Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 7137–7140
Tetrahedron Letters

Synthesis of novel fullerene amino acids and their multifullerene peptides

Louis A. Watanabe,[a] Mohammed P. I. Bhuiyan,[b] Binoy Jose,[b] Tamaki Kato[b] and Norikazu Nishino[b,*]

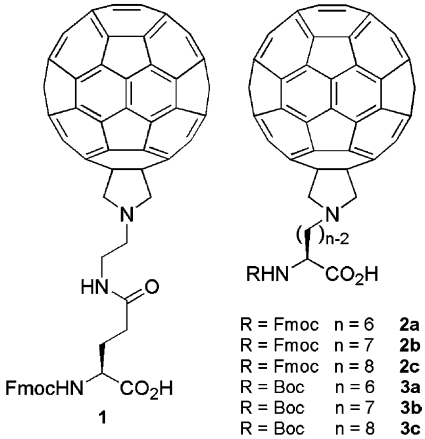
[a]*Faculty of Engineering, Kyushu Institute of Technology, Kitakyushu 808-8550, Japan*
[b]*Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan*

Received 14 May 2004; revised 7 July 2004; accepted 21 July 2004
Available online 13 August 2004

Abstract—[60]-Fullerene functionalized amino acids with 4–6 methylene spacers from α-carbon to the nitrogen atom of fulleropyrolidine and corresponding multifullerene peptides have been synthesized.

Considerable effort in fullerene chemistry has been directed to establish this novel form of carbon as a standard building block in organic synthesis.[1,2] Fullerene-based molecules have a wide variety of interesting characteristics including nonlinear optical properties, superconductivity and biological properties.[3] Biological properties of [60]-fullerene derivatives comprise of neuroprotective, enzymatic, antiapoptotic, antibacterial, DNA photocleaving, nitric oxide synthase inhibiting and chemotactic activities.[4] Fullerene amino acids and peptides are interesting targets due to their importance in biological applications and also in physicochemical studies.[5] Fullerene peptides were recently reported as antimicrobial agents[6] and also as a molecular ruler for electron transfer system.[7]

Prato and co-workers reported a C_{60}-functionalized amino acid (**1**) derived from glutamic acid and incorporated it into a peptide.[6] This is an important development to introduce fullerene moiety to bioactive peptides. The compound **1** has 6-atomed linker from the α-carbon of glutamic acid to the nitrogen atom of fulleropyrrolidine. The connecting amide could play important roles in the solubilities or in the interaction with biomolecules. On the other hand, we conveniently prepared optically active α-amino-ω-bromoalkanoic acid (Abn) as useful artificial amino acids.[8] The side chain alkyl bromide can be easily converted to various functional groups such as amino group of lysine homologues. By attaching a carboxymethyl group to the amino group, they may be reacted with C_{60} according to the literature.[6] Therefore, we attempted to prepare the novel fullerene amino acid derivatives useful in peptide synthesis (**2a–c** and **3a–c**).



Since these fullerene amino acids were expected to increase the solubility in organic solvents, the synthetic method for multifullerene peptides were also examined using **2c** or **3c** with same spacer length as **1**. To our knowledge, there is no report on the synthesis of a dipeptide or tripeptide with two or more fullerene moieties. Here, we report the synthesis of C_{60}-functionalized amino acids with different methylene spacers

Keywords: Fullerenes; Amino acids; Fullerene peptide.
* Corresponding author. Tel./fax: +81-93-695-6061; e-mail: nishino@life.kyutech.ac.jp

