1999

[60]Fullerene Amino Acids and Related Derivatives

Glenn A. Burley
University of Wollongong

Paul A. Keller
University of Wollongong, keller@uow.edu.au

Stephen G. Pyne
University of Wollongong, spyne@uow.edu.au

[60]Fullerene Amino Acids and Related Derivatives

Abstract

This paper is a review of the literature concerning the preparation of [60]fullerene amino acid and peptide derivatives. The structure and applications of these derivatives to the biological and material sciences is also presented.

Keywords

fullerene, amino acids, peptides, CMMB

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

[60]FULLERENE AMINO ACIDS AND RELATED DERIVATIVES

Glenn A. Burley, Paul A. Keller[*] and Stephen G. Pyne[*]

Department of Chemistry, University of Wollongong, Wollongong, New South Wales, 2522, Australia.

ABSTRACT

This paper is a review of the literature concerning the preparation of [60]fullerene amino acid and peptide derivatives. The structure and applications of these derivatives to the biological and material sciences is also presented.

INTRODUCTION

Initially thought to be chemically inert, [60]fullerene has been found to react with a wide range of nucleophiles and radicals and also readily undergoes cycloaddition reactions with dienes and 1,3-dipoles to produce stable derivatives[1-3]. More importantly, fullerene derivatives retain the main features of [60]fullerene such as:

a)   precise three dimensional architecture,

b)   large hydrophobic mass and,

c)   unique electronic properties.

In addition, these derivatives offer the possibility of incorporating useful functionalities for further chemical manipulation of groups to modify the physical characteristics (e.g. improved solubility in polar solvents) of [60]fullerene[1-3]. Thus the ability to derivatise [60]fullerene efficiently whilst retaining the essential characteristics of [60]fullerene have stimulated much interest in the applications of fullerenes to materials science and medicinal chemistry.

- To whom correspondence should be addressed.
- Paul_Keller@uow.edu.au; Stephen_Pyne@uow.edu.au

One of the most exciting fields of fullerene chemistry is related to their biological activity. [60]Fullerene derivatives exhibit a range of interesting biological activities including inhibition of HIV enzymes[4-12], photoinduced cytotoxicity via selective cleavage of DNA[13-17] and as a smooth muscle relaxant[18-20]. In addition, other interesting reports of fullerene derivatives of biologically significant molecules such as lipids[21], saccharides[22], oligonucleotides[23-24] and amino acids have appeared in the literature. Of these aforementioned groups, amino acid fullerene derivatives are of special interest. The covalent tethering of fullerenes to peptides and proteins has been the goal of a number of studies concerned with the application of fullerene-peptide conjugates to biological problems[25-30]. This article will report current progress in the preparation and applications of fulleryl amino acids and related derivatives.

1.0 PREPARATION OF FULLERENE AMINO ACIDS

Fulleryl amino acid and peptide derivatives have commonly been prepared by first attachment of a versatile handle widely used in amino acid chemistry, such as hydroxyl, amino or carboxylic acid functional groups to [60]fullerene followed by coupling to a protected amino acid or peptide. The methods of preparation of [60]fullerene products suitable for derivatisation with amino acids and peptides will be first outlined in this review.

1.1.0 Cycloaddition Reactions

Among the various reactions available for derivatisation of [60]fullerene, cycloaddition reactions are the most numerous. The electron deficient fullerene sphere of [60]fullerene is an excellent dienophile and dipolarophile with many resulting cycloadducts showing remarkable stability[1-3]. This section outlines the range of cycloaddition reactions used to form a suitable handle for peptide coupling, as well as the formation of fulleroprolines and a fullerylglycine derivative.

1.1.1   [2+1] Cycloaddition

The addition of the carbene or carbenoid derived from [4-*tert*-butoxycarbonylphenyl]diazomethane (**1**) to [60]fullerene and subsequent deprotection of the ester (**2**) was the first example of a fulleryl derivative with a convenient handle that was utilised for peptide functionalisation (**Scheme 1**)[31]. Acid-mediated deprotection (trifluoromethanesulfonic acid) of (**2**) formed the versatile carboxylic acid (**3**). Coupling of the pentapeptide H-(*L*-Ala-Aib)$_2$-*L*-Ala-OMe via the acyl chloride (**4**) yielded the first fullerene peptide (**5**). Using this versatile synthon (**3**), a range of peptide-fullerene derivatives could be prepared.
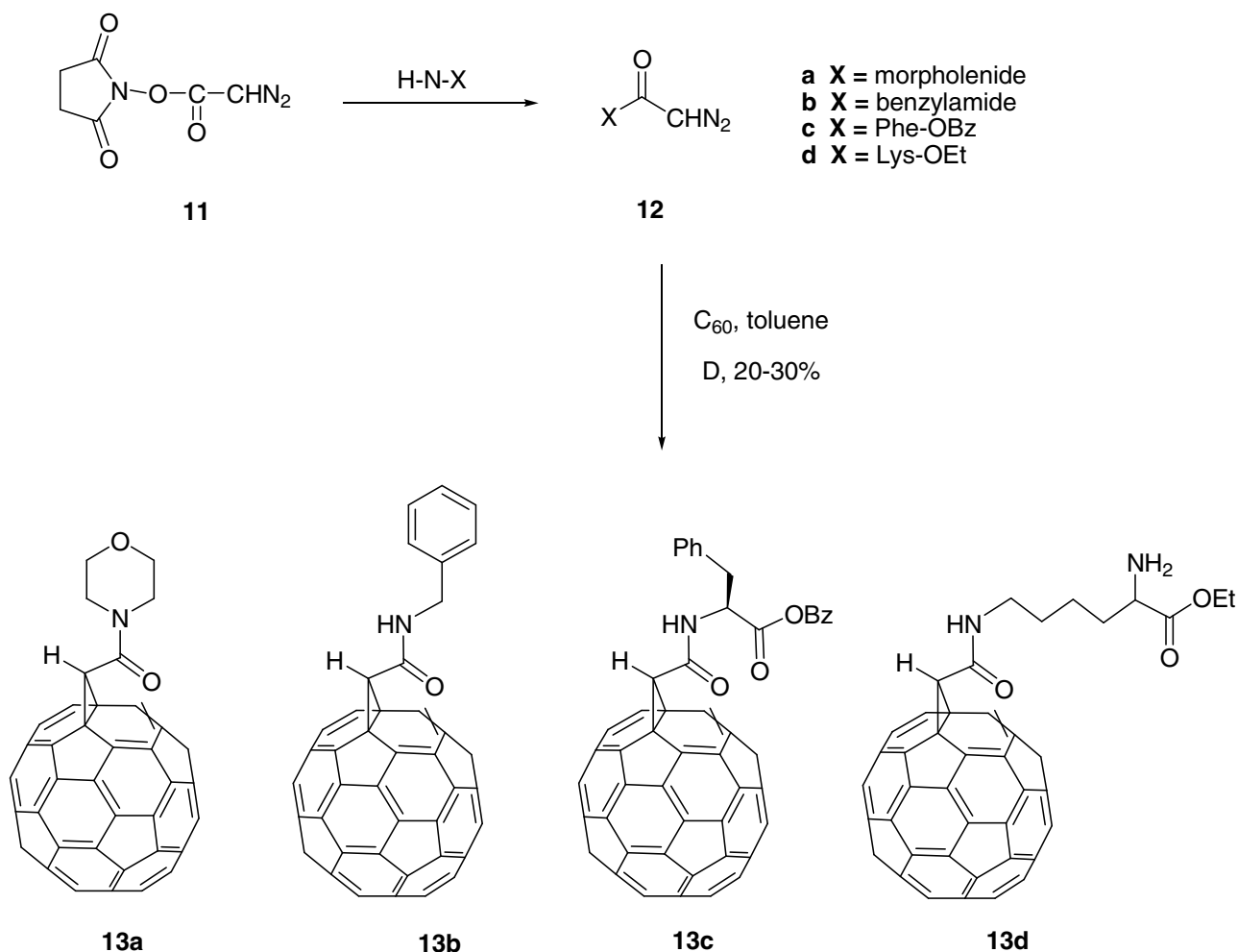
Scheme 1
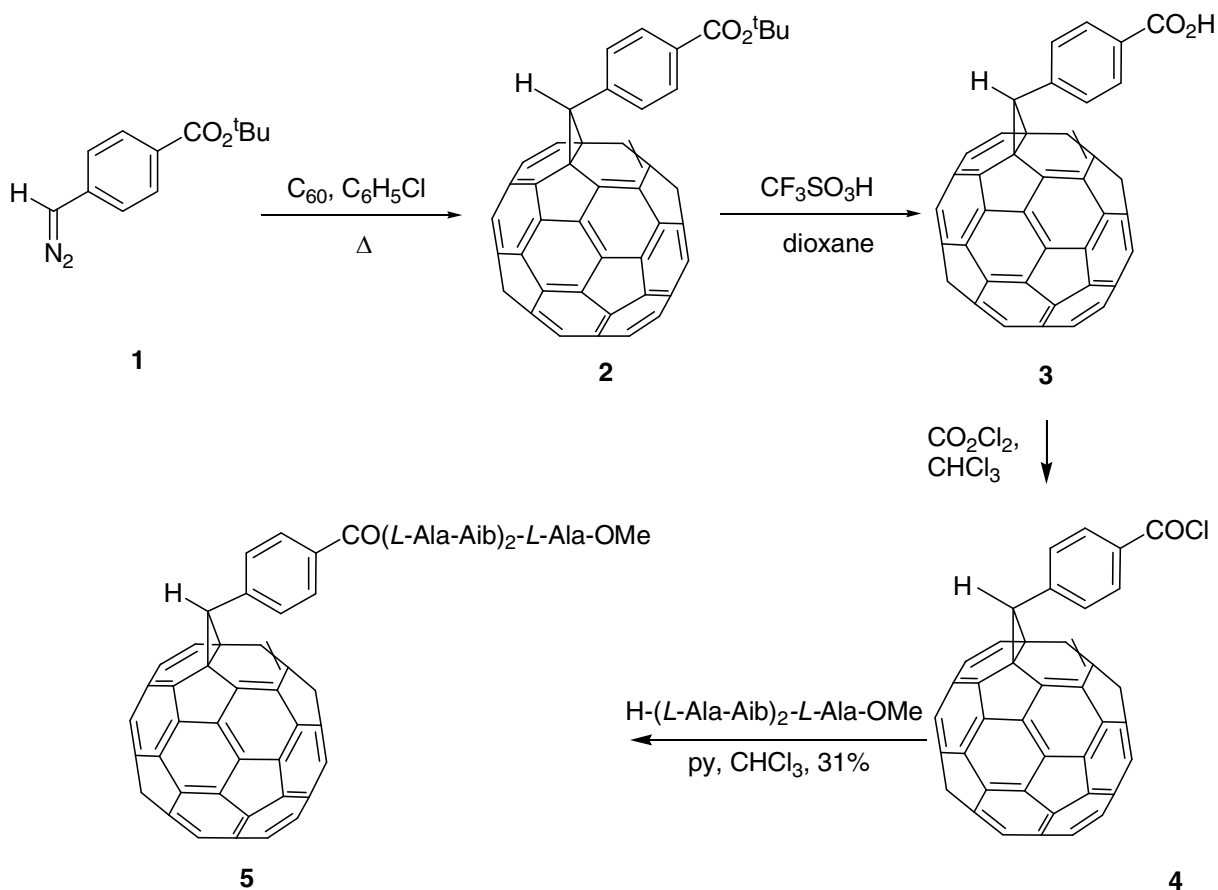
Alkyl diazoacetates were shown to be just as effective in producing another versatile synthon, the methanofullerene carboxylic acid (**8**) (**Scheme 2**)[27,32]. The 1,2-dihydro-1,2-(carboxymethano)-[60]fullerene (**7**) can be obtained either from the corresponding *tert*-butyl carboxylate (**6a**) or ethoxycarbonylmethyl carboxylate (**6b**) of the corresponding alkyl diazoacetate[32]. Isaacs and Diederich demonstrated that dicyclohexylcarbodiimide (DCC) mediated amidation of (**8**) to produce (**9**) and (**10**) was just as effective as the acyl chloride coupling methods used by Prato *et al.* (1993)[25]. The dipeptide product was poorly soluble in organic solvents while the oligopeptide (**9**) was the first water-soluble fullerene peptide to be obtained[27].

