), 4.36 (bs, 1H, N$\underline{H}$), 4.42-4.48 (m, 2H, Lys-α-proton and Leu-α-proton), 4.91 (bs, 1H, NH), 6.40 (bs, 1H, NH), 6.35 (bs, 2H, N$\underline{H}_2$), 7.36 (d, *J* = 7 Hz, 1H, NH), 7.45 (bs, 1H, NH), 7.85 (bs, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.5 (C$\underline{H}_3$, Pbf), 18.0 (C$\underline{H}_3$, Pbf), 19.2 (C$\underline{H}_3$, Pbf), 21.8 (CHCH$_2$C$\underline{H}_2$ (Lys)), 22.4 (C$\underline{H}_3$ (Leu)), 22.8 (2 x C$\underline{H}_3$ (isopentyl)), 22.9 (CHCH$_2$C$\underline{H}_2$ (Arg)), 24.8 (C$\underline{H}$(CH$_3$)$_2$ (isopentyl)), 25.0 (C$\underline{H}$(CH$_3$)$_2$ (Leu)), 25.4 (CHC$\underline{H}_2$CH$_2$ (Lys)), 28.4 (C(C$\underline{H}_3$)$_3$), 28.6 (C(C$\underline{H}_3$)$_2$ Pbf), 29.6 (C$\underline{H}_2$CH(CH$_3$)$_2$ (isopentyl)), 29.7 (CHC$\underline{H}_2$CH$_2$ (Arg)), 31.7 (C$\underline{H}_2$CH$_2$NH (Lys)), 37.2 (CH$_2$C$\underline{H}_2$NH (Lys)), 40.2 (C$\underline{H}_2$CH(CH$_3$)$_2$ (Leu)), 40.6 (NHC$\underline{H}_2$ (Lys)), 43.3 (NHC$\underline{H}_2$ (Arg)), 44.4 (NHC$\underline{H}_2$CO (Gly)), 51.2 (C$\underline{H}_2$ (Pbf)), 52.9

(NHCHCO (Leu)), 53.7 (NHCHCO (Arg)), 64.0 (OCH_2 (isopentyl)), 79.1 ($OC(CH_3)_3$), 86.3 ($OC(CH_3)_2$ Pbf), [117.5, 124.5, 132.2, 133.0, 138.3 (ArC)], 156.4 (C=N), 156.7 (CO, Boc), 158.7 (ArC), 171.7 (2 x CO), 172.2 (CO), 173.0 (CO, ester). MS (ESI +ve) *m/z* 895.6 ($[M+H]^+$, 100%), 917.6 ($[M+Na]^+$, 38%).

(10*S*,13*R*,16*R*)-Isopentyl-10-(2-(5,5-diphenylfullerenyldihydropyrrole-2-carbonyl)aminoacetamido)-16-isobutyl-2,2-dimethyl-4,11,14-trioxo-13-(3-(3-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonylguanidino)propyl)-3-oxa-5,12,15-triazaheptadecan-17-oate (272)

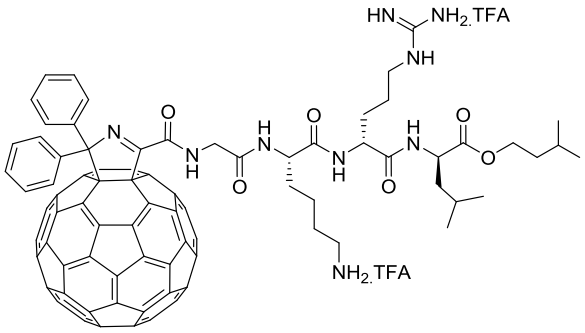


This compound was prepared *via* protocol 1, using [*60*]fullerenoproline acid **198** (75 mg, 0.067 mmol) and amine **269** (72 mg, 0.080 mmol) to yield the product **272** as a brown solid (65 mg, 53%). IR (neat, cm^{-1}): 3304 (w), [1669, 1662, 1647, 1635, 1558, 1507 (s)], [1194, 1135 (m)]; ^1H NMR (500 MHz, $CDCl_3$) δ 0.87-0.90 (m, 12H, 4 x CH_3 (Leu and isopentyl)), 0.95-1.05 (m, 2H, $CHCH_2CH_2$ (Lys)), 1.42 (s, 9H, Boc), 1.46 (s, 6H, $C(CH_3)_2$ Pbf), 1.48-1.57 (m, 4H, $CHCH_2CH_2$ (Arg) and $CHCH_2CH_2$ (Lys)), 1.63-1.66 (m, 6H, CH_2CH_2NH (Lys), OCH_2CH_2 and $CHCH_2$ (Arg)), 1.85-1.86 (m, 4H, $CH(CH_3)_2$ (isopentyl), $CHCH_2$ (Leu) and $CH(CH_3)_2$ (Leu)), 2.08 (s, 3H, CH_3 (Pbf)), 2.50 (s, 3H, CH_3 (Pbf)), 2.57 (s, 3H, CH_3 (Pbf)), 2.95 (s, 2H, CH_2 (Pbf)), 3.05 (bs, 2H, CH_2N (Arg)), 3.16 (bs, 2H, CH_2N (Lys)), 3.35 (m, 2H, NH), 3.62-3.76 (m, 2H, OCH_2), 4.07-4.09 (m, 2H, Lys-α-proton and Leu-α-proton), 4.28 (s, 2H, CH_2 (Gly)), 4.34-4.44 (m, 3H, Arg-α-proton and 2 x NH), 5.33

(bs, 1H, NH), 7.39-7.42 (m, 3H, 2 x ArCH, NH), 7.50-7.52 (m, 4H, ArCH), 7.81-7.83 (bs, 2H, NH), 8.08-8.10 (m, 4H, ArCH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.2 (CH_3, Pbf), 17.7 (CH_3, Pbf), 19.0 (CH_3, Pbf), 21.2 ($CHCH_2CH_2$ (Lys)), 22.1 (CH_3 (Leu)), 22.2 (CH_3 (Leu)), 22.8 (2 x CH_3 (isopentyl)), 22.9 ($CHCH_2CH_2$ (Arg)), 24.6 ($CH(CH_3)_2$ (isopentyl)), 24.8 ($CHCH_2CH_2$ (Lys)), 25.0 ($CH(CH_3)_2$ (Leu)), 28.3 ($C(CH_3)$), 28.6 ($C(CH_3)_2$ Pbf)), 29.2 ($CH_2CH(CH_3)_2$ (isopentyl)), 29.6 ($CHCH_2CH_2$ (Arg)), 31.7 (CH_2CH_2NH (Lys)), 37.0 (CH_2CH_2NH (Lys)), 40.2 ($CH_2CH(CH_3)_2$ (Leu)), 40.3, 40.6 ($NHCH_2$ (Lys)), 42.5 ($NHCH_2$ (Arg)), 43.0 ($NHCH_2CO$ (Gly)), 50.9 (CH_2 (Pbf)), 51.0 (NHCHCO (Leu)), 52.4, 53.3 (NHCHCO (Arg)), 53.9, 63.9 (OCH_2 (isopentyl)), 66.3, 79.2 ($C(CH_3)_3$), 82.2* ($C_{60}sp^3$), 83.3* ($C_{60}sp^3$), 86.3, 95.4 ($NCPh_2$), [117.3, 124.3 (ArC)], 128.3 (ArCH), 129.6 (ArCH), [132.2, 132.8 (ArC)], 134.6 (ArCH)]; 136.5, 138.2 (ArC), [139.0, 139.3, 139.3 ($C_{60}sp^2$)]; 140.8 (ArCH), [141.2, 141.6, 141.7, 141.8, 142.3, 142.34, 142.6, 142.65, 142.8, 144.0, 144.1, 144.7, 144.9, 145.1, 145.13, 145.2, 145.3, 145.7, 145.8, 145.89, 146.2, 146.8* (1/2 x C), 146.9* (1/2 x C), 148.2, 148.9, 153.1 ($C_{60}sp^2$)], 156.7 (CO, Boc), 158.5 (2 x C=N), 161.8 (ArC, Pbf), [162.0, 169.2, 172.0, 172.4, 172.9 (CO)]. MS (ESI +ve) *m/z* 1857 ($[M+Na]^+$, 100%), 1835 ($[M+H]^+$, 60%). HRMS (ESI +ve) calcd for $C_{118}H_{83}N_9O_{11}SNa$ 1856.5830, found 1856.5916.

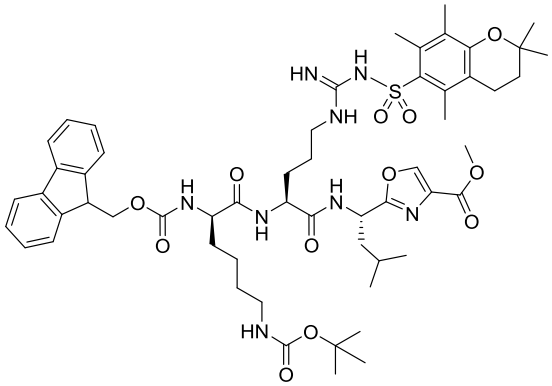
1-(*R*)-4-(*S*)-2-(2-(5,5-Diphenylfullerenyldihydropyrrole-2-carbonyl aminoacetamido)-6-ammoniohexanamido)-5-(*R*)-1-(isopentyloxy)-4-methyl-1-oxopentan-2-yl)amino-5-oxopentylguanidinium 2,2,2-trifluoroacetate (276)

This compound was prepared *via* protocol 3, using **272** (30 mg, 0.016 mmol) to yield **276** as a brown solid (17 mg, 70%). IR (neat, cm^{-1}): 3291 (w), [1734, 1669, 1647, 1635, 1558 (s)], [1186, 1158, 1146 (m)]; ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD) δ 0.83-0.89 (m, 12H, 4 x CH_3 (Leu and isopentyl)), 1.24-1.28 (m, 2H, $CHCH_2CH_2$ (Lys)), 1.42-1.54 (m,



7H, CHCH$_2$C$\underline{H}_2$ (Arg), CHC$\underline{H}_2$CH$_2$ (Lys) and CHC$\underline{H}_2$ (Leu) and C$\underline{H}$(CH$_3$)$_2$ (Leu)), 1.62-1.68 (m, 5H, C$\underline{H}_2$CH$_2$NH (Lys), OCH$_2$C$\underline{H}_2$ and C$\underline{H}$(CH$_3$)$_2$ (isopentyl)), 1.70-1.79 (m, 2H,), 2.80 (bs, 2H, C$\underline{H}_2$N (Lys)), 3.10-3.12 (m, 2H, C$\underline{H}_2$N (Arg)), 3.26 (s, 2H, C$\underline{H}_2$ (Gly)), 3.55 (bs, 1H, N$\underline{H}$), 4.00-4.02 (m, 2H, OC$\underline{H}_2$), 4.12-4.20 (m, 2H, Lys-α-proton and Leu-α-proton), 4.40-4.60 (bs, 2H, Arg-α-proton and N$\underline{H}$), 7.36 (t, 2H, *J* = 6.5 Hz, ArC$\underline{H}$), 7.47 (t, 4H, *J* = 7.5 Hz, ArC$\underline{H}$), 7.79-7.80 (bs, 1H, NH), 8.03 (d, 4H, *J* = 8.0 Hz, ArC$\underline{H}$), 8.12 (bs, 1H, NH), 8.28 (bs, 1H, NH), 9.10 (bs, 1H, NH). This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum. HRMS (ESI +ve) calcd for $C_{100}H_{59}N_9O_6$ 1482.4667, found 1482.4717.