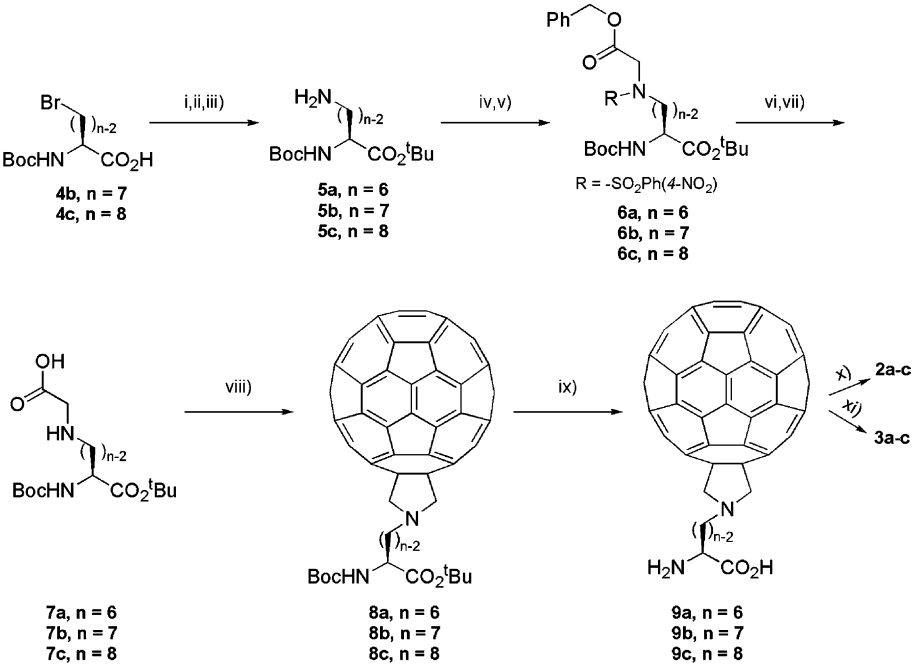
0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.07.088

(Afn, 2-amino-*n*-fulleropyrrolidinoalkanoic acid, where $n = 6–8$) and the synthesis of multifullerene peptides containing Af8s (for instance, Boc-Af8-Af8-Af8-Ala-OBzl).

The synthesis of C_{60}-functionalized amino acids was started from recently reported Boc-α-amino-ω-bromoalkanoic acids **4b** and **4c** (Boc-Abn-OH),[8] whose carboxyl group was protected with *t*-butyl group[9] and then the alkyl bromide of the side chain was transformed to the amine by the treatment with potassium phthalimide in the presence of KI and subsequent reaction with hydrazine to yield the corresponding amines **5b** and **5c**. The **5a** was obtained by the protection of the carboxyl group of Boc-Lys(Z)-OH with *t*-butyl group[9] followed by hydrogenation. The amino group of **5a–c** was then protected with 4-nitrobenzenesulfonyl (NBS) group and reacted with benzyl bromoacetate to yield **6a–c**. The NBS protection of the amine allowed 1:1 reaction with benzyl bromoacetate in quantitative yield. Reactions without protecting with NBS group cause double or triple incorporation of benzyl bromoacetate to the amino group of **5a–c** resulting in poor yields. Side chain deprotection using piperidine/*n*-BuSH[10] and subsequent benzyl ester hydrogenation yielded **7a–c**. Amino acids **7a–c** were reacted with C_{60} and paraformaldehyde to yield fullerene functionalized amino acid **8a–c** in about 60% yield. Fullerene addition reaction to **7c** was carried out in six different ratios such as, 1:1, 1.5:1, 2:1, 3:1, 4:1 and 6:1. By careful examination of the ratio of amino acid **7c** and C_{60}, we found that the use of 3 equiv of C_{60} gives the optimized yield of the desired product (see Supplementary data for details). This yield is about 20% higher than that reported for the 1,3-dipolar cycloaddition of fullerene to azomethine ylide.[6] When the ratio of fullerene is lower, such as, 1:1 or 2:1, there is a possibility of multiaddition of the amino acid to fullerene resulting in lower yield of the desired product. With a ratio of 3:1, the possibility of single addition product is high and therefore we observed a high yield. However, no further enhancement in yield was observed with increase in fullerene ratio. The excess amount of C_{60} was conveniently removed from **8a–c** by silica gel column with toluene/ethyl acetate (95/5, v/v). Compounds **8a–c** were fully characterized by ^1H and ^{13}C NMR and high-resolution FAB MS.[11] Compounds **8a–c** were deprotected using trifluoroacetic acid to afford the free fullerene amino acids (Afn) **9a–c**. The amino group of Afn was then protected to Fmoc-Afn-OH **2a–c**. Boc protection was also carried out to give Boc-Afn-OH **3a–c** (Scheme 1).