Scheme 2
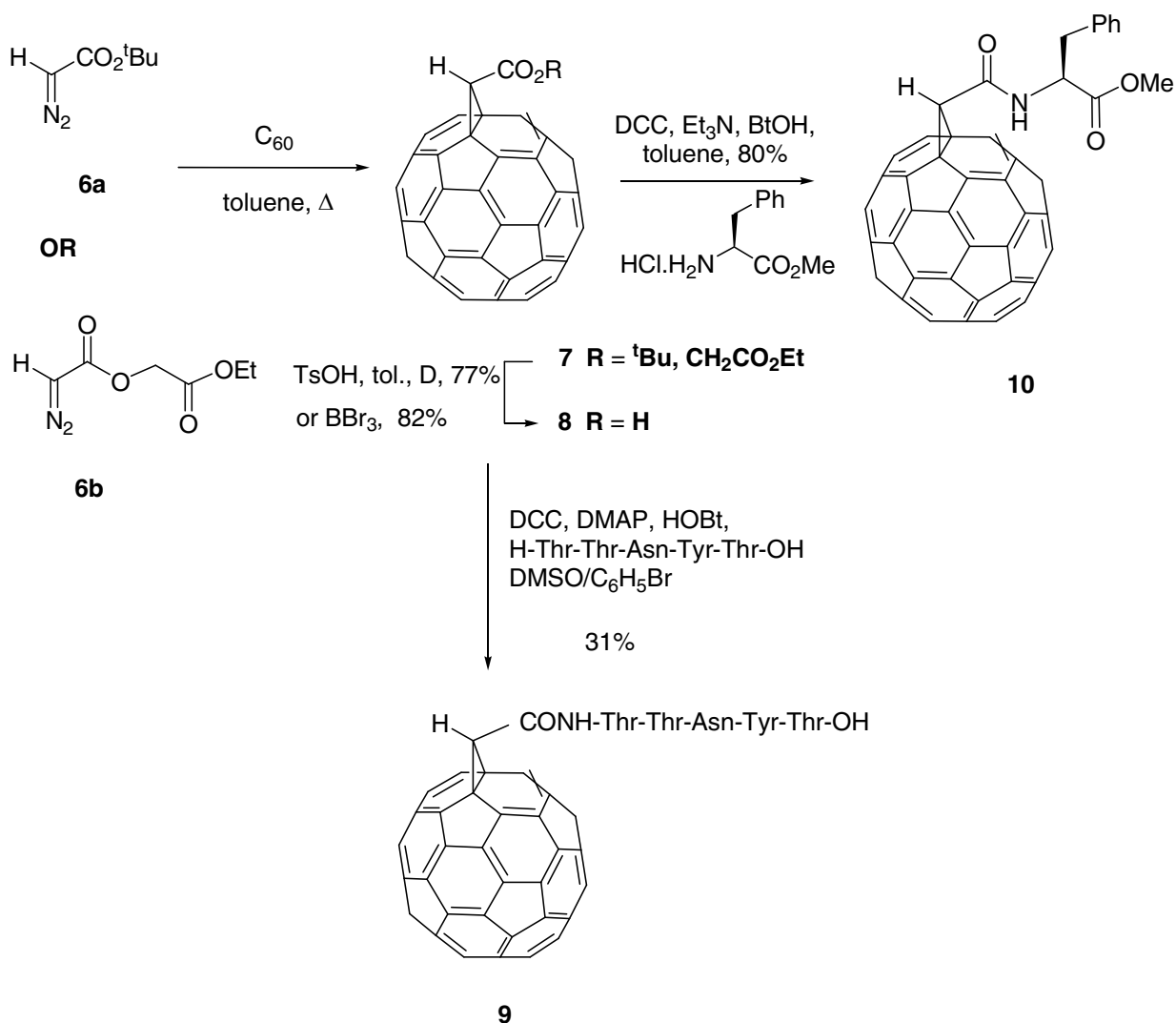
In addition to the formation of methanofullerene derivatives via diazomethanes (**Scheme 1**) and diazoacetates (**Scheme 2**), analogous reactions of [60]fullerene with diazoamides (**12a-d**) have been successfully carried out (**Scheme 3**)[26]. Diazoamide derivatives of protected peptides (**12c-d**) can be prepared in high yield by direct diazoacetylation of primary and secondary amino groups in amino acids with succinimidyl diazoacetate (**11**)[33]. The synthesis of (**13c-13d**) was achieved by refluxing stoichiometric amounts of [60]fullerene with (**11a-d**), resulting in a loss of nitrogen and the formation of predominantly the [6-6]methanofullerene amino acid derivatives (**13a-d**).

Scheme 3

11 → H-N-X → **12**

a X = morpholenide
b X = benzylamide
c X = Phe-OBz
d X = Lys-OEt

C$_{60}$, toluene

D, 20-30%

13a **13b** **13c** **13d**

The above methods using diazo compounds, however, suffer from the formation of two types of methanofullerenes products. In general, a mixture of [5-6]-position (open) and [6-6]-position (closed) products are obtained. For example, heating a solution of **6b** with [60]fullerene at reflux affords a mixture of [6-6] closed (**14a**, methanofullerene) and [5-6] open (**14b**, methanoannulene) isomers (**Scheme 4**). The thermodynamically favourable [6-6] closed adduct is obtained almost exclusively by refluxing the mixture in toluene for a further two days. The ratio of **14a** and **14b** varies with alkyl diazo substitution with purification being somewhat difficult as these regioisomers can have similar retention times on silica gel.
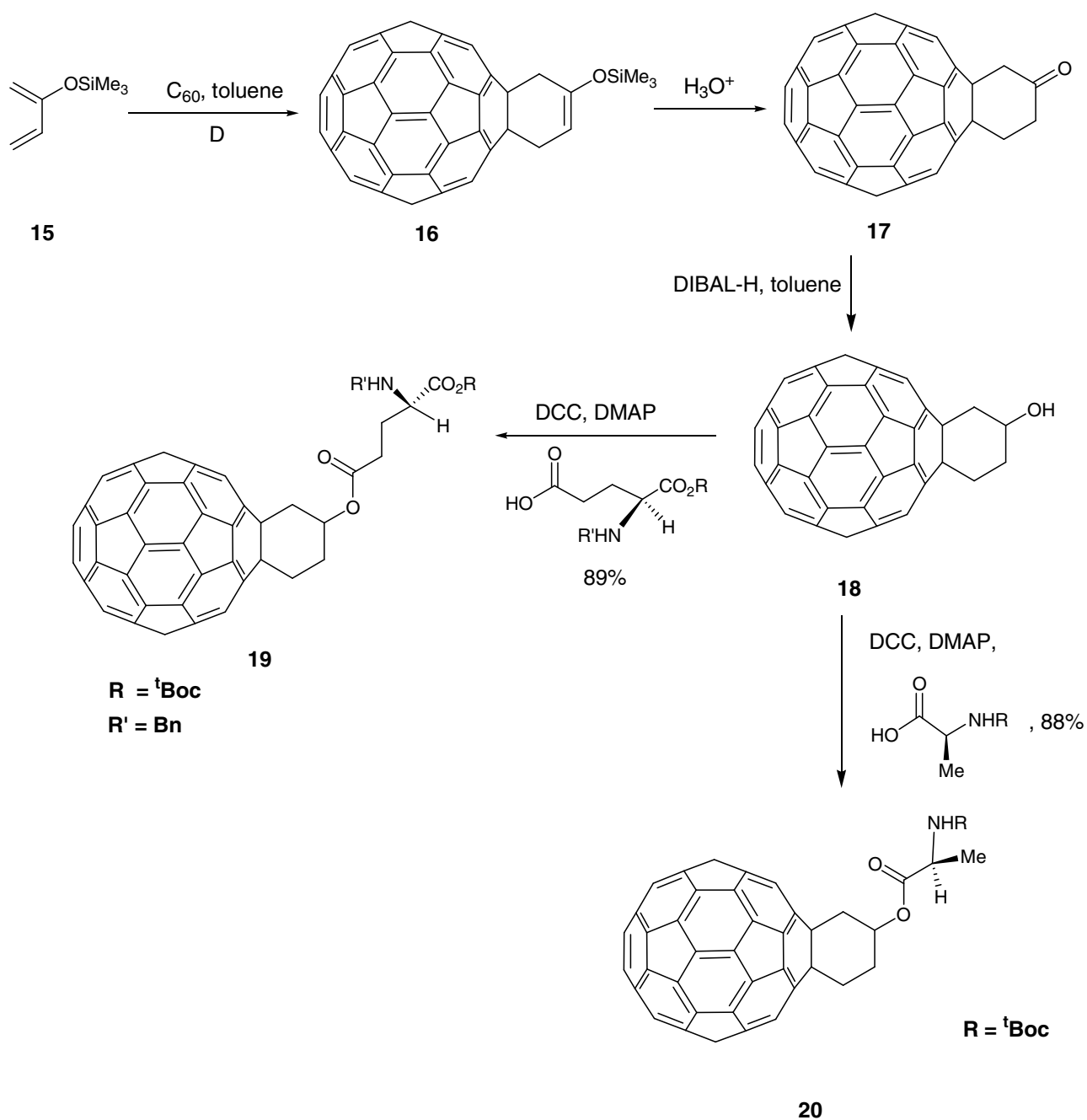
Scheme 4

6b

14a
[6-6] closed (methanofullerene)

14b
[5-6] open (methanoannulene)
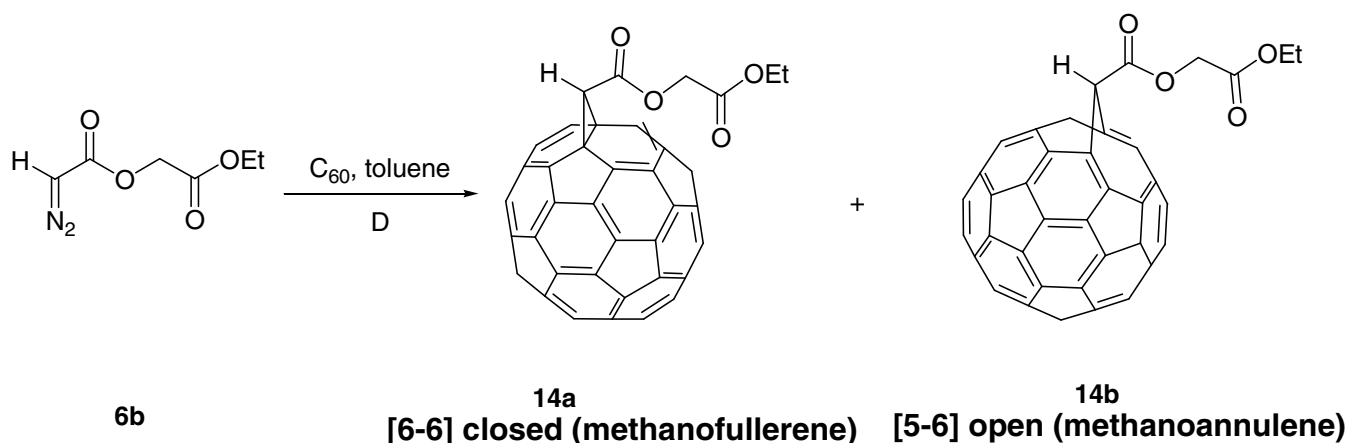
1.1.2 [4+2] Cycloaddition

Another versatile fullerene synthon for the preparation of fullerene amino acid derivatives is the racemic alcohol (**18**). This compound can be obtained by the addition of 2-trimethylsiloxy-1,3-butadiene (**15**) to [60]fullerene in refluxing toluene solution followed by hydrolysis of the cycloadduct (**16**) and then reduction of the corresponding ketone (**17**) with diisobutylaluminium hydride (DIBAL-H) (**Scheme 5**)[34]. Coupling of glutamate and alanine derivatives via DCC-mediated esterification produces adducts (**19**) and (**20**) respectively in high yields. This method provides a synthesis of amino acid derivatives of potential biological importance under mild conditions with the absence of regioisomers.