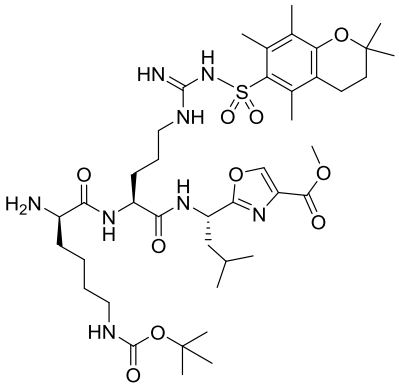
Methyl 2-((10*R*,13*S*,16*S*)-10-(9*H*-fluoren-9-yl)methoxycarbonylamino-2,2,18-trimethyl-4,11,14-trioxo-13-(3-(3-((2,2,5,7,8-pentamethylchroman-6-yl)sulfonyl)guanidino)propyl)-3-oxa-5,12,15-triazanonadecan-16-yl)oxazole-4-carboxylate (260)



This compound was prepared *via* protocol 1 using amine **256** (350 mg, 0.552 mmol) and Fmoc-(*D*)-Lys(Boc)-OH (258 mg, 0.552 mmol) to yield the desired product **260** as a white foam (515 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84-0.87 (m, 6H, 2 x C$\underline{H}_3$ (Leu)), 1.28 (s, 6H, 2 x C$\underline{H}_3$ (Pmc)), 1.39 (s, 9H, Boc), 1.46-1.65 (m, 7H,

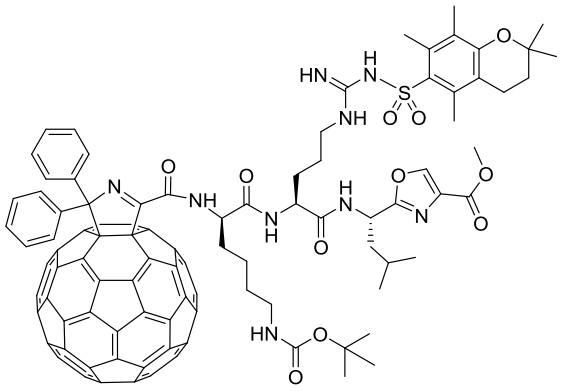
$CHCH_2C\underline{H}_2$ (Lys), $CHCH_2C\underline{H}_2$ (Arg), $C\underline{H}(CH_3)_2$ (Leu) and $C\underline{H}_2CH_2NH$ (Lys)), 1.75-1.78 (m, 6H, $CHC\underline{H}_2$ (Leu), $CHC\underline{H}_2CH_2$ (Lys) and $CHC\underline{H}_2CH_2$ (Arg)), 1.96 (bs, 1H, N<u>H</u>), 2.05 (s, 3H, $C\underline{H}_3$ (Pmc)), 2.08 (s, 3H, $C\underline{H}_3$ (Pmc)), 2.54 (s, 3H, $C\underline{H}_3$ (Pmc)), 2.59-2.60 (m, 4H, 2 x $C\underline{H}_2$ (Pmc)), 3.05 (bs, 2H, $C\underline{H}_2N$ (Arg)), 3.20-3.26 (m, 2H, $C\underline{H}_2N$ (Lys)), 3.80 (s, 3H, $OC\underline{H}_3$), 4.12-4.17 (m, 2H, $C\underline{H}_2$ (Fmoc)), 4.25-4.32 (m, 2H, Lys-α-proton and Arg-α-proton), 4.54-4.55 (m, 1H, C<u>H</u> (Fmoc)), 4.83 (bs, 1H, N<u>H</u>), 5.14-5.18 (m, 1H, α-C<u>H</u> to oxazole), 5.92 (bs, 1H, N<u>H</u>), 6.19 (bs, 1H, N<u>H</u>), 6.32 (bs, 2H, N<u>H</u>), 7.26-7.28 (m, 2H, ArC<u>H</u>), 7.36 (t, *J* = 7.5 Hz, 2H, ArC<u>H</u>), 7.54 (t, *J* = 8.5 Hz, 2H, ArC<u>H</u>), 7.72 (d, *J* = 7.5 Hz, 2H, ArC<u>H</u>), 8.04 (s, 1H, oxazole); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.3 ($\underline{C}H_3$, Pmc), 17.7 ($\underline{C}H_3$, Pmc), 18.7 ($\underline{C}H_3$, Pmc), 21.6 ($CHCH_2\underline{C}H_2$ (Lys)), 22.8 ($CHCH_2\underline{C}H_2$ (Arg)), 22.9 (2 x $\underline{C}H_3$ (Leu)), 24.8 ($\underline{C}H(CH_3)_2$ (Leu)), 26.9 ($CH\underline{C}H_2$ (Lys)), 27.0 ($C(\underline{C}H_3)_2$ (Pmc)), 27.2 ($\underline{C}H_2$ (Pmc)), 28.6 ($C(\underline{C}H_3)_3$), 29.8 ($CH\underline{C}H_2CH_2$ (Arg) and ($\underline{C}H_2CH_2NH$ (Lys)), 33.0 ($\underline{C}H_2$ (Pmc)), 40.5 ($NH\underline{C}H_2$ (Lys)), 41.8 ($NHC\underline{H}_2$ (Arg)), 46.5 ($CH\underline{C}H_2$ (Leu)), 47.2 ($CH_2\underline{C}H$ (Fmoc)), 51.8 ($O\underline{C}H_3$ ester), 52.3 (NH<u>C</u>H (Leu)), 53.6 (NH<u>C</u>HCO (Lys)), 55.2 (NH<u>C</u>HCO (Arg)), 67.3 ($\underline{C}H_2$ (Fmoc)), 73.8 ($O\underline{C}(CH_3)_2$), 79.4 ($O\underline{C}(CH_3)_3$), 118.1 (Ar<u>C</u>), 120.1 (Ar<u>C</u>H), 124.2 (Ar<u>C</u>), [125.3, 127.3, 127.9 (Ar<u>C</u>H)], [132.9, 133.6, 135.0, 135.6 (Ar<u>C</u>)], 141.4 (<u>C</u>H, oxazole), 143.9 (Ar<u>C</u>), 144.3 (Ar<u>C</u>), [153.8, 156.5 (CO)], 156.55 (C=N), 162.4 (Ar<u>C</u>), [165.7, 170.3, 171.8 (CO)]. MS (ESI +ve) *m/z* 1085.0 ($[M+H]^+$, 100%).

Methyl 2-((10*R*,13*S*,16*S*)-10-amino-2,2,18-trimethyl-4,11,14-trioxo-13-(3-(3-((2,2,5,7,8-pentamethylchroman-6-yl)sulfonyl)guanidino)propyl)-3-oxa-5,12,15-triazanonadecan-16-yl)oxazole-4-carboxylate (264)



This compound was prepared *via* protocol 2 using **260** (450 mg, 0.415 mmol) to yield the desired product **264** as a white foam (298 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87-0.90 (m, 6H, 2 x C$\underline{H}_3$ (Leu)), 1.25-1.28 (m, 2H, CHCH$_2$C$\underline{H}_2$ (Lys)), 1.29 (s, 6H, 2 x C$\underline{H}_3$ (Pmc)), 1.32-1.37 (m, 2H, CHCH$_2$C$\underline{H}_2$ (Arg)), 1.41 (s, 9H, Boc), 1.45-1.56 (m, 3H, C$\underline{H}_2$CH$_2$NH (Lys) and C$\underline{H}$(CH$_3$)$_2$ (Leu)), 1.78-1.80 (m, 6H, CHC$\underline{H}_2$ (Leu), CHC$\underline{H}_2$CH$_2$ (Lys) and CHC$\underline{H}_2$CH$_2$ (Arg)), 2.09 (s, 3H, C$\underline{H}_3$ (Pmc)), 2.55-2.62 (m, 10H, 2 x C$\underline{H}_3$ (Pmc) and 2 x C$\underline{H}_2$ (Pmc)), 3.01-3.07 (m, 4H, C$\underline{H}_2$N (Arg) and C$\underline{H}_2$N (Lys)), 3.20-3.26 (bs, 2H, NH$_2$), 3.80 (s, 3H, OC$\underline{H}_3$), 3.88 (bs, 1H, Lys-α-proton), 4.55 (bs, 1H, Arg-α-proton), 4.65 (bs, 1H, NH), 5.14-5.21 (m, 1H, α-CH to oxazole), 6.21 (bs, 1H, NH), 6.36 (bs, 2H, NH), 7.86 (bs, 1H, NH), 8.15 (s, 1H, oxazole); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.2 (C$\underline{H}_3$, Pmc), 17.5 (C$\underline{H}_3$, Pmc), 18.5 (C$\underline{H}_3$, Pmc), 21.5 (CHCH$_2$C$\underline{H}_2$ (Lys)), 21.8 (CHCH$_2$C$\underline{H}_2$ (Arg)), 22.7 (2 x C$\underline{H}_3$ (Leu)), 24.7 (C$\underline{H}$(CH$_3$)$_2$ (Leu)), 25.3 (CHC$\underline{H}_2$ (Lys)), 26.8 (C(C$\underline{H}_3$)$_2$ (Pmc)), 28.5 (C(C$\underline{H}_3$)$_3$), 29.6 (C$\underline{H}_2$ (Pmc)), 31.0 (CHC$\underline{H}_2$CH$_2$ (Arg)), 32.9 (C$\underline{H}_2$CH$_2$NH (Lys) and C$\underline{H}_2$ (Pmc)), 40.3 (NHC$\underline{H}_2$ (Lys)), 41.9 (NHC$\underline{H}_2$ (Arg)), 46.0 (CHC$\underline{H}_2$ (Leu)), 52.2 (OC$\underline{H}_3$ ester), 52.3 (NHC$\underline{H}$ (Leu)), 55.2 (NHC$\underline{H}$CO (Lys)), 58.5 (NHC$\underline{H}$CO (Arg)), 73.6 (OC(CH$_3$)$_2$), 79.6 (OC(CH$_3$)$_3$), [118.0, 124.0, 132.8, 133.6, 134.9, 135.5, 137.4 (Ar$\underline{C}$)], 144.2 (C$\underline{H}$, oxazole), 153.6 (C=N), 156.5 (CO), 162.1 (Ar$\underline{C}$), [166.0, 170.8, 171.3 (CO)]. MS (ESI +ve) *m/z* 863.0 ([M+H]$^+$, 100%).

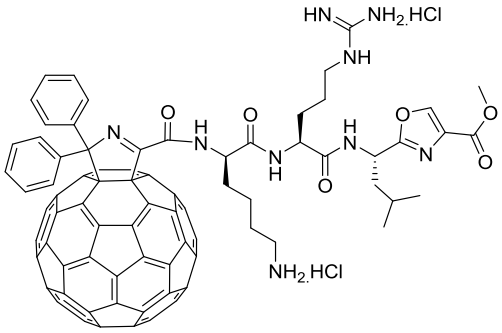
Methyl 2-((10*R*,13*S*,16*S*)-10-(5,5-diphenylfullerenyldihydropyrrole-2-carbonyl) amino-2,2,18-trimethyl-4,11,14-trioxo-13-(3-(3-((2,2,5,7,8-pentamethylchroman-6-yl)sulfonylguanidinopropyl)-3-oxa-5,12,15-triazanonadecan-16-yl)oxazole-4-carboxylate (273)



This compound was prepared *via* protocol 1, using [*60*]fullerenoproline acid **198** (95 mg, 0.099 mmol) and amine **264** (102 mg, 0.118 mmol) to yield the product as a white brown (98 mg, 55%). IR (neat, cm^{-1}): 3309 (w), [1685, 1647, 1635, 1558, 1507 (s)], 1195 (m), 1136; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84-0.87 (m, 6H, 2 x C<u>H</u>$_3$ (Leu)), 1.29 (s, 6H, 2 x C<u>H</u>$_3$ (Pmc)), 1.39 (s, 9H, Boc), 1.42-1.58 (m, 6H, CHCH$_2$C<u>H</u>$_2$ (Lys), CHCH$_2$C<u>H</u>$_2$ (Arg) and C<u>H</u>$_2$CH$_2$NH (Lys)), 1.66-1.71 (m, 2H, CHC<u>H</u>$_2$CH$_2$ (Lys)), 1.76-1.96 (m, 5H, CHC<u>H</u>$_2$ (Leu), CHC<u>H</u>$_2$CH$_2$ (Arg) and C<u>H</u>(CH$_3$)$_2$ (Leu)), 1.99 (s, 3H C<u>H</u>$_3$ (Pmc)), 2.07 (s, 3H, C<u>H</u>$_3$ (Pmc)), 2.54-2.58 (m, 7H, C<u>H</u>$_3$, 2 x C<u>H</u>$_2$ (Pmc)), 3.07 (bs, 2H, C<u>H</u>$_2$N (Arg)), 3.25-3.62 (m, 2H, C<u>H</u>$_2$N (Lys)), 3.20-3.26 (bs, 2H, NH), 3.80 (s, 3H, OC<u>H</u>$_3$), 4.60 (bs, 1H, Lys-α-proton), 4.66-4.68 (m, 1H, Arg-α-proton), 4.80 (bs, 1H, NH), 5.12 (bs, 1H, α-CH to oxazole), 6.18 (bs, 1H, NH), 6.33 (bs, 2H, NH), 7.37-7.41 (m, 2H, ArC<u>H</u>), 7.46-7.52 (m, 4H, ArC<u>H</u>), 7.60 (d, *J* = 7.0 Hz, NH), 8.05 (t, 4H, *J* = 8.5 Hz, 4H, ArC<u>H</u>), 8.08 (s, 1H, oxazole), 8.37 (bs, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.3 (<u>C</u>H$_3$, Pmc), 17.7 (<u>C</u>H$_3$, Pmc), 18.7 (<u>C</u>H$_3$, Pmc), 21.5 (CHCH$_2$<u>C</u>H$_2$ (Lys)), 21.7 (CHCH$_2$<u>C</u>H$_2$ (Arg)), 22.9 (2 x <u>C</u>H$_3$ (Leu)), 24.7 (<u>C</u>H(CH$_3$)$_2$ (Leu)), 26.9 (C(<u>C</u>H$_3$)$_2$ (Pmc) and CH<u>C</u>H$_2$ (Lys)), 28.5 (C(<u>C</u>H$_3$)$_3$), 29.6 (<u>C</u>H$_2$ (Pmc)), 32.9 (CH<u>C</u>H$_2$CH$_2$ (Arg)) and <u>C</u>H$_2$CH$_2$NH (Lys)), 40.2 (NH<u>C</u>H$_2$ (Lys)), 41.8 (NHC<u>H</u>$_2$ (Arg)), 46.7 (CH<u>C</u>H$_2$ (Leu)),