One of the major problems associated with fullerene compounds is the poor solubility in many common solvents. Therefore, we checked the solubility of the N-protected Afn in different solvents. These Afn derivatives are fairly soluble in dichloromethane, chloroform, toluene, chlorobenzene and DMF. For example, 5.5 mg of Boc-Af8-OH is soluble in 1 mL of DMF. This solubility allows the N-protected fullerene amino acids to be used in most of the coupling conditions. Even under lower solubility conditions, we observed higher yields for coupling and deprotection reactions of Afn containing peptides.

As for the peptide synthesis, initially we used solid phase peptide synthesis with Wang-PEG-resin preloaded with glycine and Fmoc-Af8-OH was coupled. However, the



Scheme 1. Reagents, conditions and yields: (i) Boc_2O, *t*-BuOH, DMAP, rt, 1 h, 80–84%; (ii) potassium phthalimide, KI, DMF, rt, 1 h, 80–85%; (iii) $NH_2–NH_2·H_2O$, EtOH, rt, 1 h, 95–100%; (iv) 4-nitrobenzenesulfonyl chloride, Et_3N, $CHCl_3$, 0 °C, 30 min, 90–95%; (v) benzyl bromoacetate, K_2CO_3, KI, DMF, 0 °C, 24 h, 90–95%; (vi) piperidine, DMF, *n*-BuSH, rt, 6 h, 85–90%; (vii) Pd–C, H_2, 6 h, 95–100%; (viii) C_{60}, $(CH_2O)_n$, toluene, reflux, 1.5 h, 60%; (ix) TFA, CH_2Cl_2, 3 h, 100%; (x) Fmoc-OSu, Na_2CO_3, H_2O/dioxane (1/1), 24 h, 95–100%; (xi) Boc_2O, H_2O/dioxane (1/1), Et_3N, 0 °C, 24 h, 100%.

tendency of the fullerene moiety to embed onto the resin made it difficult to recover the product in reasonable yield. A similar problem of solid phase peptide synthesis with fullerene amino acids was reported by Prato and co-workers.[6] Due to this drawback with solid phase synthesis of fullerene peptides, we proposed to use solution phase synthesis.

The Fmoc-Af8-OH was coupled with H-Gly-OBzl using HATU in DMF to yield Fmoc-Af8-Gly-OBzl (**10**) in almost quantitative yield. The product was characterized by high-resolution FAB MS. As we know that the common Fmoc deprotection reagents such as piperidine and morpholine result in a 1,4-addition to fullerene in the presence of oxygen, leading to the generation of bis- and tetra-adducts,[12] the deprotection of Fmoc group was carried out using 5% DBU in DMF. However, formation of bis-adduct of fullerene with DBU was observed during the deprotection reaction. Therefore, we changed the strategy to the Boc method using Boc-Af8-OH.

Boc-Af8-OH was coupled with H-Ala-OBzl using HBTU/HOBt in DMF to yield Boc-Af8-Ala-OBzl (**11**) quantitatively. The dipeptide **11** was precipitated from the reaction solvent as the coupling reaction proceeded. The product was simply filtered and washed with DMF and small volume of CH_2Cl_2. Since the product was pure enough for further step, the analytical amount was passed through a silica gel column with toluene/ethyl acetate (95/5, v/v). The Boc group of **11** was successfully removed by treatment with trifluoroacetic acid. The dipeptide benzyl ester was again coupled with Boc-Af8-OH to give Boc-Af8-Af8-Ala-OBzl (**12**) as described above. The tripeptide **12** was also precipitated from the reaction solvent in 97% yield as filtration. Repetition of the deprotection and coupling steps gave Boc-Af8-Af8-Af8-Ala-OBzl (**13**) as a precipitate from DMF in 95% yield. A part of the product was applied to silica gel column with toluene/ethyl acetate (95/5, v/v). The protected peptides were characterized by MALDI TOF MS and ^{1}H NMR using deuterized *o*-dichlorobenzene, data being given in Ref. 11 and Supplementary data. The tetrapeptide **13** resisted the deprotection with trifluoroacetic acid resulting in failure in further elongation. However, we will find out appropriate conditions in the future study.