Scheme 5

1.1.3 [3+2] Cycloaddition

A hydroxyl group can also be used for amino acid coupling in the cyclopentyl derivative (**22**) (**Scheme 6**). Thermal ring opening of 7-alkylidene-2,3-diazobicycloheptene or methylenecyclopropanone ketals (**21**) in the presence of [60]fullerene followed by silica gel hydrolysis of the ketene acetal gave a mixture of (**22**) and (**23**)[35]. The corresponding fulleryl amino ester derivatives (**26**) and (**27**) were prepared from DCC-coupled esterification of the *N*-Boc pyrrole derivative (**24**) and *N*-Boc phenylalanine (**25**) respectively, with the alcohol (**22**)[36].

Scheme 6

21

22

27%

23

34%

DCC, DMAP,

24

64%

81%

DCC, DMAP,

25

26

27
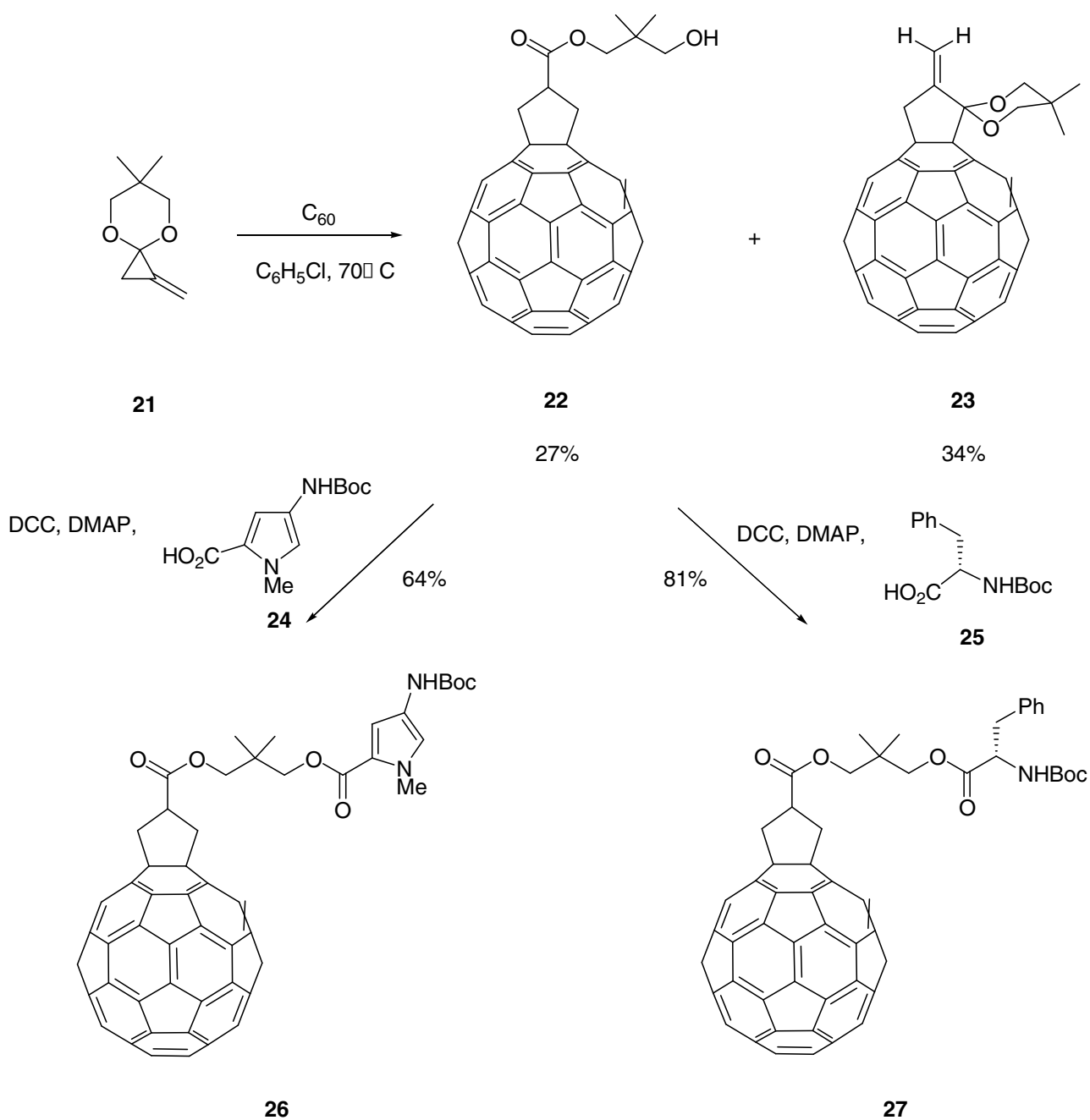
Organic azides serve as useful 1,3-dipoles and undergo [3+2] cycloaddition reactions (either photochemically or thermally) with C_{60} to yield fulleroaziridines (**Scheme 7**)[37-38]. Of these organic azides, photochemical or thermal reactions of [60]fullerene with perfluorophenyl azides (**28**) produces novel fulleroaziridines (**29**) with an activated *N*-hydroxysuccinimide ester. This active ester allows further functionalisation, such as peptide coupling resulting in the formation of (**30**).

Scheme 7

28   **29**   **30**
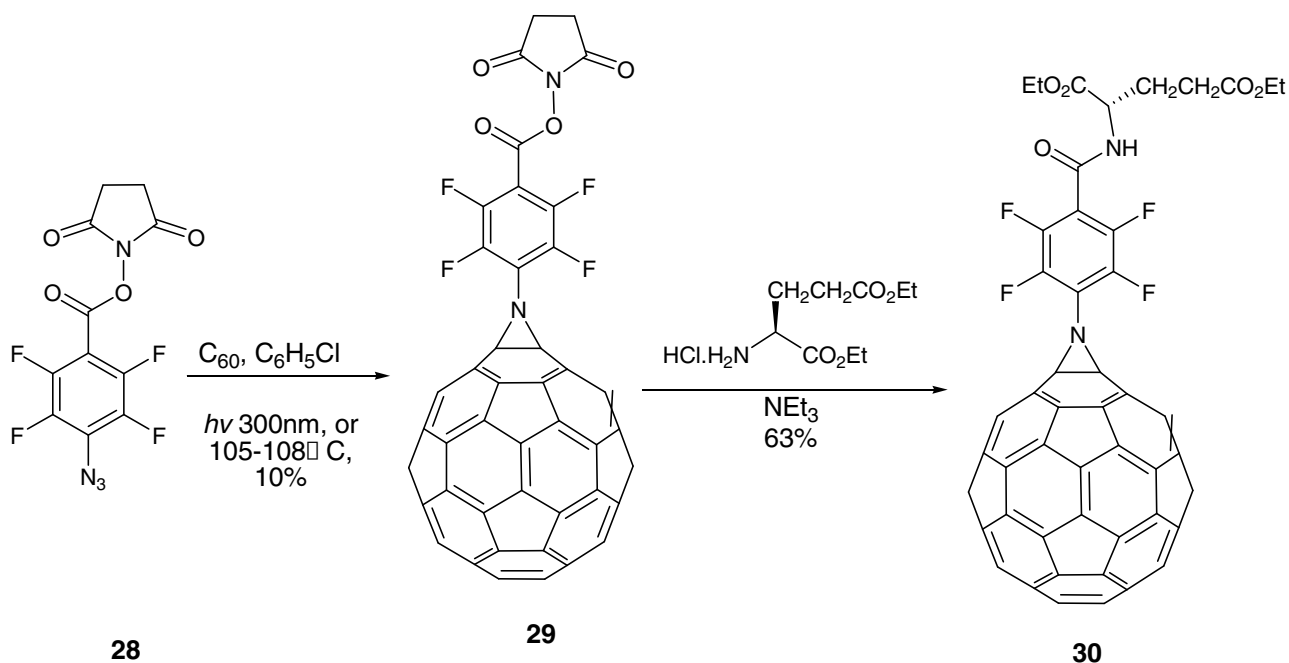
1.1.4 Addition of Azomethine Ylides

The reactions of azomethine ylides with [60]fullerene are the simplest and most efficient routes to fulleryl amino acids. These reactive 1,3-dipoles can be generated by a number of methods. The Prato group has used either thermal ring opening of electron deficient aziridines (**33**) or iminium salts derived from condensation of amino acid esters (**31**) with aldehydes (**32**) to produce azomethine ylides *in situ*[39,40]. The resulting products are exclusively [6-6] adducts, arising from addition across the [6-6] double bond of [60]fullerene (**Scheme 8**). These methods allowed the synthesis of the first fulleryl *C*-substituted amino acid, more commonly known as fulleroproline (Fpr, **34**)[41]. These reactions are versatile and a variety of substituted fulleroproline derivatives can be obtained using different aldehydes.

Scheme 8

34

Fulleroproline (Fpr)
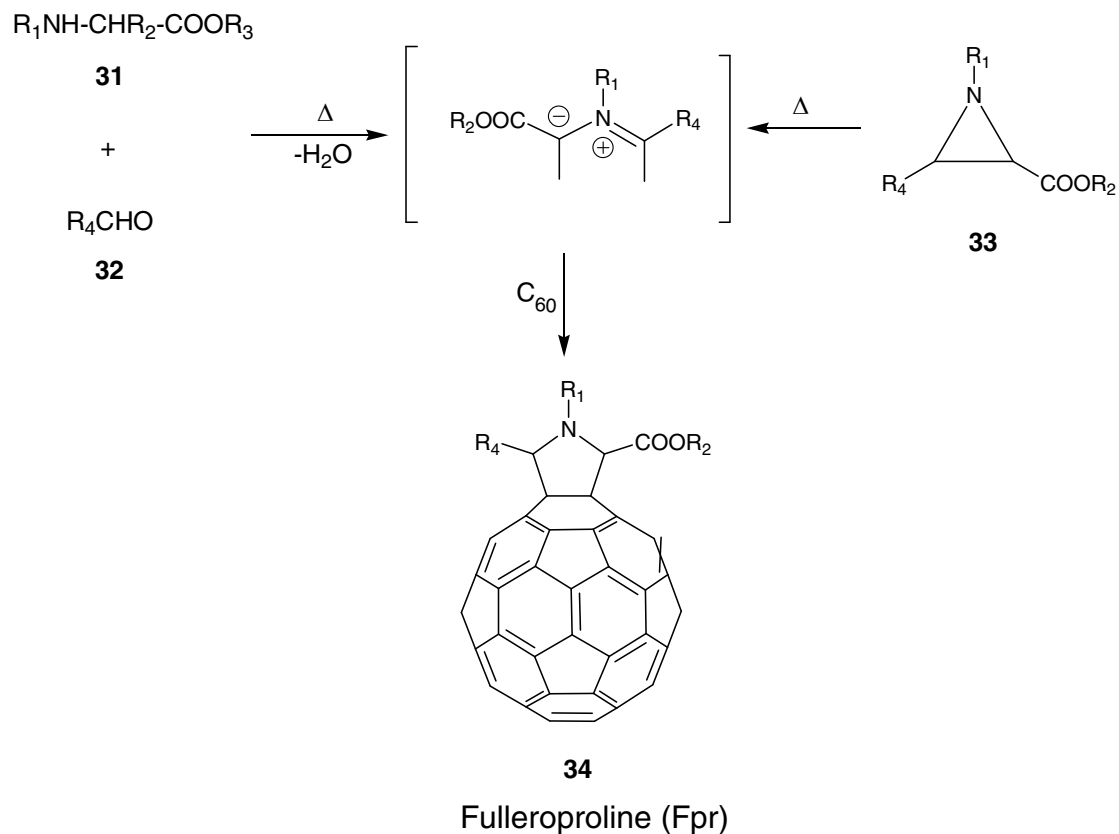
Fpr derivatives can be prepared with the pyrrolidine nitrogen protected (**Scheme 9**) or unprotected (**Scheme 10**)[42]. Selective deprotection of the *N*-protecting group (either trityl or *p*-methoxybenzyl) in (**36**) was achieved in the presence of the methyl ester group using trifluoroacetic acid (TFA) (**Scheme 9**). This allows Fpr to be incorporated into peptides using conventional peptide coupling techniques. The unprotected adduct (**40**) cannot be isolated as a pure solid due to the reactivity of the free amino group, but can be stored in the dark and in dilute solutions[39,42]. Compound (**40**) however can be used to prepare either *N*-terminal peptides [(**43**) and (**44**)] or *C*-terminal peptides [(**45**) and (**46**)] as shown in **Scheme 10**.