52.3 (O<u>C</u>H_3 ester), 54.2 (NH<u>C</u>H (Leu)), 55.2 (NH<u>C</u>HCO (Lys)), 58.5 (NH<u>C</u>HCO (Arg)), 73.7 (O<u>C</u>$(CH_3)_2$), 82.2 (O<u>C</u>$(CH_3)_3$), 95.8 (N<u>C</u>Ph_2), 118.3 (Ar<u>C</u> (Pmc)), 124.1 (Ar<u>C</u> (Pmc)), [128.5, 128.6, 129.9 (Ar<u>C</u>H)], 133.0 (Ar<u>C</u>), [134.9, 135.6 ($C_{60}sp^2$)], [136.7, 138.2 (Ar<u>C</u>, Pmc)], [139.3, 139.5 ($C_{60}sp^2$)], [139.8, 140.8 (Ar<u>C</u>)], 141.0 (<u>C</u>H, oxazole), [141.3, 141.8, 141.9, 142.0, 142.5, 142.8, 142.9, 143.0, 143.08, 144.2, 144.3, 144.4, 145.0, 145.2, 145.3, 145.5, 145.6, 145.9, 146.4* (1/2 x C), 146.5* (1/2 x C), 147.0, 147.2, 148.3, 148.6, 149.0, 153.4, ($C_{60}sp^2$)], 153.7 (C=N), 156.5 (CO, Boc), 157.8 (C=N), [161.5, 161.9 (CO)], 162.3 (Ar<u>C</u>), 171.7 (2 x CO)]. Two $C_{60}sp^3$ signals were not observed due to poor signal to noise. HRMS (ESI +ve) calcd for $C_{116}H_{75}N_9O_{11}SNa$ 1824.5204, found 1824.5183.

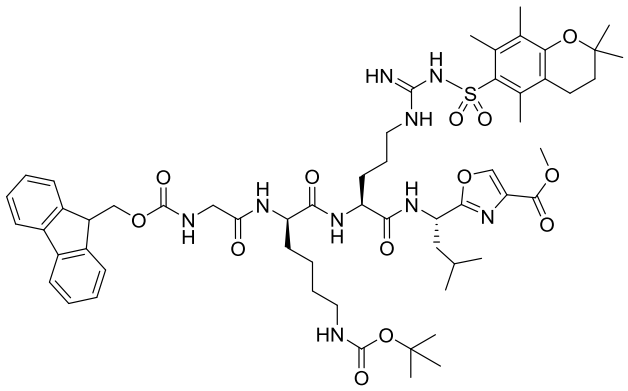
1-(*S*)-4-(*R*)-2-(5,5-Diphenylfullerenyldihydropyrrole-2-carbonylamino-6-ammoniohexanamido)-5-(*S*)-1-(4-(methoxycarbonyloxazol-2-yl)-3-methylbutylamino)-5-oxopentyl)guanidinium chloride (277)



This compound was prepared *via* protocol 3, using **273** (60 mg, 0.033 mmol) to yield **277** as a brown solid (25 mg, 53%). IR (neat, cm^{-1}): 3336 (w), [1647, 1635, 1507 (s)], 1162 (m); ^{1}H NMR (500 MHz, DMSO-d6): δ 0.77-0.86 (m, 6H, 2 x C<u>H</u>$_3$ (Leu)), 1.22-1.66 (m, 6H, CHCH$_2$C<u>H</u>$_2$ (Lys), CHCH$_2$C<u>H</u>$_2$ (Arg) and C<u>H</u>$_2$CH$_2$NH (Lys))), 1.67-1.79 (m, 4H, CHC<u>H</u>$_2$CH$_2$ (Lys) and CHC<u>H</u>$_2$CH$_2$ (Arg)), 1.85-1.88 (m, 3H, CHC<u>H</u>$_2$ (Leu) and C<u>H</u>$(CH_3)_2$ (Leu)), 2.73 (bs, 2H, NH_2), 3.05 (bs, 2H, C<u>H</u>$_2$N (Arg)), 3.34-3.62 (m, 2H, C<u>H</u>$_2$N (Lys)), 3.75 (s, 3H, OC<u>H</u>$_3$), 4.17 (bs, 1H, Lys-α-proton), 4.36 (t, 1H, *J* = 6.5 Hz, NH), 4.45 (bs, 1H, Arg-α-proton), 4.62 (t, 1H, *J* = 7.5 Hz, NH), 4.97-4.98 (m, 1H, α-CH to oxazole), 7.41-7.45 (m, 2H, ArC<u>H</u>), 7.53-7.58 (m,

4H, ArCH), 8.06-8.10 (m, 4H, ArCH), 8.17 (d, 1H, *J* = 7.5 Hz, NH), 8.38 (d, 1H, *J* = 8.0 Hz, NH), 8.43 (d, 1H, *J* = 8.0 Hz, NH), 8.56 (d, 1H, *J* = 8.0 Hz, NH), 8.68 (s, 1H, oxazole), 9.50-9.55 (m, 2H, NH_2 (Arg)). This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum. HRMS (ESI +ve) calcd for $C_{97}H_{49}N_9O_6$ 1436.3884, found 1436.3822.

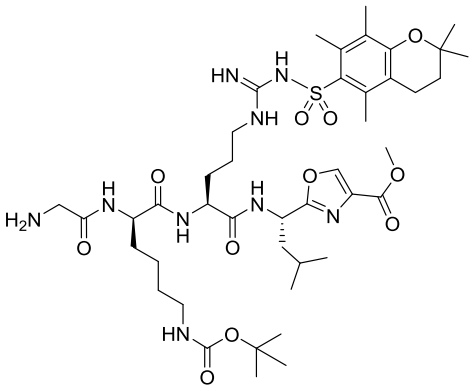
Methyl 2-((8*R*,11*S*,14*S*)-8-(4-(*tert*-butoxycarbonyl)aminobutyl)-1-(9*H*-fluoren-9-yl)-16-methyl-3,6,9,12-tetraoxo-11-(3-(3-((2,2,5,7,8-pentamethylchroman-6-yl)sulfonyl)guanidino)propyl)-2-oxa-4,7,10,13-tetraazaheptadecan-14-yl)oxazole-4-carboxylate (267)



This compound was prepared *via* protocol 1 using **264** (136 mg, 0.157 mmol) and Fmoc-Gly-OH (47 mg, 0.157 mmol) to yield the desired product **267** as a white foam (128 mg, 71%). ^1H NMR (500 MHz, $CDCl_3$): δ 0.82 (d, *J* = 6.0 Hz, 6H, 2 x CH_3 (Leu)), 1.26 (s, 6H, 2 x CH_3 (Pmc)), 1.38 (s, 9H, Boc), 1.43-1.66 (m, 7H, $CHCH_2CH_2$ (Lys), $CHCH_2CH_2$ (Arg), $CH(CH_3)_2$ (Leu) and CH_2CH_2NH (Lys)), 1.74-1.76 (m, 6H, $CHCH_2$ (Leu), $CHCH_2CH_2$ (Lys) and $CHCH_2CH_2$ (Arg)), 1.96 (bs, 1H, NH), 2.06 (s, 3H, CH_3 (Pmc)), 2.53 (s, 3H, CH_3 (Pmc)), 2.55 (s, 3H, CH_3 (Pmc)), 2.57-2.58 (m, 4H, 2 x CH_2 (Pmc)), 3.01 (bs, 2H, CH_2N (Arg)), 3.18-3.26 (m, 3H, CH_2N (Lys) and NH), 3.70 (s, 3H, OCH_3), 3.90-4.13 (m, 3H, CH_2 (Fmoc) and NH), 4.22 (bs, 2H, Lys-α-proton and Arg-α-proton), 4.53 (bs, 2H, CH_2 (Gly)), 4.54-4.55 (m, 1H, CH (Fmoc)), 4.83 (bs, 1H, NH), 5.14-5.18 (m, 1H, α-CH to oxazole), 5.92 (bs, 1H, NH), 6.19 (bs, 1H, NH), 6.32 (bs, 2H, NH), 7.26-7.28

(m, 2H, ArCH), 7.36 (t, *J* = 7.5 Hz, 2H, ArCH), 7.54 (t, *J* = 8.5 Hz, 2H, ArCH), 7.72 (d, *J* = 7.5 Hz, 2H, ArCH), 8.04 (s, 1H, oxazole); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.3 (CH_3, Pmc), 17.7 (CH_3, Pmc), 18.7 (CH_3, Pmc), 21.6 ($CHCH_2CH_2$ (Lys)), 22.8 ($CHCH_2CH_2$ (Arg)), 22.88 (2 x CH_3 (Leu)), 24.8 ($CH(CH_3)_2$ (Leu)), 26.9 ($CHCH_2$ (Lys)), 26.98 ($C(CH_3)_2$ (Pmc)), 27.2 (CH_2 (Pmc)), 28.6 ($C(CH_3)_3$), 29.8 ($CHCH_2CH_2$ (Arg) and (CH_2CH_2NH (Lys)), 33.0 (CH_2 (Pmc)), 40.5 ($NHCH_2$ (Lys)), 41.8 ($NHCH_2$ (Arg)), 44.2 (CH_2 (Gly)), 46.5 ($CHCH_2$ (Leu)), 47.2 (CH_2CH (Fmoc)), 52.3 (NHCH (Leu)), 53.6 (NHCHCO (Lys)), 55.2 (NHCHCO (Arg)), 67.3 (CH_2, (Fmoc)), 73.8 ($OC(CH_3)_2$), 79.4 ($OC(CH_3)_3$), 118.1 (ArC), 120.1 (ArCH), 124.2 (ArC), [125.3, 127.3, 127.9 (ArCH)], [132.9, 133.6, 135.0, 135.6 (ArC)], 141.4 (CH, oxazole), 143.9 (ArC), 144.3 (ArC), [153.8, 156.5 (CO)], 156.6 (C=N), 165.7 (ArC), 170.6 (2 x CO), 171.8 (CO)]. MS (ESI +ve) *m/z* 1142.0 ($[M+H]^+$, 100%).