In order to collect some information about the conformation of **13**, we tried to measure CD spectra. However, we could not measure the CD spectrum of **13** due to the poor solubility in THF. Though the induced CD was observed for chiral fullerene-bound polymer in very low temperature condition in the literature,[13] the rather short peptide **13** with fullerenes too far from the chiral α-methine exhibited no induced CD in *o*-dichlorobenzene. Therefore, to obtain the structural characteristics of the multifullerene peptide, we carried out energy minimization studies on the tetrapeptide **13** using a Molecular Operating Environment (MOE) software of Chemical Computing Group Inc. to see a conformational outline. The calculation was carried out using the force field MMFF94 without considering the solvent effect. One of the possible conformations was obtained as shown in Figure 1 ($\phi_1 = -75.1°$, $\psi_1 = 68.0°$; $\phi_2 = -172°$, $\psi_2 = -83.6°$; $\phi_3 = -68.5°$, $\psi_3 = 179°$; $\phi_4 = -29.8°$, $\psi_4 = -177°$). The main chain conformation is not similar to α-helix or β-strand. The fullerene moieties of the peptide may disrupt the conformation of **13** in the energy-minimized structure.

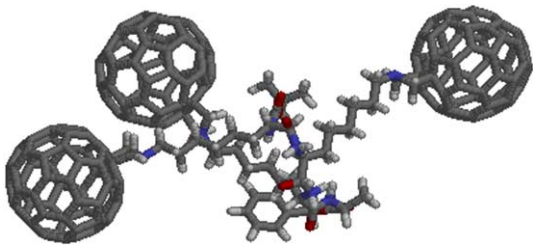


Figure 1. Conformational outline of **13**.

In summary, we described the side chain modification of α-amino-ω-bromoalkanoic acid to lysine homologues and to the fullerene amino acids. Further, we successfully synthesized multifullerene peptides using Boc protected fullerene amino acids by solution phase method. The attraction of this synthetic approach is the generality of the method for the preparation of fullerene amino acids with different spacer lengths between the α-carbon and the nitrogen atom of the fulleropyrolidine. These novel amino acids have potential as building blocks for the synthesis of peptides with interesting biological and physicochemical properties.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.07.088. This contains ^{1}H and ^{13}C NMR spectra of selected compounds and optimization data for fullerene to amino acid coupling reaction.

References and notes

1. (a) Diederich, F.; Thilgen, C. *Science* **1996**, *271*, 317–323; (b) Diederich, F.; Kessinger, R. *Acc. Chem. Res.* **1999**, *32*, 537–545.
2. (a) Hirsch, A. *The Chemistry of the Fullerenes*; Thieme: New York, 1994; (b) Hirsch, A. *Fullerenes Related Struct. Top. Curr. Chem.* **1999**, *199*, 1–65.
3. (a) Guldi, D. M.; Prato, M. *Acc. Chem. Res.* **2000**, *33*, 695–703; (b) Martin, N.; Sànchez, L.; Illescas, B.; Pèrez, I. *Chem. Rev.* **1998**, *98*, 2527–2547; (c) Guldi, D. M. *Chem. Commun.*, **2000**, 321–327; (d) Prato, M. *J. Mater. Chem.* **1997**, *7*, 1097–1109.
4. (a) Friedman, S. H.; De Camp, D. L.; Sijbesma, P. R.; Srdanov, G.; Wudl, F.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6506–6509; (b) McGovern, S. L.; Caselli, E.; Grigorieff, N.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 1712–1722; (c) Cusan, C.; Da Ros, T.; Spalluto, G.; Foley, S.; Janot, J. M.; Seta, P.; Larroque, C.; Tomasini, M. C.; Antonelli, T.; Feraro, L.; Prato, M. *Eur. J. Org. Chem.* **2002**, *17*, 2928–2932; (d) Zhu, Z.; Schuster, D. I.; Tuckerman, M. E. *Biochemistry* **2003**, *42*, 1326–1333;

(e) Nakamura, E.; Isobe, H. *Acc. Chem. Res.* **2003**, *36*, 807–815; (f) Bosi, S.; Da Ros, T.; Spalluto, G.; Prato, M. *Eur. J. Med. Chem.* **2003**, *38*, 913–923.