Scheme 9

35 → o-Cl₂C₆H₄ / C₆₀, D, 30% → **36**

$o\text{-}Cl_2C_6H_4$

C_{60}, D, 30%

36 → TFA → **37**

37 → Ac₂O, NEt₃, 76% → **38**

Ac_2O, NEt_3,

76%

35

36

37

38
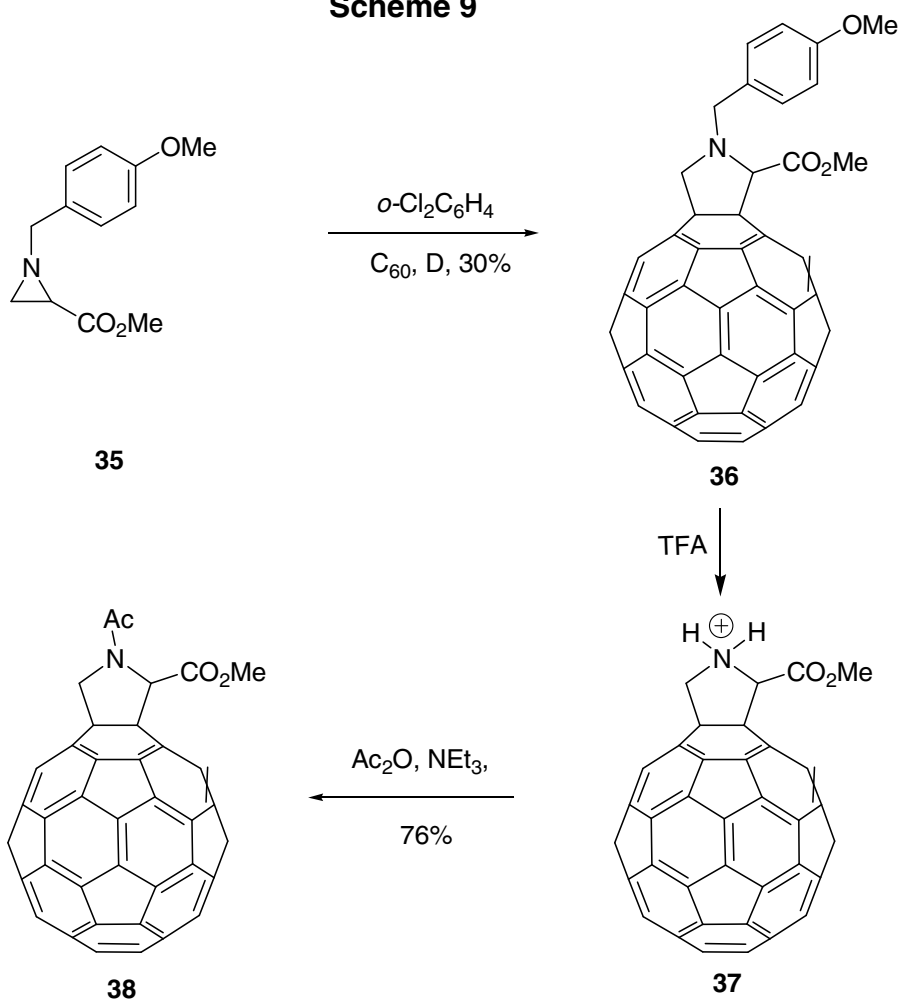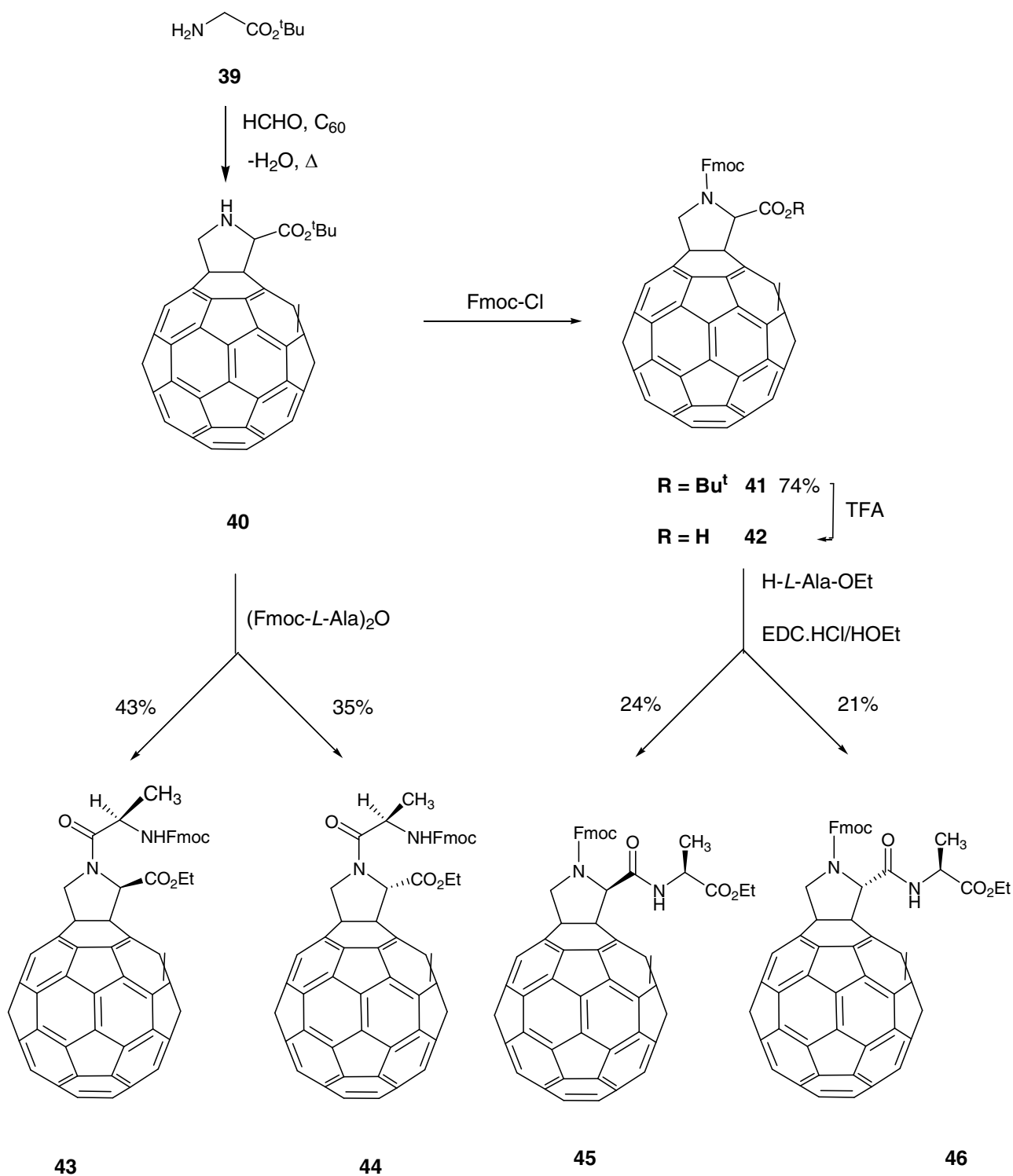
Scheme 10

Diastereoselective additions of azomethine ylides to [60]fullerene have also been reported (**Scheme 11**). For example, the reaction of [60]fullerene with the chiral *O*-protected *L*-4-hydroxyproline derivative (**47**) and formaldehyde gives the fulleroproline derivatives (**48a** and **48b**) in a diastereoselective fashion[43]. The presence of the bulky *tert*-butyldiphenylsiloxy (OTBDPS) group favours attack of the 1,3-dipole with the bulky OTBDPS group facing away from the fullerene sphere, resulting in the major product being adduct (**48b**).

Scheme 11

(CH₂O)ₙ

+

47

TBDPS = Bu^{t}Ph₂Si

toluene, Δ
C₆₀

48a
11%

48b
24%

Scheme 12

PhCH=NCH₂EWG

49

toluene
Δ

PhCH=NCH₂EWG

anti

syn

EWG =	*trans*	*cis*
CO₂CH₃	21%	40%
CONHCH₂CO₂Et	12%	45%
PO(OEt)₂	20%	58%

C₆₀

C₆₀

trans

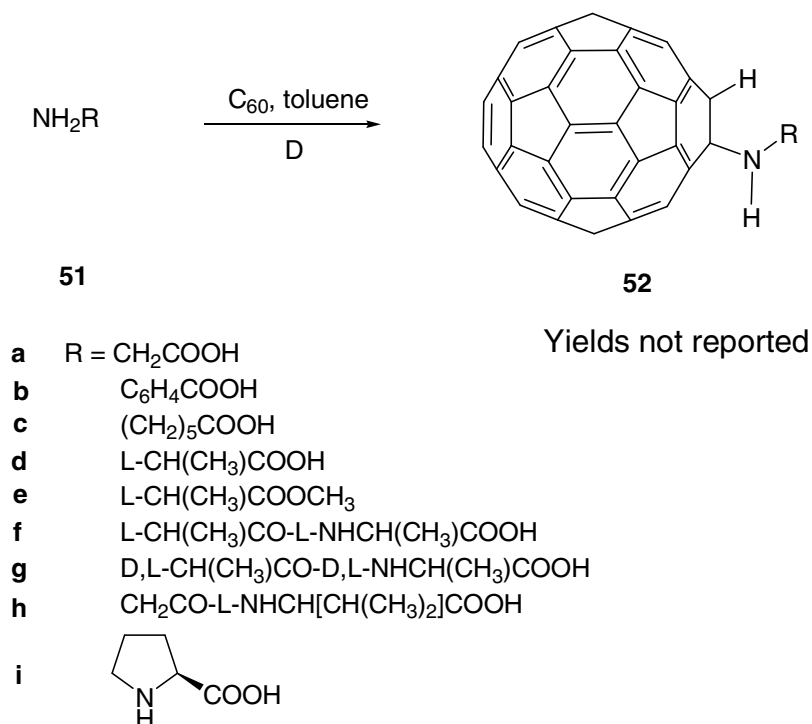**50a**

minor

cis

50b

major

Other routes for the preparation of fulleroproline derivatives via azomethine ylides are shown in **Scheme 12**. *N*-Benzylidine imines of amino acids (**49**) also produce azomethine ylides *in situ* and afford Fpr adducts by refluxing a toluene solution of these imines in the presence of [60]fullerene[44-46]. The *anti* and the *syn* intermediates produced are in thermal equilibrium with the imine (**49**) and react with [60]fullerene to produce (**50a**) and (**50b**) respectively. The *anti* intermediate is thermodynamically less stable than the corresponding *syn* intermediate since the phenyl group in the former is not coplanar with the nitrogen atom for steric reasons and thus adducts of the type **50b** are favoured over **50a** in these reactions[46]. Although pyrrolidino[60]fullerenes are much less basic than their pyrrolidine counterparts, alkylation of pyrrolidino[60]fullerenes has recently been achieved under mild solvent-free phase transfer conditions in the presence of microwave radiation[47].

1.2.0 Nucleophilic Addition

1.2.1 Addition of Amines

It has been well documented that primary and secondary amines undergo nucleophilic additions with the electron deficient [60]fullerene. It has been suggested that these adducts are formed by a stepwise mechanism that involves initial electron transfer followed by radical recombination[48-51]. Natural amino acids have a highly nucleophilic primary amine (except for Pro) and these have been reported to react readily with [60]fullerene to form water-soluble products (**52**) (**Scheme 13**)[52-59]. The yields for these adducts, however, were not reported. The formation of monoaddition adducts from the reaction of [60]fullerene with simple amines however, is rare.