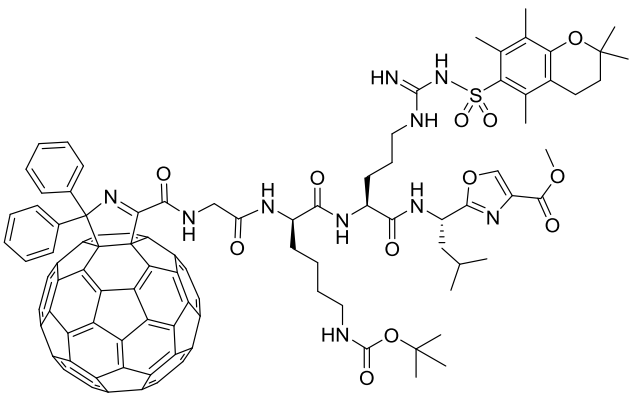
Methyl 2-((10*R*,13*S*,16*S*)-10-(2-aminoacetamido)-2,2,18-trimethyl-4,11,14-trioxo-13-(3-(3-(2,2,5,7,8-pentamethylchroman-6-yl)sulfonylguanidino)propyl)-3-oxa-5,12,15-triazanonadecan-16-yl)oxazole-4-carboxylate (270)



This compound was prepared *via* protocol 2 using **267** (120 mg, 0.105 mmol) to yield the desired product **270** as a white foam (85 mg, 88%). IR (neat, cm^{-1}): 3303 (w), 3064, [1683, 1647, 1558, 1507 (s)], 1141 (m), 1106; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.82 (d, *J* = 6.0 Hz, 6H, 2 x CH_3), 1.25 (s, 6H, 2 x CH_3 (Pmc)), 1.39 (s, 9H, Boc), 1.41-1.68 (m, 7H, $CHCH_2CH_2$ (Lys), $CHCH_2CH_2$ (Arg), CH_2CH_2NH (Lys) and $CH(CH_3)_2$ (Leu)), 1.74-1.76 (m, 6H, $CHCH_2$ (Leu), $CHCH_2CH_2$ (Lys) and $CHCH_2CH_2$ (Arg)), 1.96 (bs, 1H,

NH), 2.08 (s, 3H, C<u>H</u>$_3$ (Pmc)), 2.51 (s, 3H, C<u>H</u>$_3$ (Pmc)), 2.56 (s, 3H, C<u>H</u>$_3$ (Pmc)), 2.57-2.58 (m, 4H, 2 x C<u>H</u>$_2$ (Pmc)), 3.01 (bs, 2H, C<u>H</u>$_2$N (Arg)), 3.18-3.26 (m, 3H, C<u>H</u>$_2$N (Lys) and NH), 3.80 (s, 3H, OC<u>H</u>$_3$), 3.90-4.13 (m, 3H, C<u>H</u>$_2$ (Gly) and Lys-α-proton), 4.22 (bs, 2H, NH$_2$), 4.53 (bs, 2H, Arg-α-proton and NH), 4.83 (1H, NH), 5.14-5.18 (m, 1H, α-C<u>H</u> to oxazole), 5.92 (bs, 1H, NH), 6.19 (bs, 1H, NH), 6.32 (bs, 2H, NH), 8.04 (s, 1H, oxazole); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.3 (<u>C</u>H$_3$, Pmc), 17.7 (<u>C</u>H$_3$, Pmc), 18.7 (<u>C</u>H$_3$, Pmc), 21.6 (CHCH$_2$<u>C</u>H$_2$ (Lys)), 21.9 (CHCH$_2$<u>C</u>H$_2$ (Arg)), 22.8 (2 x <u>C</u>H$_3$ (Leu)), 24.8 (<u>C</u>H(CH$_3$)$_2$ (Leu)), 26.9 (CH<u>C</u>H$_2$ (Lys)), 26.98 (C(<u>C</u>H$_3$)$_2$ (Pmc)), 28.6 (C(<u>C</u>H$_3$)$_3$), 29.8 (<u>C</u>H$_2$ (Pmc)), 33.0 (CH<u>C</u>H$_2$CH$_2$ (Arg)), 33.1 (<u>C</u>H$_2$CH$_2$NH (Lys) and <u>C</u>H$_2$ (Pmc)), 40.5 (NH<u>C</u>H$_2$ (Lys)), 41.8 (NHC<u>H</u>$_2$ (Arg)), 42.3 (<u>C</u>H$_2$ (Gly)), 46.5 (CH<u>C</u>H$_2$ (Leu)), 52.3 (O<u>C</u>H$_3$ ester), 53.6 (NH<u>C</u>H (Leu)), 55.2 (NH<u>C</u>HCO (Lys) and (NH<u>C</u>HCO (Arg)), 73.8 (O<u>C</u>(CH$_3$)$_2$), 79.4 (O<u>C</u>(CH$_3$)$_3$), [118.1, 124.2, 125.3, 132.9, 133.6, 135.0, 135.6 (Ar<u>C</u>)], 141.4 (<u>C</u>H, oxazole), [153.8, 156.4, 165.7, 171.8 (2 x CO) (CO)]. MS (ESI +ve) *m/z* 920.0 ([M+H]$^+$, 100%).

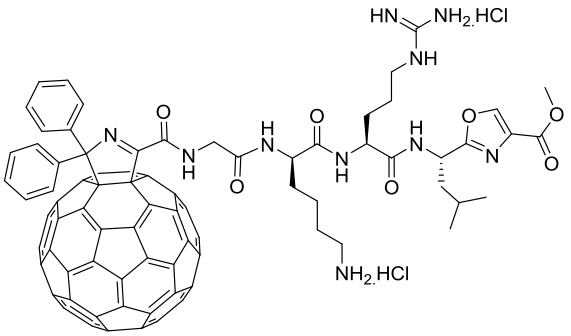
Methyl-2-((10*R*,13*S*,16*S*)-10-(2-(5,5-diphenylfullerenyldihydropyrrole-2-carbonyl) aminoacetamido)-2,2,18-trimethyl-4,11,14-trioxo-13-(3-(3-(2,2,5,7,8-pentamethylchroman-6-yl)sulfonylguanidino)propyl)-3-oxa-5,12,15-triazanonadecan-16-yl)oxazole-4-carboxylate (274)



This compound was prepared *via* protocol 1 using **270** (75 mg, 0.0816 mmol) and fullerenoproline acid **198** (65 mg, 0.0679 mmol) to yield the desired product **274** as a brown solid (64 mg, 51%). IR (neat,

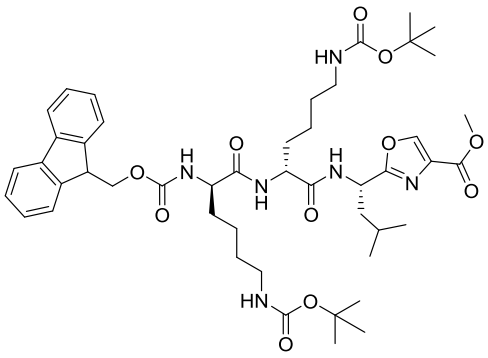
cm^{-1}): 3285 (w), [1685, 1635, 1653, 1507 (s)], [1253, 1164, 1100 (m)]; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86-0.90 (m, 6H, 2 x C$\underline{H}_3$ (Leu)), 1.30 (s, 6H, 2 x C$\underline{H}_3$ (Pmc)), 1.41 (s, 9H, Boc), 1.45-1.51 (m, 4H, CHCH$_2$C$\underline{H}_2$ (Lys) and CHCH$_2$C$\underline{H}_2$ (Arg)), 1.62-1.68 (m, 4H, C$\underline{H}_2$CH$_2$NH (Lys) and CHC$\underline{H}_2$CH$_2$ (Lys)), 1.79 (t, *J* = 6.5 Hz, 2H, CHC$\underline{H}_2$ (Leu)), 1.78-1.88 (m, 3H, CHC$\underline{H}_2$CH$_2$ (Arg) and C$\underline{H}$(CH$_3$)$_2$ (Leu)), 2.08 (s, 3H, C$\underline{H}_3$, (Pmc)), 2.54 (s, 3H, C$\underline{H}_3$, (Pmc)), 2.56 (s, 3H, C$\underline{H}_3$, (Pmc)), 2.61 (t, *J* = 6.5 Hz, 2H, C$\underline{H}_2$ (Pmc)), 3.02 (bs, 2H, C$\underline{H}_2$N (Arg)), 3.14-3.18 (m, 2H, C$\underline{H}_2$N (Lys)), 3.83 (s, 3H, OC$\underline{H}_3$), 4.30 (d, *J* = 8.0 Hz, 2H (Gly)), 4.37-4.41 (m, 3H, Lys-α-proton, Arg-α-proton and NH), 5.16-5.17 (m, 1H, α-CH to oxazole), 7.38 (t, *J* = 6.5 Hz, 2H, ArC$\underline{H}$), 7.45-7.50 (m, 4H, ArC$\underline{H}$), 7.75 (bs, 1H, NH), 7.84 (bs, 1H, NH), 8.05 (t, *J* = 7.5 Hz, 4H, ArC$\underline{H}$), 8.13 (s, 1H, oxazole), 8.90 (bs, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.1 (C$\underline{H}_3$, Pmc), 17.5 (C$\underline{H}_3$, Pmc), 18.5 (C$\underline{H}_3$, Pmc), 21.4 (CHCH$_2$C$\underline{H}_2$ (Lys)), 21.7 (CHCH$_2$C$\underline{H}_2$ (Arg)), 22.7 (2 x C$\underline{H}_3$ (Leu)), 24.6 (C$\underline{H}$(CH$_3$)$_2$ (Leu)), 25.5 CHC$\underline{H}_2$ (Lys)), 26.8 (C(C$\underline{H}_3$)$_2$ (Pmc)), 28.4 (C(C$\underline{H}_3$)$_3$), 29.6 (C$\underline{H}_2$ (Pmc)), 32.8 (CHC$\underline{H}_2$CH$_2$ (Arg)) and C$\underline{H}_2$CH$_2$NH (Lys)), 40.2 (NHC$\underline{H}_2$ (Lys)), 41.8 (NHC$\underline{H}_2$ (Arg)), 44.4 (C$\underline{H}_2$ (Gly)), 46.0 (CHC$\underline{H}_2$ (Leu)), 52.3 (OC$\underline{H}_3$ ester), 52.5 (NHC$\underline{H}$ (Leu) and (NHC$\underline{H}$CO (Lys)), 58.7 (NHC$\underline{H}$CO (Arg)), 73.7 (OC(CH$_3$)$_2$), 79.2 (OC(CH$_3$)$_3$), 82.2* ($C_{60}sp^3$), 89.5* ($C_{60}sp^3$), 95.6 (NC$\underline{P}$h$_2$), [117.9 124.0 (Ar$\underline{C}$ (Pmc)], 128.4 (Ar$\underline{C}$H), 129.8 (2 x Ar$\underline{C}$H), [132.8, 133.5, 134.7 (Ar$\underline{C}$)], [134.9, 135.5, 135.6, 136.6 ($C_{60}sp^2$)], [139.1, 139.5, 140.9 (Ar$\underline{C}$)], 141.3 (Ar$\underline{C}$H, oxazole), [141.8, 141.9, 142.4, 142.5, 142.7, 142.8, 143.0, 143.1, 144.2, 144.3, 144.9, 145.1, 145.3, 145.4, 145.5, 145.6, 145.8, 146.0, 146.1, 146.38* (1/2 x C), 146.4* (1/2 x C), 146.9, 147.1, 148.3, 149.5, 153.6, ($C_{60}sp^2$)], 155.9 (C=N), 156.5 (CO, Boc), 157.8 (C=N), 162.0 (Ar$\underline{C}$, oxazole), [163.9 (CO), 167.3 (2 x CO), 171.5 (2 x CO). HRMS (ESI +ve) calcd for $C_{118}H_{78}N_{10}O_{12}SNa$ 1881.5419, found 1881.5436.