Scheme 13

NH$_2$R $\xrightarrow[\text{D}]{\text{C}_{60}\text{, toluene}}$

51

52

Yields not reported

a   R = CH$_2$COOH
b   C$_6$H$_4$COOH
c   (CH$_2$)$_5$COOH
d   L-CH(CH$_3$)COOH
e   L-CH(CH$_3$)COOCH$_3$
f   L-CH(CH$_3$)CO-L-NHCH(CH$_3$)COOH
g   D,L-CH(CH$_3$)CO-D,L-NHCH(CH$_3$)COOH
h   CH$_2$CO-L-NHCH[CH(CH$_3$)$_2$]COOH

i

1.2.2 Addition of Carbon Nucleophiles

The cyclopropanation of [60]fullerene, first discovered by Bingel, proceeds via the deprotonation of α–haloesters with base in the presence of [60]fullerene[60]. This is one of the most widely used reactions in fullerene chemistry. With the development of a one-pot cyclopropanation reaction by Camps and Hirsch, this reaction is also one of the most efficient with yields averaging 50% (**Scheme 14**)[61]. The reaction involves the formation of the α–haloester (**54**) *in situ* by deprotonation of one methylene proton and bromination using carbon tetrabromide (CBr$_4$). The resultant α–haloester is deprotonated by a second equivalent of DBU and undergoes nucleophilic attack of [60]fullerene. Cyclopropanation is complete with the incipient anion of C$_{60}$ attacking the electron deficient carbon of the α–haloester and the bromine acting as a leaving group. Alternatively, the deprotonated α–haloester may undergo α–elimination of the halide to give the corresponding carbene (**55**) that undergoes [2+1] cycloaddition across the [6-6] double bond of fullerene[62].

Scheme 14
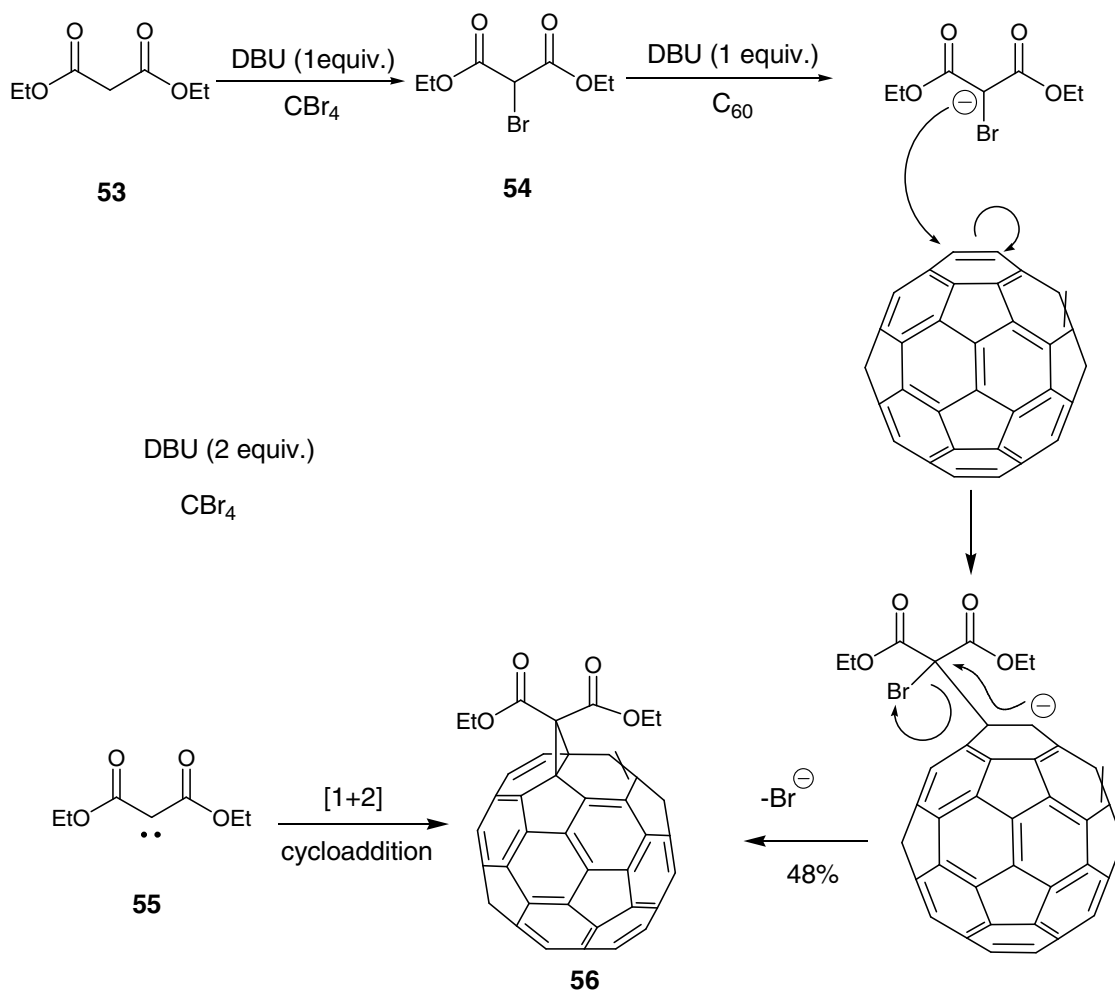
Up until recently, the versatility of this reaction was confined primarily to malonate derivatives[61]. Our group has shown that fulleryl glycine imino esters can be synthesised from commercially available glycine imino esters under Hirsch cyclopropanation conditions (**Scheme 15**). For example, treatment of a solution of [60]fullerene with *tert*-butyl *N*-diphenylmethyleneglycinate (**57**), carbon tetrabromide and diazabicyclo[5.4.0]undec-7-ene (DBU) gave the cyclopropane imino ester (**58**)[63]. As opposed to the literature findings[64], (**58**) was shown to be remarkably stable under acidic and hydrogenolysis conditions. Treatment of (**58**) under mild reductive conditions using sodium cyanoborohydride in mildly acidic media (pH=4), formed not the reduced imine compound but a novel ring opened 1,2-dihydro[60]fullerylglycine derivative (**59**). The two fullerene carbons alpha to C-1 (C-6 and C-9) and C-2 (C-3 and C-12) were observed downfield of other fullerene resonances and occurred in the region δ154.4-152.4 (**Figure 1**) in HMBC experiments. Interestingly, C-6 and C-9 and C-3 and C-12 appeared as diastereotopic pairs due to the stereogenicity of C-61. This compound, like Fpr, is only the second example (albeit protected) of a *C*-substituted fulleryl amino acid. We are currently developing methods to deprotect the *t*-butyl ester or the diphenyl amine groups in (**59**).
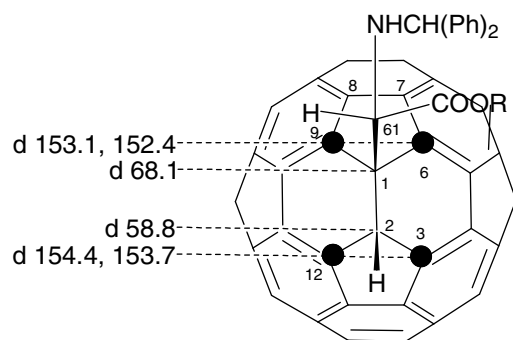
Scheme 15

57

C₆₀, DBU, CBr₄ / C₆H₅Cl, RT

58

NaCNBH₃, pH 4 / THF   R = Bu^t, 52%   R = Et, 79%

R = Bu^t

44%

59

Figure 1. ^{13}C NMR (d[6]-benzene/CS₂, 1 : 1) chemical shifts and assignments for **59**, R = Bu^t
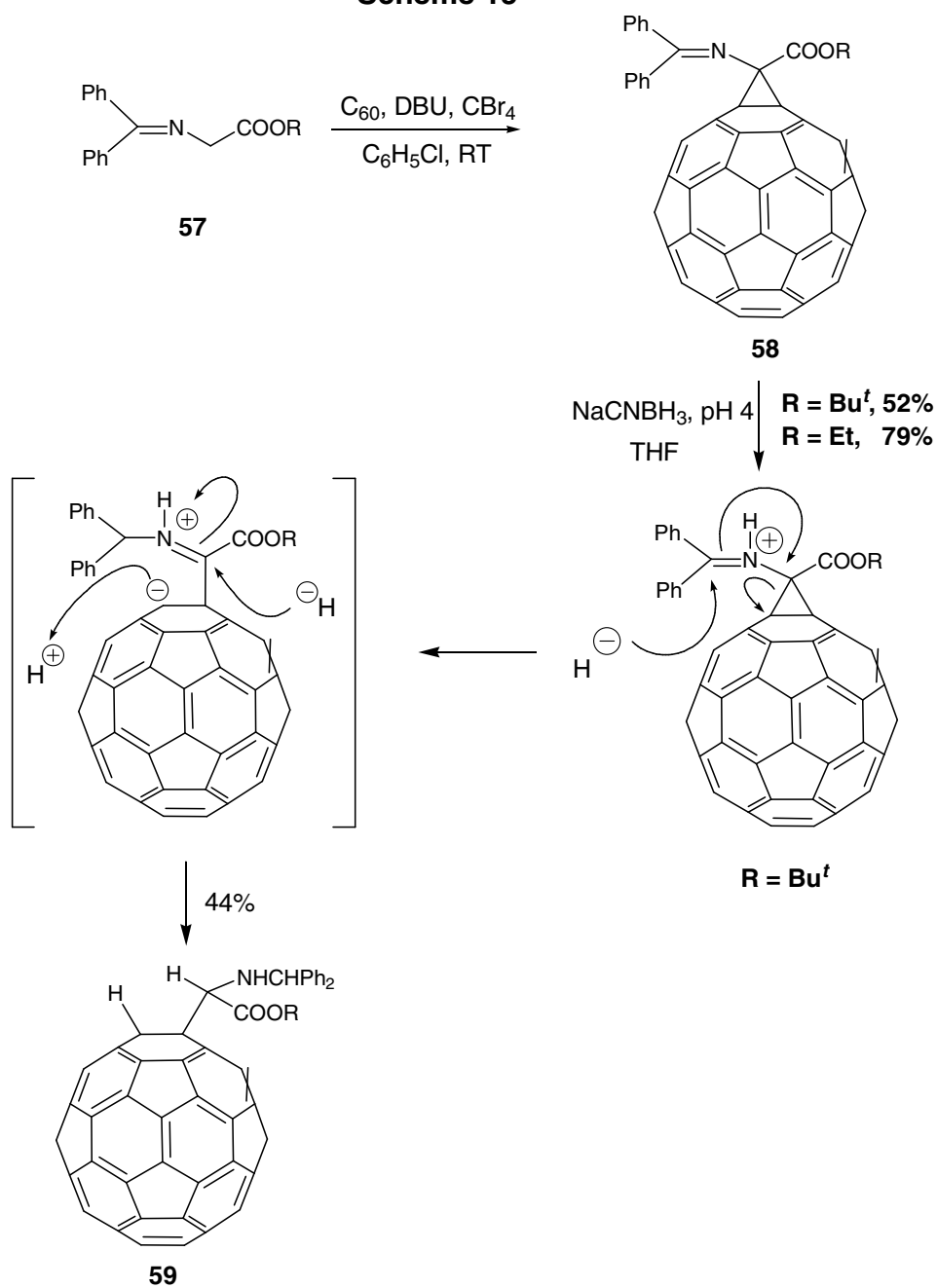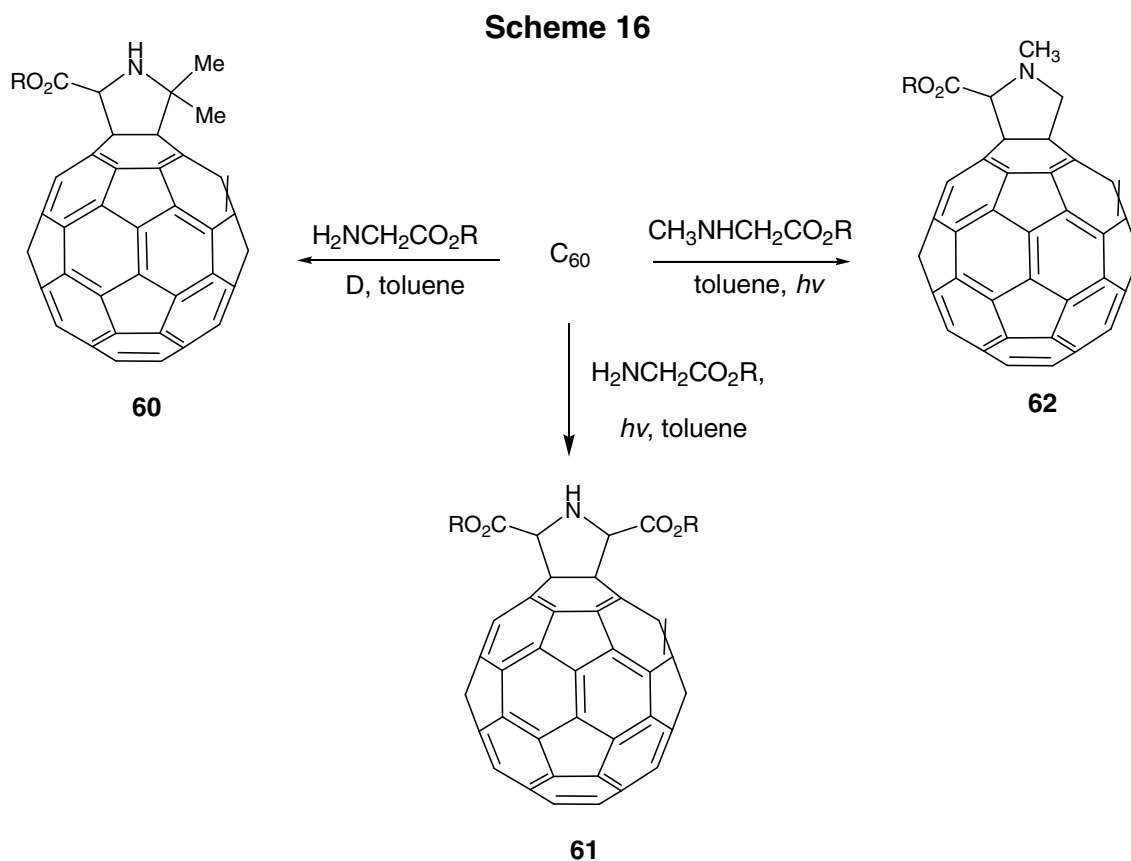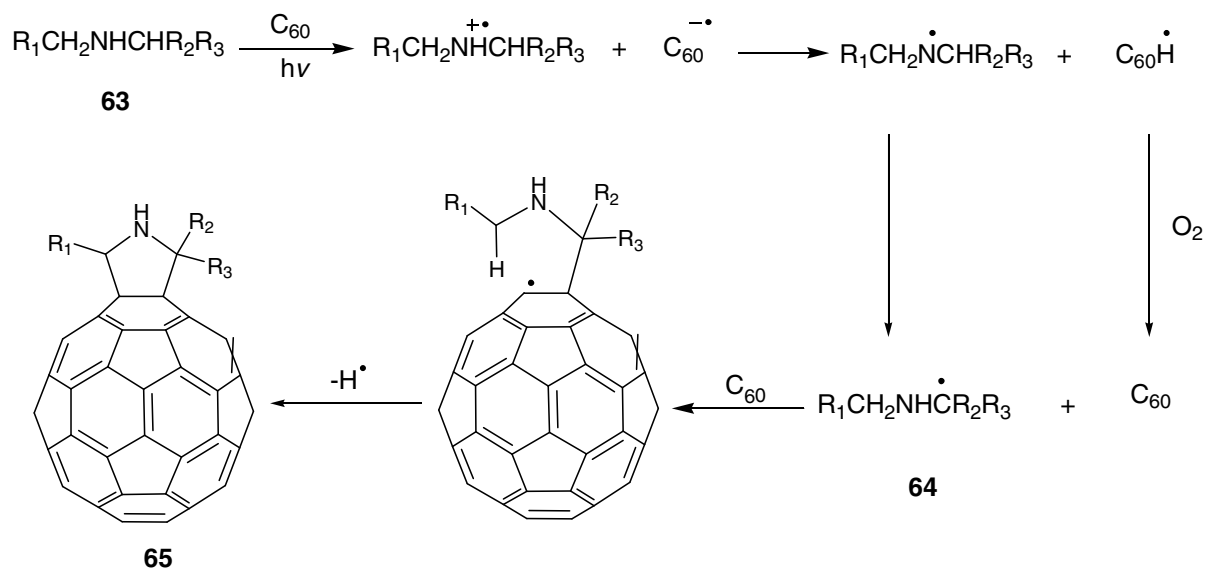
Scheme 15

Figure 1. ^{13}C NMR (d^6-benzene/CS$_2$, 1 : 1) chemical shifts and assignments for **59**, R = Bu^t
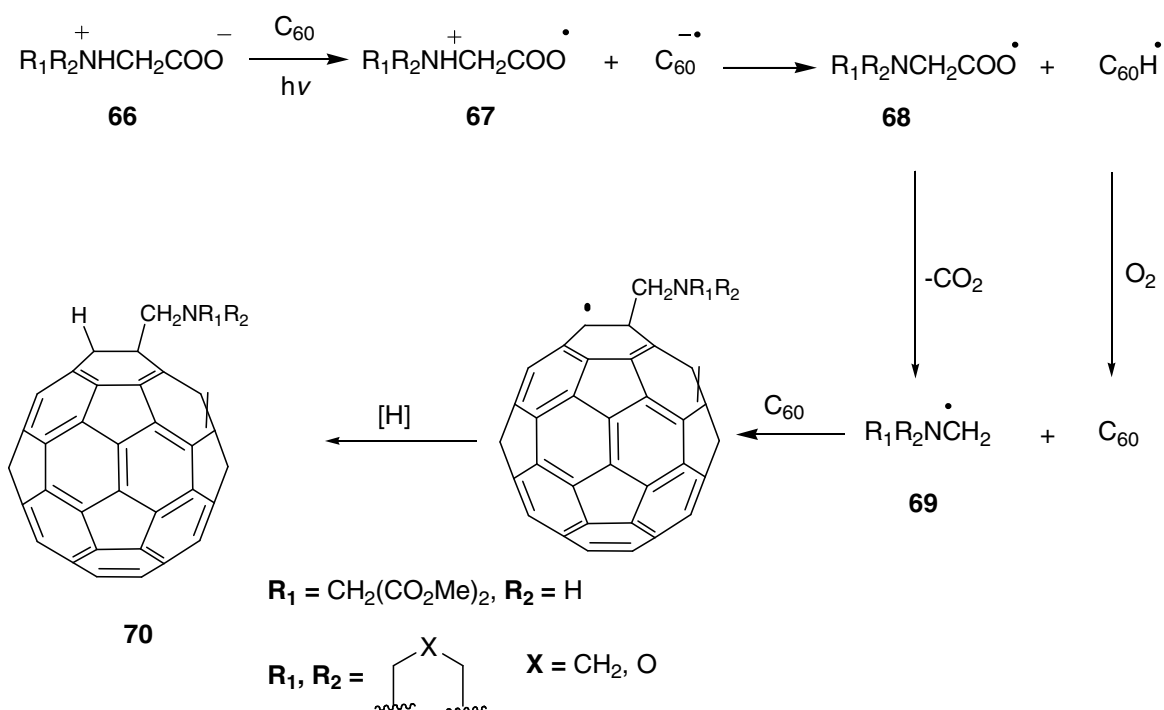
1.3.0 Radical Additions

Photolysis reactions are another method commonly utilised by fullerene chemists to produce fullerene amino acid derivatives. Fulleroproline derivatives can be formed by photolysis of primary and secondary amino acid esters (**Scheme 16**)[65-67]. The initial key step in these photoreactions is presumed to be the formation of an α−carbon-centred radical (**64**) which attacks the fullerene (**Scheme 17**). The presence of O_2 in the reaction vessel appears to accelerate this photoreaction. Another interesting result using photolysis of amino acids with [60]fullerene is the photolytic decarboxylation of $(MeOOC)_2CH_2NCH_2COOH$ to form a 1,2-dihyro[60]fullerene [(**70**), $R_1=CH_2(CO_2Me)_2$, $R_2=H$] (**Scheme 18**)[42]. The mechanism, similar to the reaction pathway of the amino acid ester (**Scheme 17**), involves the formation of the carboxyl radical (**67**). Decarboxylation of (**67**) forms the aminomethyl radical (**69**) which undergoes addition to [60]fullerene and H-abstraction from the environment to yield (**70**).

Scheme 16

H₂NCH₂CO₂R
D, toluene

C₆₀

CH₃NHCH₂CO₂R
toluene, *hv*

H₂NCH₂CO₂R,
hv, toluene

60

62

61

Scheme 17

$R_1CH_2NHCHR_2R_3 \xrightarrow[h\nu]{C_{60}} R_1CH_2\overset{+\bullet}{N}HCHR_2R_3 \ + \ \overset{-\bullet}{C_{60}} \longrightarrow R_1CH_2\overset{\bullet}{N}CHR_2R_3 \ + \ C_{60}\overset{\bullet}{H}$

63

65

$\xleftarrow{C_{60}} R_1CH_2NH\overset{\bullet}{C}R_2R_3 \ + \ C_{60}$

64

$\downarrow$ $\qquad$ $\downarrow O_2$

Scheme 18

$\overset{+}{R_1R_2N}HCH_2COO^{-} \xrightarrow[h\nu]{C_{60}} R_1R_2\overset{+}{N}HCH_2\overset{\bullet}{COO} \ + \ \overset{-\bullet}{C_{60}} \longrightarrow R_1R_2NCH_2\overset{\bullet}{COO} \ + \ C_{60}\overset{\bullet}{H}$

66 $\qquad\qquad\qquad$ **67** $\qquad\qquad\qquad$ **68**

$\downarrow -CO_2 \qquad \downarrow O_2$

70

$\xleftarrow{C_{60}} R_1R_2\overset{\bullet}{N}CH_2 \ + \ C_{60}$

69

$\mathbf{R_1} = CH_2(CO_2Me)_2, \ \mathbf{R_2} = H$

$\mathbf{R_1, R_2} =$ (ring with X) $\qquad \mathbf{X} = CH_2, O$

2.0 APPLICATIONS OF FULLERENE AMINO ACIDS

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 to date.

2.1 Fullerene Peptide Structure

Since the pioneering work by Prato *et al.*[25], several fullerene peptide studies have been carried out[27,68]. Spectroscopic data of adduct (**5**) (**Scheme 1**) showed this compound exhibited both fullerene and peptide properties. Using an optically active alanine residue in the peptide chain, the first example of a chiral fullerene derivative was synthesised. Conformational analysis using ^{1}H-NMR and FT-IR revealed that an ordered secondary helical structure is adopted, predominantly a 3_{10}-helix rather than α–helix. This result is consistent with literature studies of a variety of short Aib (α–aminoisobutyric acid) rich peptides[69]. The secondary structure of this peptide sequence appears not to be affected by the [60]fullerene moiety.

In contrast to the pentapeptide adduct (**9**), fulleroproline peptide structures incorporate a chiral C_α directly bonded to the fullerene sphere[42]. The stereochemistry of C_α in these compounds was determined by circular dichroism (CD). The CD spectra of adducts (**43-46**) were not particularly successful due to insolubility problems, however, transformation of these adducts into the corresponding 2,5-dioxopiperazines solved the stereochemical dilemma. CD revealed a negative Cotton effect corresponding to an *S*-configuration of C_α in Fpr, whereas a positive Cotton effect corresponds to an *R*-configuration. The Fpr dipeptide adducts (**43-46**) were shown to be poorly soluble in organic solvents, thus analyses of their secondary structure were not forthcoming. Conformational characterisation was achieved with the small Fpr peptides (**71**) and (**72**)[68]. In all these cases, the *N*-terminus of Fpr was blocked by acylation using isobutyroyl chloride (Ibu-Cl) and triethylamine (NEt$_3$) to prepare di- and tripeptide derivatives (**71**) and (**72**). X-ray diffraction techniques reveal both peptides (**71**) and (**72**) are folded in a type-II β-turn conformation, characterised by a weak H-bond between the carbonyl of the Ibu group and amide proton of *D*-alanine. This type of secondary structure is analogous to the known behaviour of the *L*-Pro-*D*-Ala parent sequence[70-73].