1-(*S*)-4-(*R*)-2-(2-(5,5-Diphenylfullerenyldihydropyrrole-2-carbonyl aminoacetamido-6-ammoniohexanamido)-5-(*S*)-1-(4-(methoxycarbonyl)oxazol-2-yl-3-methylbutylamino)-5-oxopentylguanidinium chloride (278)



This compound was prepared *via* protocol 3, using **274** (35 mg, 0.0188 mmol) to yield **278** as a brown solid (13 mg, 46%). IR (neat, cm^{-1}): 3305 (w), [1675, 1655, 1651, 1507 (s)], [1258, 1162, 1107 (m)]; ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD (4:1)): δ 0.84-0.88 (m, 6H, 2 x C<u>H</u>$_3$ (Leu)), 1.24-1.28 (m, 2H, CHCH$_2$C<u>H</u>$_2$ (Lys)), 1.56-1.79 (m, 7H, CHCH$_2$C<u>H</u>$_2$ (Arg), C<u>H</u>$_2$CH$_2$NH (Lys), CHC<u>H</u>$_2$CH$_2$ (Lys) and C<u>H</u>(CH$_3$)$_2$ (Leu)), 1.83-1.87 (m, 6H, CHC<u>H</u>$_2$CH$_2$ (Arg), CHC<u>H</u>$_2$ (Leu) and CH$_2$N<u>H</u>$_2$ (Lys)), 2.10 (s, 1H, NH), 2.65 (bs, 1H, NH), 2.88 (bs, 2H, C<u>H</u>$_2$N (Lys)), 2.93 (bs, 1H, NH), 3.08 (bs, 2H, C<u>H</u>$_2$N (Arg)), 3.30 (s, 3H, OC<u>H</u>$_3$), 3.40-3.41 (m, 2H, NH$_2$), 3.80 (s, 2H (Gly)), 4.19 (d, 1H, *J* = 7.5 Hz, NH), 4.34-4.40 (m, 3H, Lys-α-proton, Arg-α-proton and α-CH to oxazole), 7.40 (t, 2H, *J* = 7.5 Hz, ArC<u>H</u>), 7.51 (m, 4H, ArC<u>H</u>), 7.85 (s, 1H, NH), 8.11-8.15 (m, 4H, ArC<u>H</u>), 8.41 (s, 1H, oxazole); This compound was not sufficiently soluble to generate an adequate ^{13}C NMR spectrum. HRMS (ESI +ve) calcd for $C_{99}H_{52}N_{10}O_7$ 1493.4099, found 1493.4077.

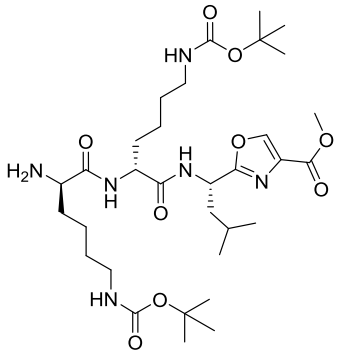
Methyl 2-((10*R*,13*R*,16*S*)-10-(9*H*-fluoren-9-yl)methoxycarbonylamino-13-(4-*tert*-butoxycarbonylaminobutyl)-2,2,18-trimethyl-4,11,14-trioxo-3-oxa-5,12,15-triazanonadecan-16-yl)oxazole-4-carboxylate (261)



This compound was prepared *via* protocol 1 using **257** (150 mg, 0.340 mmol) and Fmoc-(*D*)-Lys(Boc)-OH (159 mg, 0.340 mmol) to yield the desired product **261** as a white foam (220 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.91-0.94 (m, 6H, 2 x C$\underline{H}_3$ (Leu)), 1.34-1.49 (m, 7H, 2 x CHCH$_2$C$\underline{H}_2$ (Lys), C$\underline{H}$(CH$_3$)$_2$ (Leu) and C$\underline{H}_2$CH$_2$NH (Lys)), 1.41 (s, 9H, Boc), 1.42 (s, 9H, Boc), 1.65-1.69 (m, 2H, C$\underline{H}_2$CH$_2$NH (Lys)), 1.75-1.78 (m, 2H, CHC$\underline{H}_2$ (Leu)), 1.82-1.88 (m, 4H, 2 x CHC$\underline{H}_2$CH$_2$ (Lys)), 3.04 (bs, 2H, C$\underline{H}_2$N (Lys)), 3.20 (bs, 2H, C$\underline{H}_2$N (Lys)), 3.77 (s, 3H, OC$\underline{H}_3$), 4.18 (t, *J* = 7 Hz, 1H, C$\underline{H}$, Fmoc), 4.36-4.37 (m, 2H, C$\underline{H}_2$, Fmoc), 4.51 (bs, 1H, NH), 4.73 (bs, 1H, Lys-α-proton), 4.80 (bs, 1H, Lys-α-proton), 5.20-5.29 (m, 1H, α-C$\underline{H}$ to oxazole), 5.95 (bs, 1H, NH), 7.08 (bs, 1H, NH), 7.26-7.30 (m, 3H, 2 x ArC$\underline{H}$, NH), 7.36-7.40 (m, 2H, ArC$\underline{H}$), 7.51 (d, *J* = 7.5 Hz, 2H, ArC$\underline{H}$), 7.57 (d, *J* = 7.5 Hz, 1H, NH), 7.74 (d, *J* = 7.5 Hz, 2H, ArC$\underline{H}$), 8.00 (s, 1H, oxazole); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.6 (CHCH$_2$C$\underline{H}_2$ (Lys)), 22.4 (CHCH$_2$C$\underline{H}_2$ (Lys)), 22.8 (2 x C$\underline{H}_3$ (Leu)), 24.7 (C$\underline{H}$(CH$_3$)$_2$ (Leu)), 28.4 (2 x C(C$\underline{H}_3$)$_3$), 29.4 (CHC$\underline{H}_2$ (Lys)), 29.6 (CHC$\underline{H}_2$ (Lys)), 31.2 (2 x C$\underline{H}_2$CH$_2$NH (Lys)), 39.5 (NHC$\underline{H}_2$ (Lys)), 40.1 (NHC$\underline{H}_2$ (Lys)), 46.0 (CHC$\underline{H}_2$ (Leu)), 47.0 (CH$_2$C$\underline{H}$ (Fmoc)), 52.0 (OC$\underline{H}_3$ ester), 52.9 (NHC$\underline{H}$ (Leu)), 55.7 (2 x NHC$\underline{H}$CO (Lys)), 67.2 (C$\underline{H}_2$, Fmoc), 79.2 (2 x OC(CH$_3$)$_3$), [119.9, 124.9, 125.0, 127.0 (ArC$\underline{H}$)], 132.9 (ArC, oxazole), 141.2 (C$\underline{H}$, oxazole), 141.3

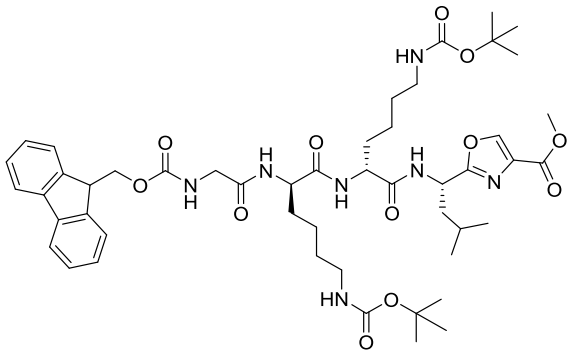
(ArC), 143.9 (ArC), 156.5 (2 x CO, Boc), 161.3 (ArC, oxazole), [166.0, 171.4 (2 x CO), 172.2 (CO)]. MS (ESI +ve) *m/z* 891.0 ($[M+H]^+$, 100%).

Methyl 2-((10*R*,13*R*,16*S*)-10-amino-13-(4-((*tert*-butoxycarbonyl)amino)butyl)-2,2,18-trimethyl-4,11,14-trioxo-3-oxa-5,12,15-triazanonadecan-16-yl)oxazole-4-carboxylate (265)



This compound was prepared *via* protocol 2 using **261** (200 mg, 0.224 mmol) to yield the desired product **265** as a white foam (128 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93-0.95 (m, 6H, 2 x C$\underline{H}_3$ (Leu)), 1.34-1.39 (m, 4H, 2 x CHCH$_2$C$\underline{H}_2$ (Lys)), 1.43 (s, 18H, 2 x Boc), 1.50-1.57 (m, 5H, 2 x C$\underline{H}_2$CH$_2$NH (Lys) and C$\underline{H}$(CH$_3$)$_2$ (Leu)), 1.75-1.76 (m, 6H, CHC$\underline{H}_2$ (Leu) and 2 x CHC$\underline{H}_2$CH$_2$ (Lys)), 1.90-1.96 (m, 1H, NH), 3.11 (bs, 4H, 2 x C$\underline{H}_2$N (Lys)), 3.48 (bs, 1H, Lys-α-proton), 3.87 (s, 3H, OC$\underline{H}_3$), 4.42-4.44 (m, 2H, NH$_2$), 4.62-4.66 (m, 2H, Lys-α-proton and NH), 5.18-5.24 (m, 1H, α-CH to oxazole), 7.18 (bs, 1H, NH), 7.82 (bs, 1H, NH), 8.12 (s, 1H, CH, oxazole); ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.0 (CHCH$_2$C$\underline{H}_2$ (Lys)), 22.9 (CHCH$_2$C$\underline{H}_2$ (Lys)), 23.0 (2 x C$\underline{H}_3$ (Leu)), 23.07 (C$\underline{H}$(CH$_3$)$_2$ (Leu)), 25.0 (CHC$\underline{H}_2$ (Lys)), 28.6 (2 x C(C$\underline{H}_3$)$_3$), 29.8 (C$\underline{H}_2$CH$_2$NH (Lys)), 30.0 (C$\underline{H}_2$CH$_2$NH (Lys), 40.3 (NHC$\underline{H}_2$ (Lys)), 42.7 (NHC$\underline{H}_2$ (Lys)), 46.1 (CHC$\underline{H}_2$ (Leu)), 52.3 (NHC$\underline{H}$ (Leu)), 52.4 (NH$_2$C$\underline{H}$CO (Lys)), 55.1 (NHC$\underline{H}$CO (Lys)), 79.2 (2 x OC(CH$_3$)$_3$), 133.3 (ArC, oxazole), 144.1 (ArCH, oxazole), 156.2 (2 x CO, Boc), 161.7 (ArC, oxazole), [165.7, 171.8, 176.4 (CO)]. MS (ESI +ve) *m/z* 669.0 ($[M+H]^+$, 100%).

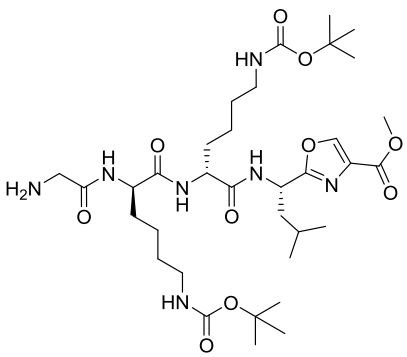
Methyl 2-(8*R*,11*R*,14*S*)-8,11-bis(4-*tert*-butoxycarbonylaminobutyl-1-(9*H*-fluoren-9-yl)-16-methyl-3,6,9,12-tetraoxo-2-oxa-4,7,10,13-tetraazaheptadecan-14-yl)oxazole-4-carboxylate (268)



This compound was prepared *via* protocol 1 using **265** (65 mg, 0.0973 mmol) and Fmoc-Gly-OH (30 mg, 0.0973 mmol) to yield the desired product **268** as a white foam (76 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88-0.89 (m, 6H, 2 x C$\underline{H}_3$ (Leu)), 1.30-1.45 (m, 4H, 2 x CHCH$_2$C$\underline{H}_2$ (Lys)), 1.41 (s, 18H, 2 x Boc), 1.60-1.95 (m, 7H, C$\underline{H}$(CH$_3$)$_2$ (Leu), 2 x C$\underline{H}_2$CH$_2$NH (Lys) and CHC$\underline{H}_2$ (Leu)), 2.61 (bs, 1H, NH), 3.05 (bs, 4H, 2 x C$\underline{H}_2$N (Lys)), 3.81 (s, 3H, OC$\underline{H}_3$), 4.05 (bs, 2H, C$\underline{H}_2$ (Gly)), 4.18 (bs, 1H, C$\underline{H}$, Fmoc), 4.22-4.37 (m, 2H, C$\underline{H}_2$, Fmoc), 4.55 (bs, 1H, NH), 4.62 (bs, 1H, Lys-α-proton), 4.90 (bs, 1H, Lys-α-proton), 5.08 (bs, 1H, NH), 5.20-5.29 (m, 1H, α-CH to oxazole), 6.50 (bs, 1H, NH), 7.25 (t, *J* = 7.0 Hz, 2H, ArC$\underline{H}$), 7.36 (t, *J* = 7.5 Hz, 2H, ArC$\underline{H}$), 7.73 (d, *J* = 7.5 Hz, 2H, ArC$\underline{H}$), 7.77 (m, 3H, 2 x ArC$\underline{H}$ and NH), 8.09 (s, 1H, C$\underline{H}$, oxazole); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.9 (CHCH$_2$C$\underline{H}_2$ (Lys)), 22.8 (CHCH$_2$C$\underline{H}_2$ (Lys)), 22.9 (2 x C$\underline{H}_3$ (Leu)), 24.9 (C$\underline{H}$(CH$_3$)$_2$ (Leu)), 28.6 (2 x C(C$\underline{H}_3$)$_3$), 29.8 (2 x CHC$\underline{H}_2$ (Lys)), 31.9 (2 x C$\underline{H}_2$CH$_2$NH (Lys)), 40.1 (NHC$\underline{H}_2$ (Lys)), 40.5 (NHC$\underline{H}_2$ (Lys)), 42.6 (C$\underline{H}_2$ (Gly)), 44.7 (CHC$\underline{H}_2$ (Leu)), 47.2 (CH$_2$C$\underline{H}$ (Fmoc)), 52.3 (OC$\underline{H}_3$ ester), 53.1 (NHC$\underline{H}$ (Leu)), 54.2 (2 x NHC$\underline{H}$CO (Lys)), 67.3 (OC$\underline{H}_2$, Fmoc), 79.2 (2 x OC(CH$_3$)$_3$), [120.1, 125.3, 127.2, 127.9 (ArC$\underline{H}$)], 133.1 (Ar$\underline{C}$, oxazole), 141.4 (C$\underline{H}$, oxazole), 143.9 (Ar$\underline{C}$), 144.1 (Ar$\underline{C}$), 156.6 (2 x CO, Boc), 161.7

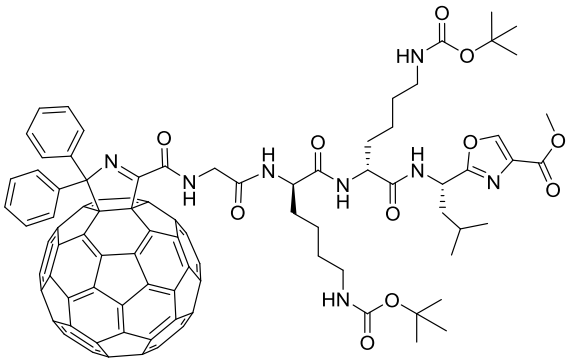
(ArC, oxazole), [165.9 (CO), 171.6 (2 x CO), 171.7 (2 x CO)]. MS (ESI +ve) *m/z* 948.0 ($[M+H]^+$, 100%).

Methyl-2-((10*R*,13*R*,16*S*)-10-(2-aminoacetamido)-13-(4-*tert*-butoxycarbonylamino)butyl-2,2,18-trimethyl-4,11,14-trioxo-3-oxa-5,12,15-triazanonadecan-16-yl)oxazole-4-carboxylate (271)



This compound was prepared *via* protocol 2 using **268** (60 mg, 0.0633 mmol) to yield the desired product **271** as a white foam (39 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.91-0.95 (m, 6H, CH($C\underline{H}_3$)$_2$ (Leu)), 1.24-1.58 (m, 9H, 2 x $CHCH_2C\underline{H}_2$ (Lys), 2 x $C\underline{H}_2CH_2NH$ (Lys) and $C\underline{H}(CH_3)_2$ (Leu)), 1.42 (s, 18H, 2 x Boc), 1.60-1.68 (m, 2H, $CHC\underline{H}_2$ (Leu)), 1.75-1.80 (m, 2H, $CHC\underline{H}_2CH_2$ (Lys)), 1.82-1.88 (m, 2H, $CHC\underline{H}_2CH_2$ (Lys)), 1.90-1.96 (m, 1H, NH), 2.22-2.40 (bs, 2H, $N\underline{H}_2$), 3.07 (t, *J* = 6.0 Hz, 4H, $C\underline{H}_2N$ (Lys)), 3.36-3.45 (m, 2H, ($C\underline{H}_2$ (Gly)), 3.87 (s, 3H, $OC\underline{H}_3$), 4.30-4.35 (m, 1H, Lys-α-proton), 4.48-4.55 (m, 1H, Lys-α-proton), 4.85-4.95 (m, 1H, NH), 5.20-5.38 (m, 1H, α-C<u>H</u> to oxazole), 7.28 (bs, 1H, NH), 7.48 (bs, 1H, NH), 7.88 (bs, 1H, NH), 8.15 (s, 1H, C<u>H</u>, oxazole); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.7 ($CHCH_2\underline{C}H_2$ (Lys)), 22.7 ($CHCH_2\underline{C}H_2$ (Lys)), 22.8 (2 x $\underline{C}H_3$ (Leu)), 24.7 ($\underline{C}H(CH_3)_2$ (Leu)), 28.4 (2 x C($\underline{C}H_3)_3$), 29.4 ($\underline{C}H_2CH_2NH$ (Lys)), 29.6 ($\underline{C}H_2CH_2NH$ (Lys), 31.1 ($CH\underline{C}H_2$ (Lys)), 31.3 ($CH\underline{C}H_2$ (Lys)), 40.0 ($NH\underline{C}H_2$ (Lys)), 40.1 ($NH\underline{C}H_2$ (Lys)), 44.6 ($CH\underline{C}H_2$ (Leu)), 45.9 ($\underline{C}H_2$ (Gly)), 52.1 (NH<u>C</u>H (Leu)), 52.7 (NH<u>C</u>HCO (Lys)), 53.8 (NH<u>C</u>HCO (Lys)), 79.0 (2 x O<u>C</u>(CH$_3$)$_3$), 132.9 (Ar<u>C</u>, oxazole), 143.9 (<u>C</u>H, oxazole), 156.2 (2 x CO, Boc), 161.5 (Ar<u>C</u>, oxazole), [165.9, 171.4, 172.0, 174.1 (CO)]. MS (ESI +ve) *m/z* 726.0 ([M+H]+, 100%).

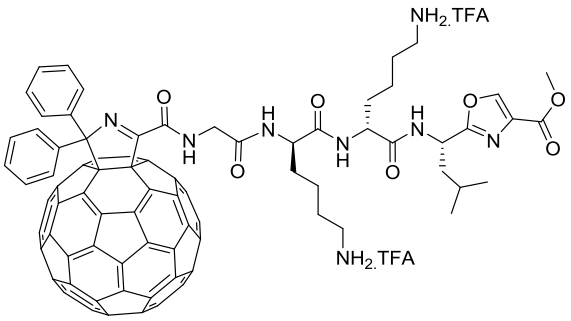
Methyl-2-((10*R*,13*R*,16*S*)-10-(2-(5,5-diphenylfullerenyldihydropyrrole-2-carbonyl)aminoacetamido)-13-(4-((*tert*-butoxycarbonyl)amino)butyl)-2,2,18-trimethyl-4,11,14-trioxo-3-oxa-5,12,15-triazanonadecan-16-yl)oxazole-4-carboxylate (275)



To a suspension of acid **198** (46 mg, 0.048 mmol) in $CH_2Cl_2/CHCl_3$ (1:1), (100 mL) was added HOBt (7.7 mg, 0.057 mmol) and EDCI (11 mg, 0.057 mmol) and the mixture was stirred for 15 min before a solution of **271** (35 g, 0.048 mmol) and triethylamine (8.0 µL, 0.057 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The resulting solution was stirred at rt for a further 4 h before the solvent was removed under reduced pressure. The crude residue was then subjected to flash silica gel chromatography, elution with MeOH/CH_2Cl_2 (1:49) provided the title compound **275** as a brown solid (52 mg, 65%). IR (neat, cm^{-1}): 3321 (w), [1695, 1647, 1635 (s)], [1363, 1248, 1153 (m)]; ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD (9:1)): δ 0.91-0.94 (m, 6H, $CH(C\underline{H}_3)_2$ (Leu)), 1.34-1.51 (m, 8H, 2 x $CHCH_2C\underline{H}_2$ (Lys) and 2 x $C\underline{H}_2CH_2NH$ (Lys)), 1.42 (s, 18H, 2 x Boc), 1.58-1.76 (m, 4H, 2 x $CHC\underline{H}_2CH_2$ (Lys)), 1.79-1.88 (m, 2H, $CHC\underline{H}_2$ (Leu)), 1.95 (bs, 1H, $C\underline{H}(CH_3)_2$ (Leu)), 3.07 (bs, 4H, $C\underline{H}_2N$ (Lys)), 3.37 (s, 2H, ($C\underline{H}_2$ (Gly))), 3.83 (s, 3H, $OC\underline{H}_3$), 4.20-4.32 (m, 1H, NH), 4.34 (bs, 2H, Lys-α-proton and NH), 4.50 (bs, 1H, Lys-α-proton), 5.18-5.20 (m, 1H, α-C<u>H</u> to oxazole), 5.26 (bs, 1H, NH), 5.34 (bs, 1H, NH), 7.34, (s, 1H, C<u>H</u>, oxazole), 7.39-7.43 (m, 2H, ArC<u>H</u>), 7.48-7.52 (m, 4H, ArC<u>H</u>), 7.69 (d, *J* = 7.0 Hz, 1H, NH), 7.92 (d, *J* = 7.5 Hz, 1H, NH), 8.03 (d, *J* = 8 Hz, 4H, ArC<u>H</u>), 8.08 (bs, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$ +

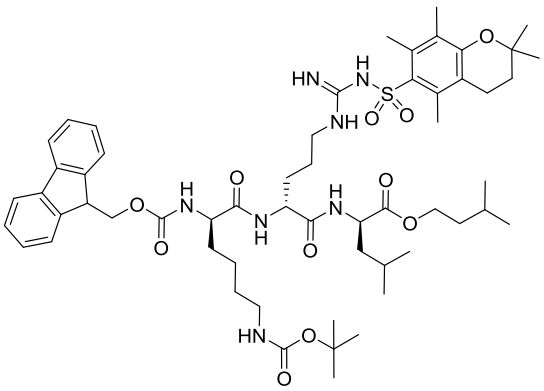
CD_3OD (9:1)): δ 21.5 ($CHCH_2\underline{C}H_2$ (Lys)), 22.9 (2 x $\underline{C}H_3$ (Leu)), 24.8 ($\underline{C}H(CH_3)_2$ (Leu)), 28.4 (2 x $C(\underline{C}H_3)_3$), 29.5 ($\underline{C}H_2CH_2NH$ (Lys) and ($CH\underline{C}H_2$ (Lys)), 42.6 ($NH\underline{C}H_2$ (Lys)), 46.0 ($CH\underline{C}H_2$ (Leu)), 48.6 ($\underline{C}H_2$ (Gly)), 48.8 ($NH\underline{C}H_2$ (Lys)), 49.0 ($NH\underline{C}H$ (Leu)), 49.7 ($NH\underline{C}HCO$ (Lys)), 52.3 ($NH\underline{C}HCO$ (Lys)), 95.7 ($\underline{C}Ph_2$), [128.5, 128.6, 129.9 ($Ar\underline{C}H$)], 134.7, ($Ar\underline{C}$, oxazole), [134.9, 136.7, 139.2, 139.3, 139.5, 139.6, 140.9, 141.0, 141.4, 141.9 ($C_{60}sp^2$)], 142.0 ($\underline{C}H$, oxazole), 142.5 ($Ar\underline{C}$), [142.6, 142.9, 143.1, 143.2, 144.2, 144.4, 144.9, 145.0, 145.2, 145.3, 145.5, 145.6, 145.9, 146.1, 146.5, 147.0* (1/2 x C), 147.2* (1/2 x C), 148.4, 149.1 ($C_{60}sp^2$)], 153.2 (C=N), 153.3 (2 x CO, Boc), 161.6 ($Ar\underline{C}$, oxazole), [166.2 (2 x CO), 171.0 (2 x CO), 171.2 (CO, ester). Two $C_{60}sp^3$ signals and Boc *tert*-carbons were not observed due to poor signal to noise. HRMS (ESI +ve) calcd for $C_{109}H_{68}N_8O_{11}$ 1665.5085, found 1665.5359.

(*R*)-5-(2-(5,5-Diphenylfullerenyldihydropyrrole-2-carbonyl)-aminoacetamido)-6-(*R*)-6-ammonio-1-(*S*)-1-(4-(methoxycarbonyloxazol-2-yl)-3-methylbutylamino-1-oxohexan-2-yl)amino-6-oxohexan-1-aminium 2,2,2-trifluoroacetate (279)



This compound was prepared *via* protocol 3, using **275** (35 mg, 0.0210 mmol) to yield **279** as a brown solid (17 mg, 56%). IR (neat, m^{-1}): 3309 (w), [1685, 1647, 1558 (s)], [1457, 1193, 1135 (m)]; HRMS (ESI +ve) calcd for $C_{99}H_{52}N_8O_7$ 1465.4037, found 1465.4045. This compound was not sufficiently soluble to generate an adequate ^1H NMR and ^{13}C NMR spectrum.