71   **72**   **73**
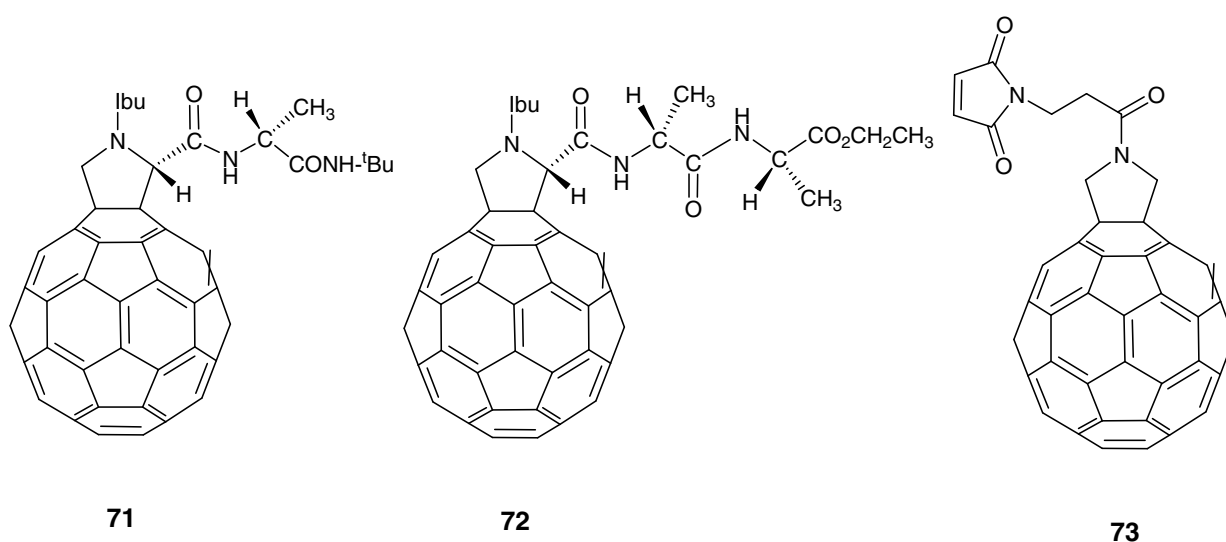
Although not a Fpr in the strict sense, the fullerene derivative (**73**) is worthy of mention[74]. The [60]fullerene cage is able to reversibly accept up to a maximum of six electrons in optimal conditions and thus can be detected electrochemically and thus could have uses as a biological probe[75]. By coupling the thiol-selective maleimide derivative (**73**) to the thiol unit in the small copper-containing protein azurin mutant (containing one surface cysteine residue), the fullerene-modified protein was detected by UV-vis spectroscopy. Cyclic voltammetry of the fullerene-modified protein revealed the presence of a new redox couple at −302mV, assigned to the first reduction and reoxidation of [60]fullerene. A shift to a more negative potential was observed for the copper containing azurin protein compared to the native protein. The authors suggest this pronounced change in potential maybe due to communication between the fullerene and the copper centre.

2.2 Biological Activity

The Prato group using the methanofullerene carboxylic acid (**8**) shown in **Scheme 2** reported the first bioactive peptide (**9**)[27,32]. The pentapeptide sequence H-Thr-Thr-Asn-Tyr-Thr-OH is a known activator of human monocyte chemotaxis. The biological activity of the coupled fulleryl peptide (**9**) was evaluated compared to the underivatised peptide, revealing potent chemotaxic activity, higher than of the underivatised pentapeptide.

Anti-HIV-1 protease (HIV-P) activities were also conducted on compound (**9**). From studies of Friedman *et al.* the [60]fullerene sphere acts as an ideal hydrophobic substrate in the centre of HIV-P[4,5,11]. Activity of the pentapeptide coupled fullerene adduct was shown to be moderate with K_i values ~100μM level. More recent [60]fullerene derivatives have revealed HIV-P activity in the nanomolar range (**Table 1**)[11]. These findings have shown the potential for water soluble [60]fullerene derivatives to be viable biological agents for diseases such as cancer therapy. Although the mode of action of water-soluble [60]fullerene derivatives such as (**74**) have been hotly debated in the literature, they have shown a potential use for photodynamic therapy[76-78]. Compound (**74**) was shown to be cytotoxic to the HeLa S3 cell line upon short irradiation periods with fluorescent light. No cytotoxic activity was observed with the absence of fluorescent light (**Figure 2**).

Table 1: Inhibition constants of fullerene derivatives active against HIVP
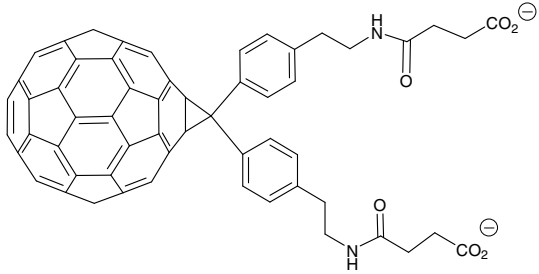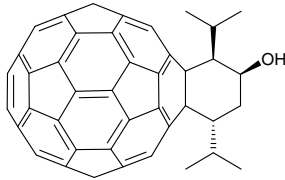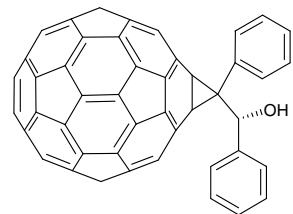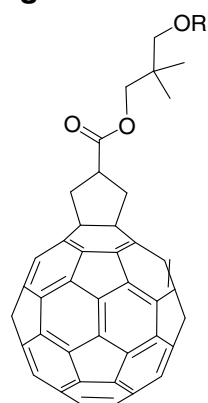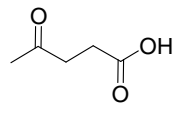
Compound	K_i (mM)
	5 mM
	150 nM
	103 nM

Figure 2: *In vitro* cytotoxicity of **74** against HeLa S3 cells

74

R	IC_{50} (mM)	
	dark	**light**
	>100	6.3
mitomycin C	0.06	0.06

2.3 Molecular Recognition

[60]Fullerene based HPLC materials have numerous practical applications in both chromatography and supramolecular chemistry. Fpr derivatives have been successfully coupled to

solid supports on silica gel (**Scheme 19**)[79,80]. Packing this solid [60]fullerene matrix as a new HPLC stationary phase allowed investigation of its chromatographic properties. Initial studies showed that this new stationary phase is both effective in organic and aqueous media, however differences were observed with the [60]fullerene stationary silica phase with respect to conventional silica stationary phases. These differences are due to solute molecules interacting with the fullerene sphere, thus affecting retention times compared with conventional silica. This is particularly apparent with cavity-containing molecules such as calix[n]arenes and cyclodextrins. Molecules with a cavity complementary to the diameter of the [60]fullerene sphere were effectively separated.

Scheme 19
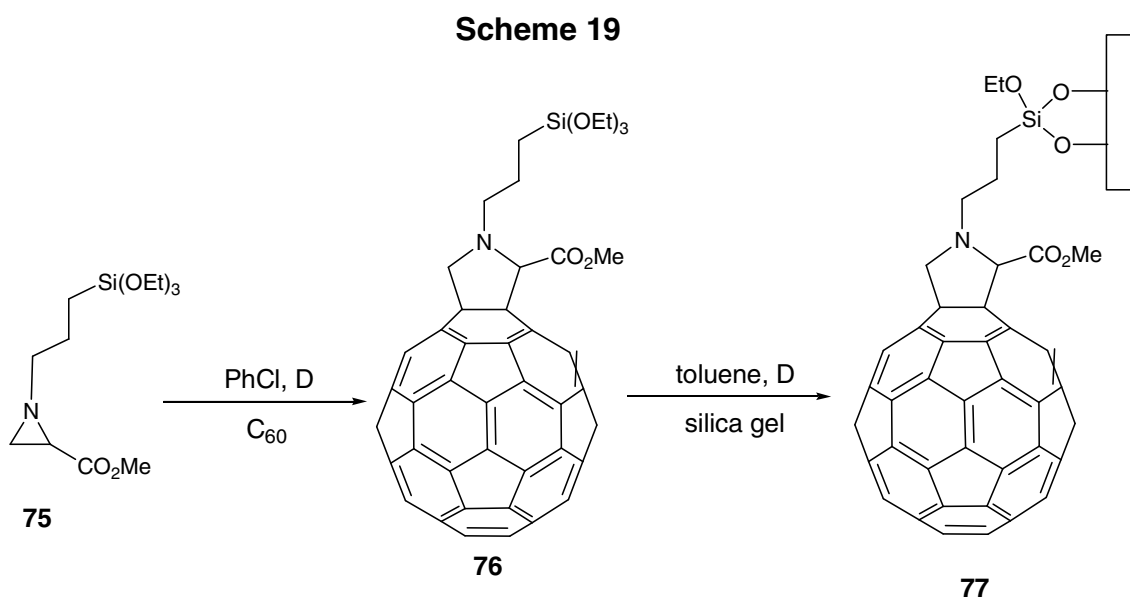
Molecular recognition of small peptides was also achieved using this silica gel Fpr matrix. A number of small helical-shaped peptides containing hydrophobic cavities of various sizes were successfully separated in aqueous media using the Fpr solid matrix. Helical proteins that comprise a hydrophobic cleft complementary to the diameter of the grafted [60]fullerene were selectively retained on the stationary phase.

CONCLUDING REMARKS

In conclusion, a number of novel reactions have been developed to prepare amino acid [60]fullerene derivatives and their corresponding peptides. Further developments in this area will see increasing application of these compounds in a variety of areas including materials and biological sciences.

Notes Added in Proof

Recently published (Da Ros, T., Prato, M. *Chem. Commun.*, **1999**, 663-669) was a concise review describing the medicinal chemistry of some fullerenes. It included a short section on fulleryl amino acids and peptides.

REFERENCES

1. Hirsch, A. "The Chemistry of Fullerenes"; Thieme: Stuttgart, 1994.

2. Diederich, F.; Isaacs, L.; Philp, D., Chem. Soc. Rev. 1994, 23, 243-255.

3. Hirsch, A. Synthesis, 1995, 895-913.

4. Friedman, S.H.; DeCamp, D.L.; Sijbesma, R.P.; Srdanov, G.; Wudl, F.; Kenyon, G.L., J. Am. Chem. Soc. 1993, 115, 6506-6509.

5. Sijbesma, R.P.; Srdanov, G.; Wudl, F.; Castoro, J.A.; Wilkins, C.; Friedman, S.H.; DeCamp, D.L.; Kenyon, G.L., J. Am. Chem. Soc. 1993, 115, 6510-6512.

6. Schinazi, R.F.; Sijbesma, R.P.; Srdanov, G.; Hill, C.L.; Wudl, F., Antimicob. Agents Chemother. 1993, 37, 1707-1710.

7. Prato, M.; Bianco, A.; Maggini, M.; Scorrano, G.; Toniolo, C.; Wudl, F., J. Org. Chem. 1993, 58, 5578-5580.

8. Toniolo, C.; Bianco, A.; Maggini, M.; Scorrano, G.; Prato, M.; Marastoni, M.; Tomatis, R.; Spisani, S.; Palu, G.; Blair, E.D., J. Med. Chem. 1994, 37, 4558-4562.