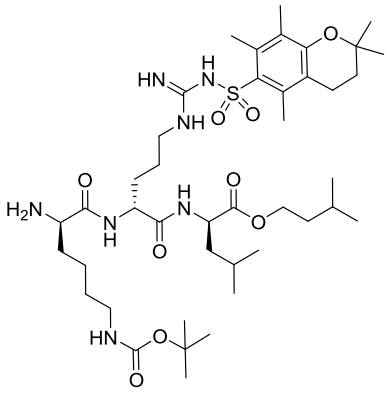
(10*R*,13*R*,16*R*)-Isopentyl 10-(9H-fluoren-9-yl)methoxycarbonylamino-16-isobutyl-2,2-dimethyl-4,11,14-trioxo-13-(3-(3-((2,2,5,7,8-pentamethylchroman-6-yl)sulfonyl)guanidino)propyl)-3-oxa-5,12,15-triazaheptadecan-17-oate (258)



This compound was prepared *via* protocol 1 using **254** (220 mg, 0.353 mmol) and Fmoc-(*S*)-Lys(Boc)-OH (165 mg, 0.353 mmol) to yield the desired product **258** as a white foam (300 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84-0.88 (m, 12H, 4 x C<u>H</u>$_3$ (Leu and isopentyl)), 1.40 (s, 9H, Boc), 1.43 (s, 6H, C(C<u>H</u>$_3$)$_2$, (Pmc)), 1.46-1.49 (m, 4H, CHCH$_2$C<u>H</u>$_2$ (Arg) and CHC<u>H</u>$_2$CH$_2$ (Lys)), 1.57-1.72 (m, 6H, C<u>H</u>$_2$CH$_2$NH (Lys), OCH$_2$C<u>H</u>$_2$ and CHC<u>H</u>$_2$ (Arg)), 1.76 (bs, 1H, C<u>H</u>(CH$_3$)$_2$ (isopentyl)), 1.92 (m, 2H, CHC<u>H</u>$_2$ (Leu)), 1.99 (bs, 1H, C<u>H</u>(CH$_3$)$_2$ (Leu)), 2.07 (s, 3H, C<u>H</u>$_3$, (Pmc)), 2.50 (s, 3H, C<u>H</u>$_3$, (Pmc)), 2.57 (s, 3H, C<u>H</u>$_3$, (Pmc)), 2.92 (m, 4H, C<u>H</u>$_2$, (Pmc)), 3.06 (bs, 2H, C<u>H</u>$_2$N (Arg)), 3.23 (bs, 2H, C<u>H</u>$_2$N (Lys)), 4.05-4.10 (m, 2H, Lys-α-proton and C<u>H</u>CH$_2$ (Fmoc)), 4.16-4.20 (m, 2H, CO$_2$C<u>H</u>$_2$), 4.32-4.34 (m, 2H, CHC<u>H</u>$_2$ (Fmoc)), 4.43-4.45 (m, 1H, Arg-α-proton), 4.56-4.57 (m, 1H, Leu-α-proton), 4.82 (bs, 1H, NH), 5.29 (s, 1H, NH), 5.82 (bs, 1H, NH), 6.29 (bs, 2H, NH), 7.26-7.29 (m, 3H, 2 x ArC<u>H</u>, NH), 7.35-7.38 (m, 2H, ArC<u>H</u>), 7.56-7.58 (m, 2H, ArC<u>H</u>), 7.73 (d, *J* = 7.5 Hz, 2H, ArC<u>H</u>); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.8 (<u>C</u>H$_3$, Pmc), 18.3 (<u>C</u>H$_3$, Pmc), 19.6 (<u>C</u>H$_3$, Pmc), 21.9 (CHCH$_2$<u>C</u>H$_2$ (Lys)), 22.6 (2 x <u>C</u>H$_3$ (isopentyl)), 22.7 (2 x <u>C</u>H$_3$ (Leu)), 22.8 (2 x <u>C</u>H$_3$ (isopentyl)), 23.0 (CHCH$_2$<u>C</u>H$_2$ (Arg) and (<u>C</u>H(CH$_3$)$_2$ (isopentyl)), 25.0 (<u>C</u>H(CH$_3$)$_2$ (Leu)), 25.2 (CH<u>C</u>H$_2$CH$_2$ (Lys)), 28.7 (C(<u>C</u>H$_3$)$_3$), 28.8 (2 x <u>C</u>H$_3$ (Pmc) and (<u>C</u>H$_2$ (Pmc)), 29.6 (CH<u>C</u>H$_2$CH$_2$ (Arg)), 29.8 (<u>C</u>H$_2$CH$_2$NH (Lys)), 31.7 (<u>C</u>H$_2$ (Pmc)), 37.5

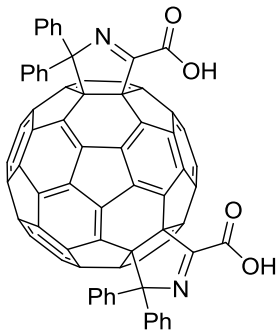
($\underline{C}H_2CH(CH_3)_2$ (isopentyl)), 40.3 ($\underline{C}H_2CH(CH_3)_2$ (Leu)), 40.6 (NH$\underline{C}H_2$ (Lys)), 43.9 (NHC$\underline{H}_2$ (Arg)), 51.6 ($CH_2\underline{C}H$ (Fmoc)), 52.7 (NH$\underline{C}$HCO (Leu)), 53.7 (NH$\underline{C}$HCO (Lys)), 55.5 (NH$\underline{C}$HCO (Arg)), 64.3 (O$\underline{C}H_2$ (isopentyl)), 67.4 ($\underline{C}H_2$CH (Fmoc)), 79.6 (O$\underline{C}(CH_3)_3$), 86.6 (O$\underline{C}(CH_3)_2$), 117.7 (Ar$\underline{C}$, Pmc), 120.2 (Ar$\underline{C}$H), 124.8 (Ar$\underline{C}$, Pmc), [125.4, 125.5, 128.0 (Ar$\underline{C}$H)], [132.5, 138.6, 141.4, 141.5 (Ar$\underline{C}$, Pmc)], [143.9, 144.1 (Ar$\underline{C}$)], 156.6 (CO, Boc), [159.0, 171.9, 172.5 (CO)], 173.4 (CO, ester); MS (ESI +ve) *m/z* 1074.0 ([M+H]+, 100%).

(10*R*,13*R*,16*R*)-Isopentyl 10-amino-13-(3-(3-((2,2-dimethylchroman-6-yl)sulfonyl) guanidino)propyl)-16-isobutyl-2,2-dimethyl-4,11,14-trioxo-3-oxa-5,12,15-triazaheptadecan-17-oate (262)



This compound was prepared *via* protocol 2 using **258** (275 mg, 0.256 mmol) to yield the desired product **262** as a white foam (185 mg, 85%). This compound was carried through the subsequent reaction without any further purification.

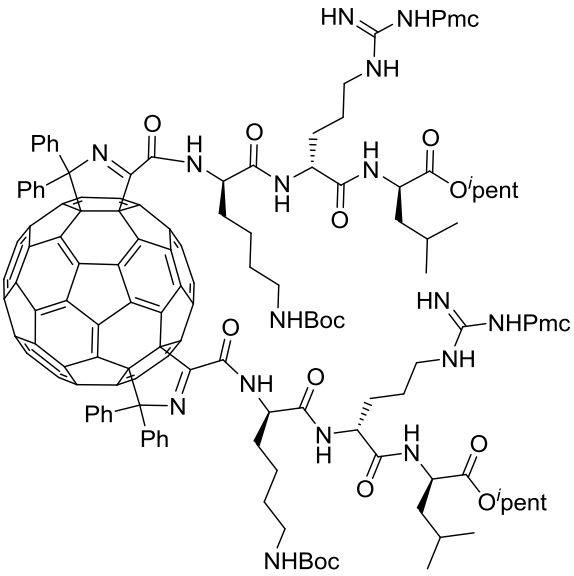
***trans*-4-Bis-(5,5-diphenylfullerenyldihydropyrrole-2-carboxylic acid) (284)**



To a solution of the bis ester **283** (0.128 g, 0.098 mmol) in 1,2-dichloroethane (80 mL) at rt was added $(CH_3)_3SnOH$ (0.077 g, 0.392 mmol) and the solution was heated at 80 ^oC for 4 h, then $(CH_3)_3SnOH$ (0.077 g, 0.392 mmol) was added at the same temperature and the reaction was continued for 4 h. The reaction mixture was evaporated under reduced pressure and the resulting residue was taken in

CH_2Cl_2 (100 mL). The organic layer was washed with 5% HCl (3 x 50 mL), brine (3 x 50 mL), dried (Mg_2SO_4) and evaporated under reduced pressure to yield **284** (0.109 g, 93%) as a brown solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28-7.29 (m, 1H, ArC<u>H</u>), 7.39 (t, 2H, *J* = 7.2 Hz, ArC<u>H</u>), 7.46 (t, 1H, *J* = 7.2 Hz, ArC<u>H</u>), 7.56 (t, 2H, *J* = 7.2 Hz, ArC<u>H</u>), 7.94 (d, 2H, *J* = 7.2 Hz, ArC<u>H</u>), 8.08 (d, 2H, *J* = 7.2 Hz, ArC<u>H</u>), 8.71 (bs, 1H, CO_2H). ESI-MS (+ve): *m/z* 1195 (40%, $[M+H]^+$), 1200 (100%, $[M+Li]^+$).

trans-4-Bis-(isopentyl (10*R*,13*R*,16*R*)-10-(5,5-diphenylfullerenyldihydropyrrole-2-carboxamido)-16-isobutyl-2,2-dimethyl-4,11,14-trioxo-13-(3-(3-((2,2,5,7,8-pentamethylchroman-6-yl)sulfonyl)guanidino)propyl))-3-oxa-5,12,15-triazaheptadecane-17-oate (285)



To a suspension of bis[*60*]fullerenoproline acid **284** (96 mg, 0.080 mmol) in $CHCl_3$/pyridine (2:1) (90 mL) was added HOBt (27 mg, 0.200 mmol) and EDCI (45 mg, 0.240 mmol) and the mixture was stirred for 15 min before a solution of **262** (171 mg, 0.200 mmol) in $CHCl_3$/pyridine (2:1) (15 mL) was added dropwise. The resulting solution was stirred at rt for a further 4 h, before the solvent was removed under reduced pressure. The crude residue was then subjected to flash silica gel chromatography, elution with MeOH/CH_2Cl_2 (1:49) provided the title compound **285** as a brown solid (0.115 g, 50%). IR (neat, cm^{-1}): 3277 (w), [1685, 1647, 1635, 1558 (s)], [1260, 1187, 1156 (m)]; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.79-0.90 (m, 24H, 8 x C<u>H</u>$_3$ (Leu and isopentyl)), 1.23-1.25 (m, 4H, 2 x CHC<u>H</u>$_2$CH$_2$ (Lys)), 1.28 (s, 18H, 6 x C<u>H</u>$_3$ (2

x Boc)), 1.30-1.38 (m, 12H, 4 x C$\underline{H}_3$ (Pmc)), 1.42-1.48 (m, 14H, 2 x CHCH$_2$C$\underline{H}_2$ (Arg), 2 x C$\underline{H}_2$CH$_2$NH (Lys), 2 x OCH$_2$C$\underline{H}_2$ and 2 x C$\underline{H}$(CH$_3$)$_2$ (isopentyl)), 1.55-1.68 (m, 10H, 2 x CHC$\underline{H}_2$ (Arg), 2 x CHC$\underline{H}_2$CH$_2$ (Lys) and 2 x C$\underline{H}$(CH$_3$)$_2$ (Leu)), 1.77 (t, *J* = 6.5 Hz, 4H, 2 x CHC$\underline{H}_2$ (Leu)), 2.05 (m, 4H, 2 x C$\underline{H}_2$ (Pmc)), 2.07 (s, 6H, 2 x C$\underline{H}_3$, (Pmc)), 2.31 (t, *J* = 7.5 Hz, 4H, 2 x C$\underline{H}_2$ (Pmc)), 2.52-2.59 (m, 12H, 4 x C$\underline{H}_3$, (Pmc)), 3.01-3.08 (m, 4H, C$\underline{H}_2$N, (Lys)), 4.00-4.10 (m, 4H, 2 x OC$\underline{H}_2$), 4.53-4.56 (m, 6H, 2 x Leu-α-proton and C$\underline{H}_2$N (Arg)), 4.65-4.68 (m, 2H, 2 x Lys-α-proton), 5.38 (bs, 4H, NH), 5.50-5.58 (m, 2H, 2 x Arg-α-proton)), 6.24 (bs, 4H, NH), 7.08-7.16 (m, 2H, NH), 7.33-7.37 (m, 6H, ArC$\underline{H}$), 7.44-7.46 (m, 2H, ArC$\underline{H}$), 7.51-7.54 (m, 4H, ArC$\underline{H}$), 7.80-7.90 (m, 4H, ArC$\underline{H}$), 8.02-8.05 (m, 4H, ArC$\underline{H}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.1 (C$\underline{H}_3$, Pmc), 14.1 (C$\underline{H}_3$, Pmc), 17.5 (C$\underline{H}_3$, Pmc), 18.5 (C$\underline{H}_3$, Pmc), 19.7 (C$\underline{H}_3$, Pmc), 21.5 (CHCH$_2$C$\underline{H}_2$ (Lys)), 21.6 (CHCH$_2$C$\underline{H}_2$ (Lys)), 22.5 (2 x C$\underline{H}_3$ (isopentyl)), 22.6 (2 x C$\underline{H}_3$ (isopentyl)), 22.7 (4 x C$\underline{H}_3$ (Leu)), 23.2 (CHCH$_2$C$\underline{H}_2$ (Arg)), 24.8 (C$\underline{H}$(CH$_3$)$_2$ (isopentyl)), 24.9 (C$\underline{H}$(CH$_3$)$_2$ (Leu)), 25.0 (C$\underline{H}$(CH$_3$)$_2$ (Leu)), 25.1 (CHC$\underline{H}_2$CH$_2$ (Lys)), 26.8 (CHC$\underline{H}_2$CH$_2$ (Lys)), 28.4 (C(C$\underline{H}_3$)$_3$), 28.5 (C(C$\underline{H}_3$)$_3$), 29.3 (C$\underline{H}_2$ (Pmc)), 29.7 (C$\underline{H}_2$ (Pmc)), 30.0 (CHC$\underline{H}_2$CH$_2$ (Arg)), 30.1 (CHC$\underline{H}_2$CH$_2$ (Arg)), 31.9 (C$\underline{H}_2$CH$_2$NH (Lys)), 32.8 (C$\underline{H}_2$ (Pmc)), 37.1 (C$\underline{H}_2$CH(CH$_3$)$_2$ (isopentyl)), 37.14 (C$\underline{H}_2$CH(CH$_3$)$_2$ (isopentyl)), 40.5 (C$\underline{H}_2$CH(CH$_3$)$_2$ (Leu) and (NHC$\underline{H}_2$ (Lys)), 51.0 (NHC$\underline{H}$CO (Leu)), 52.8 (NHC$\underline{H}$CO (Leu)), 54.3 (NHC$\underline{H}$CO (Lys) and (NHC$\underline{H}$CO (Arg)), 62.9 (OC$\underline{H}_2$ (isopentyl)), 64.0 (OC$\underline{H}_2$ (isopentyl)), 73.6 (2 x C(CH$_3$)$_2$), 79.2 (2 x C(CH$_3$)$_3$), [82.2*, 83.4*, ($C_{60}sp^3$)], 96.2 (C$\underline{P}$h$_2$), [117.9, 121.0, 124.0, 128.2 (Ar$\underline{C}$, Pmc)], [128.4, 129.7, 129.8, 130.0 (Ar$\underline{C}$H)], [134.9, 135.5 (Ar$\underline{C}$, Pmc)], [136.6, 139.0, 139.4, 140.7, 140.9, 141.2, 141.5, 141.7, ($C_{60}sp^2$)], [141.8, 141.9, 1 (Ar$\underline{C}$)], [142.39* (1/2 x C), 142.4, 142.6, 142.7, 142.9, 143.9, 144.2, 144.8, 145.1, 145.3, 145.39, 145.4* (1/2 x C), 145.9, 146.0, 146.3, 146.9, 147.0, 147.5, 148.4* (1/2 x C), 148.8, 149.0, 150* (1/2 x C) ($C_{60}sp^2$)],

153.2 (C=N), 153.3 (C=N), [153.5, 156.3 (CO, Boc), 160.8, 161.5, 162.0, 171.2, 171.4, 172.8, 173.3 (CO)]. HRMS (ESI +ve) calcd for $C_{174}H_{164}N_{16}O_{20}S_2Na$ 2884.1647, found 2884.1675.

References

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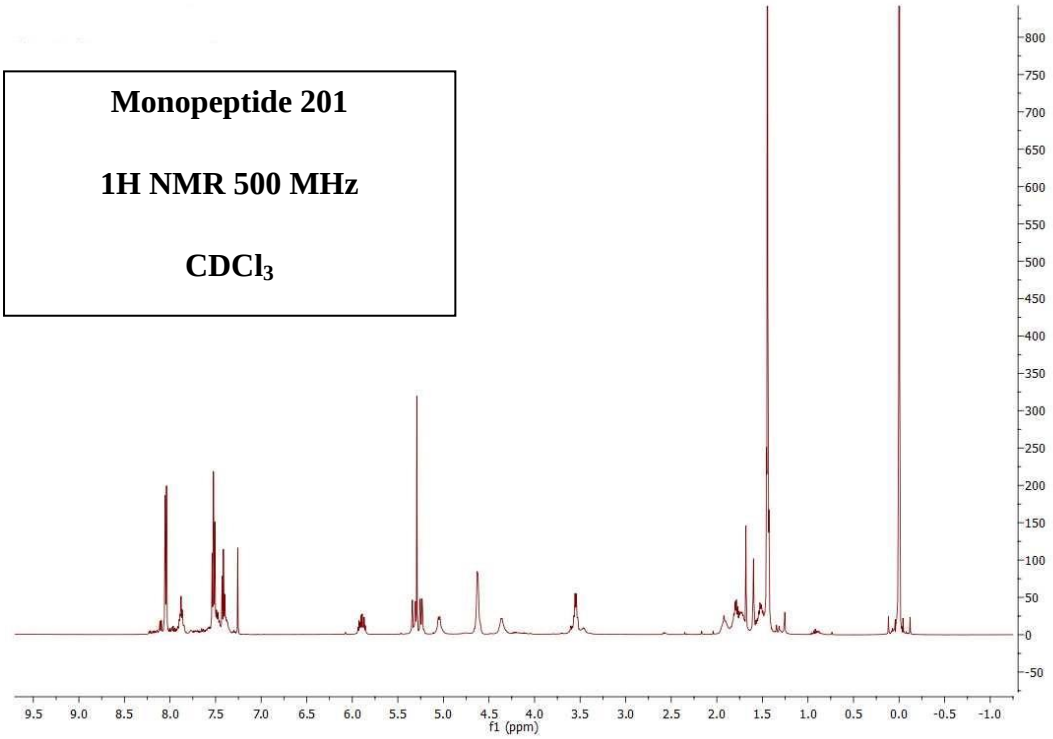
Appendix

Monopeptide 201

1H NMR 500 MHz

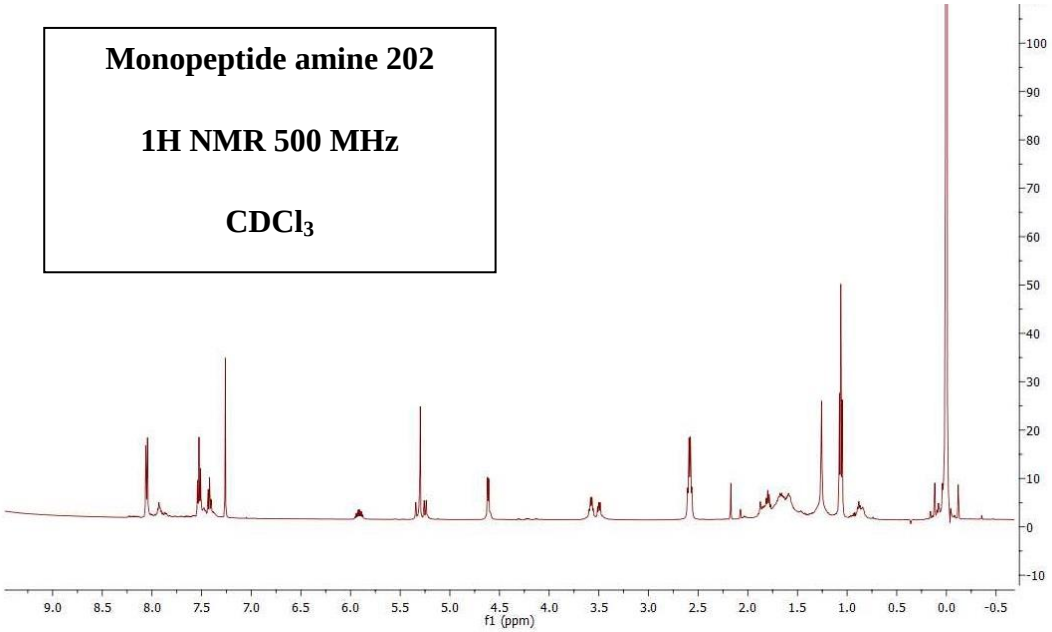
$CDCl_3$



Monopeptide amine 202

1H NMR 500 MHz

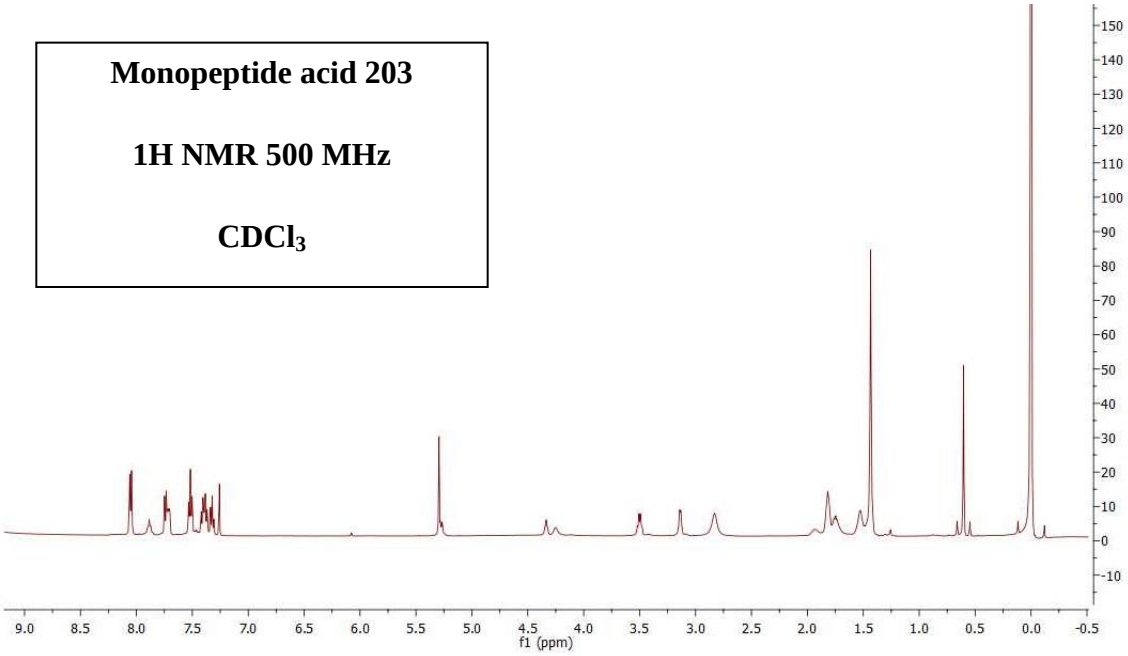
$CDCl_3$



Monopeptide acid 203

1H NMR 500 MHz

$CDCl_3$



Dipeptide 204

1H NMR 500 MHz

$CDCl_3$