9. Da Ros, T.; Prato, M.; Novello, F.; Maggini, M.; Banfi, E., J. Org. Chem. 1996, 61, 9070-9072.

10. Schuster, D.I.; Wilson, S.R.; Schinazi, R.F., Bioorg. Med. Chem. Lett. 1996, 6, 1253-1256.

11. Friedman, S.H.; Ganapathi, P.S.; Rubin, Y.; Kenyon, G.L., J. Med. Chem. 1998, 41, 2424-2429.

12. Rajagopalan,P.; Wudl, F.; Schinazi, R., F.; Boudinot, F.D., Antimicob. Agents Chemother., 1996, 40, 2262-2264.

13. Nakamura, E.; Tokuyama, H.; Yamago, S.; Shiraki, T.; Sugiura, Y., Bull. Chem. Soc. Jpn. 1996, 69, 2143-2151.

14. Tokuyama, H.; Yamago, S.; Nakamura, E., J. Am. Chem. Soc. 1993, 115, 7918-7919.

15. Bouterine, A., S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Hélène, C., Angew. Chem. Int. ed. Eng. 1994, 33, 2462-2465.

16. An, Y-Z.; Chen, C-H., B.; Anderson, J.L.; Sigman, D.S.; Foote, C.S.; Rubin, Y., Tetrahedron 1996, 52, 5179-5189.

17. Irie, K.; Nakamura, Y.; Ohigashi, H.; Tokuyama, H.; Yamago, S.; Nakamura, E., Biosci. Biotech. Biochem. 1996, 60, 1359-1361.

18. Satoh, M.; Matsuo, K.; Takanashi, Y.; Takayanagi, I., Gen. Pharmac. 1995, 26, 1533-1538.

19. Satoh, M.; Matsuo, K.; Kiriya, H.; Mashino, T.; Hirobe, M.; Takayanagi, I., Gen. Pharmac. 1997, 29, 345-351.

20. Satoh, M.; Matsuo, K.; Kiriya, H.; Mashino, T.; Nagano, T.; Hirobe, M.; Takayanagi, I. , Eur. J. Parmac. 1997, 327, 175-181.

21. Murukami, H.; Watanabe, Y.; Nakashima, N., J. Am. Chem. Soc., 118, 4484-4485.

22. Vasalla, A.; Uhlmann, P.; Waldraff, C.A.A.; Diederich, F.; Thilgen, C., Angew. Chem. Int. ed. Eng. 1992, 31, 1388-1390.

23. Bouterine, A., S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Hélène, C., Angew. Chem. Int. ed. Eng. 1994, 33, 2462-2465.

24. An, Y-Z.; Chen, C-H., B.; Anderson, J.L.; Sigman, D.S.; Foote, C.S.; Rubin, Y., Tetrahedron 1996, 52, 5179-5189.

25. Prato, M.; Bianco, A.; Maggini, M.; Scorrano, G.; Toniolo, C.; Wudl, F., J. Org. Chem. 1993, 58, 5578-5580.

26. Skiebe, A.; Hirsch, A.,J. Chem. Commun. 1994, 335-336.

27. Toniolo, C.; Bianco, A.; Maggini, M.; Scorrano, G.; Prato, M.; Marastoni, M.; Tomatis, R.; Spisani, S.; Palu, G.; Blair, E.D., J. Med. Chem. 1994, 37, 4558-4562.

28. Bianco, A.; Maggini, M.; Scorrano, G.; Toniolo, C.; Marconi, G.; Villani, C.; Prato, M., J. Am. Chem. Soc. 1996, 118, 4072-4080.

29. Kurz, A.; Halliwell, C.M.; Davis, J.J.; Hill, H.A.O.; Canters, G.W., Chem. Commun. 1998, 433-434.

30. Bianco, A.; Bertolini, T.; Crisma, M.; Valle, G.; Toniolo, C.; Maggini, M.; Scorrano, G.; Prato, M., J. Peptide Res. 1997, 50, 159-170.

31. Prato, M.; Bianco, A.; Maggini, M.; Scorrano, G.; Toniolo, C.; Wudl, F., J. Org. Chem. 1993, 58, 5578-5580.

32. Isaacs, L.; Diederich, F., Helvetica Chimica Acta 1993, 76, 2454-2464.

33. Quihia, A.; Rene, L.; Guilhem, J.; Pascaed, C.; Badet, B., J. Org. Chem. 1993, 58, 1641-1649.

34. An, Y-Z.; Anderson, J.L.; Rubin, Y., J. Org. Chem. 1993, 58, 4799-4801.

35. Prato, M.; Suzuki, T.; Foroudian, H.; Li, Q.; Khemani, K.; Wudl, F.; Leonetti, J.; Little, R.D.; White, T.; Rickborn, B.; Yamago, S.; Nakamura, E., J. Am. Chem. Soc. 1993, 115, 1594-1598.

36. Yamago, S.; Tokuyama, H.; Nakamura, E.; Prato, M.; Wudl, F., J. Org. Chem. 1993, 58, 4796-4801.

37. Prato, M.; Li, Q.; Wudl, F.; Lucchini, V., J. Am. Chem. Soc. 1993, 115, 1148-1150.

38. Yan, M.; Cai, S-X.; Keana, J.F.W., J. Org. Chem. 1994, 59, 5951-5954.

39. Maggini, M.; Scorrano, G.; Bianco, A.; Toniolo, C.; Sijbesma, R., P.; Wudl, F.; Prato, M., Chem. Commun. 1994, 305-306.

40. Maggini, M.; Scorrano, G.; Prato, M., J. Am. Chem. Soc. 1993, 115, 9798-9799.

41. Prato, M.; Maggini, M.; Scorrano, G., Synth. Metals 1996, 77, 89-91.

42. Bianco, A.; Maggini, M.; Scorrano, G.; Toniolo, C.; Marconi, G.; Villani, C.; Prato, M. J., Am. Chem. Soc. 1996, 118, 4072-4080.

43. Novello, F.; Prato, M.; DaRos, T.; DeAmici, M.; Bianco, A.; Toniolo, C.; Maggini, M., Chem. Commun. 1996, 903-904.

44. Shu, L-H.; Wang, G-W.; Wu, S-H.; Wu, H-M.; Lao, X-F., Tetrahedron Lett. 1995, 36, 3871-3874.

45. Ohno, M.; Kojima, S.; Shirakawa, Y.; Eguchi, S., Heterocycles 1997, 46, 49-52.

46. Wu, S-H.; Sun, W-Q.; Zhang, D-W.; Shu, L-H.; Wu, H-M.; Xu, J-F.; Lao, X-F., J. Chem. Soc. Perkin Trans. 1, 1998, 1733-1738.

47. De la Cruz, P.; de la Hoz.; A.; font, L.M.; Langa, F.; Perez-Rodriguez, M.C., Tetrahedron Lett. 1998, 39, 6053-6056.

48. Wudl, F.; Hirsch, A.; Khemani, K.C.; Suzuki, T.; Allemand, P-M.; Koch, A.; Eckert, H.; Srdanov, G.; Webb, H. In Fullerenes: Synthesis, Properties and Chemistry of Large carbon Clusters; Hammons, G.S.; Kuck, V.J., Eds.: American Chemical Society Symposium Series 481; 1992; p61.

49. Hirsch, A.; Li, Q.; Wudl, F. Angew. Chem. Int. ed. Eng. 1991, 30, 1309.

50. Shedari, R.; Govindaraj, A.; Nagarajan, R.; Pradeep, T.; Rao, C.N.R., Tetrahedron Lett. 1992, 23, 2069-2071.

51. Skiebe, A.; Hirsch, A.; Klose, H.; Gotschy, B., Chem. Phys. Lett. 1994, 220, 138-140.

52. Romanova, V.S.; Tsyryapkin, V.A.; Lyakhovetsky, Y.I.; Parnes, Z.N.; Volpin, M.E., Russ. Chem. Bull. 1994, 43, 1090-1091.

53. Kotelnikova, R.A.; Kotelnikov, A.I.; Bogdanov, G.N.; Romanova, V.S.; Kuleshova, E.F.; Parnes, Z.N.; Volpin, M.E., FEBS Lett. 1996, 389, 111-114.

54. Belavsteva, E.M.; Kichenko, E.V.; Romanova, V.S.; Parnes, Z.N.; Volpin, M.E., Russ. Chem. Bull. 1996, 45, 831-832.

55. Timofeeva, G.I.; Romanova, V.S.; Lopanova, L.A., Russ. Chem. Bull. 1996, 45, 834-835.

56. Timofeeva, G.I.; Kuleshova, E.F.; Romanova, V.S., Russ. Chem. Bull. 1997, 46, 472-473.

57. Volpin, M.E.; Parnes, Z.N.; Romanova, V.S., Russ. Chem. Bull. 1998, 47, 1021-1022.

58. Zhou, D.J.; Gan, L.B.; Xu, L.B.; Luo, C.P.; Huang, C.H. Fullerene Sci. Technol. 1995, 3, 127-132.

59. Gan, L.B; Zhou, D.J.; Luo, C.P.; Huang, C.P.; Pan, J.Q.; Lu, M.J., Wu, Y., Chinese Chem. Lett. 1995, 6, 199-206.

60. Bingel, C. Chem. Ber. 1993, 126, 1957-1961.

61. Camps, X.; Hirsch, A., J. Chem. Soc. Perkin. Trans. 1, 1997, 1595-1596.

62. Moody, C.J. and Whitham, G.H. in 'Reactive Intermediates" 1992 Oxford Science Publications.

63. Burley, G.A.; Keller; P.A.; Pyne S.G.; Ball, G.E. Chem. Commun., 1998, 2539-2540.

64. Tarzia, G.; Balsammi, C.; Spadoni, G.; Duranti, E., Synthesis, 1988, 514-517.

65. Zhou, D.J.; Tan, H.; Luo, C.P.; Gan, L.B.; Huang, C.H.; Pan, J.Q.; Lu, M.J., Wu, Y., Tetrahedron Lett. 36, 9169-9170.

66. Gan, L.B.; Zhou, D.J.; Luo, C.P.; Tan, H.; Huang, C.H.; Lu, M.J.; Pan, J.Q.; Wu, Y., J. Org. Chem. 1996, 61, 1954-1961.

67. Gan, L.B.; Jiang, J.F.; Zhang, W.; Su, Y.; Huang, C.H.; Pan, J.Q.; Lu, M.J., Wu, Y., J. Org. Chem. 1998, 63, 4240-4247.

68. Bianco, A.; Bertolini, T.; Crisma, M.; Valle, G.; Toniolo, C.; Maggini, M.; Scorrano, G.; Prato, M., J. Peptide Res. 1997, 50, 159-170.

69. Toniolo, C. and Benedetti, E., Macromolecules, 1991, 24, 4004-4009.

70. Aubry, A.; Protas, J.; Boussard, G.; Marraud, M., Acta Crystallogr., Sect. B 1997, 33, 2399-2406.

71. Boussard, G.and Marraud, M., J. Am. Chem. Soc. 1985, 107, 1825-1218.

72. Boussard, G., Marraud, M.; Aubry, A., Biopolymers 1979, 18, 1297-1331.

73. Aubry, A.; Cung, M.T., Marraud, M., J. Am. Chem. Soc. 1985, 107, 7640-7647.

74. Kurz, A.; Halliwell, C.M.; Davis, J.J.; Hill, H.A.; Canters, G.W. Chem. Commun. 1998, 433-434.

75. Ohsawa, Y.and Saji, T., Chem. Commun. 1992, 781-782.

76. Tokutama, H.; Yamago, S.; Nakamura, E., J. Am. Chem. Soc. 1993, 115, 7918-7919.

77. Irie, K.; Nakamura, Y.; Ohigashi, H.; Tokuyama, H.; Yamago, S.; Nakamua, E., Biosci. Biotech. Biochem. 1996, 60, 1359-1361.

78. An, Y-Z.; Chen, C-H.; Anderson, J.L.; Sigman, D.S.; Foote, C.S.; Rubin, Y., Tetrahedron, 1996, 52, 5179-5189.

79. Bianco, A.; Gasparrini, F.; Maggini, M.; Misiti, D.; Polese, A.; Prato, M.; Scorrano, G.; Toniolo, C.; Villani, C., J. Am. Chem. Soc. 1997, 119, 7550-7554.

80. Prato, M.and Maggini, M., Acc. Chem. Res. 1998, 31, 519-526.