5. (a) Sofou, P.; Elemes, Y.; Panou, P. E.; Stavrakoudis, A.; Tsikaris, V.; Sakarellos, C.; Sakarellos, D. M.; Maggini, M.; Formaggio, F.; Toniolo, C. *Tetrahedron* **2004**, *60*, 2823–2828; (b) Bianco, A.; Da Ros, T.; Prato, M.; Toniolo, C. *J. Pept. Sci.* **2001**, *7*, 208–219.
6. (a) Pellarini, F.; Pantarotto, D.; Da Ros, T.; Giangaspero, A.; Tossi, A.; Prato, M. *Org. Lett.* **2001**, *3*, 1845–1848; (b) Giangaspero, A.; Zelensky, I.; Briand, J.-P.; Prato, M. *J. Am. Chem. Soc.* **2002**, *124*, 12543–12549.
7. Polese, A.; Mondini, S.; Bianco, A.; Toniolo, C.; Scorano, G.; Guldi, D. M.; Maggini, M. *J. Am. Chem. Soc.* **1999**, *121*, 3446–3452.
8. Watanabe, L. A.; Jose, B.; Kato, T.; Nishino, N.; Yoshida, M. *Tetrahedron Lett.* **2004**, *45*, 491–494.
9. Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y. *Synthesis* **1994**, 1063–1066.
10. Samukov, V. V.; Pozdnyakov, P. P.; Sabirov, A. N. *Abstracts of Posters, Novosibirisk Institute of Organic Chemistry*, 1998; p 155.
11. All the new compounds were characterized by NMR and mass spectral data. Selected spectroscopic data: **8a**: ^{1}H NMR (500 MHz, CD_2Cl_2): δ 5.05 (d, 1H), 4.41 (s, 4H), 4.22 (m, 1H), 3.09 (m, 2H), 1.95 (m, 2H), 1.82 (m, 2H), 1.69 (m, 2H), 1.49 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (CD_2Cl_2): δ 172.3, 155.8, 147.6, 146.6, 146.4, 146.1, 145.8, 145.7, 145.0, 143.5, 143.0, 142.7, 142.5, 142.3, 142.2, 140.5, 136.6, 82.0, 79.8, 71.2, 68.4, 55.1, 33.3, 29.0, 28.5, 28.2, 23.8; high-resolution FAB MS $(M+1)^+$ 1049.2374, calcd for $C_{77}H_{33}N_2O_4$ 1049.2440; **8b**: ^{1}H NMR (500 MHz, CD_2Cl_2): δ 5.06 (d, 1H), 4.4 (s, 4H), 4.18 (m, 1H), 3.07 (m, 2H), 1.97–1.85 (m, 4H), 1.76–1.61 (m, 2H), 1.58–1.48 (m, 2H), 1.45 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (CD_2Cl_2): δ 172.4, 155.8, 147.7, 146.6, 146.5, 146.4, 146.1, 145.7, 145.6, 144.9, 143.5, 143.0, 142.7, 142.5, 142.3, 140.5, 136.6, 81.9, 79.8, 71.2, 68.4, 55.1, 33.3, 29.1, 28.5, 28.2, 27.7, 25.6; high-resolution FAB MS $(M+1)^+$ 1063.2517, calcd for $C_{78}H_{35}N_2O_4$ 1063.2597; **8c**: ^{1}H NMR (500 MHz, CD_2Cl_2): δ 5.04 (d, 1H), 4.40 (s, 4H), 4.14 (m, 1H), 3.06 (m, 2H), 2.00–1.90 (m, 2H), 1.85–1.80 (m, 2H), 1.71–1.62 (m, 2H), 1.58–1.49 (m, 4H), 1.47 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (CD_2Cl_2): δ 172.4, 155.8, 147.7, 146.7, 146.6, 146.4, 146.1, 145.7, 145.6, 144.9, 143.5, 142.9, 142.7, 142.5, 142.3, 140.5, 136.6, 81.8, 79.8, 71.2, 68.4, 55.2, 33.3, 29.7, 29.2, 28.5, 28.2, 27.9, 25.7; high-resolution FAB MS $(M+1)^+$ 1077.2719, calcd for $C_{79}H_{37}N_2O_4$ 1077.2753; **9a**: high-resolution FAB MS $(M+1)^+$ 893.1256, calcd for $C_{68}H_{17}N_2O_2$ 893.1290; **9b**: high-resolution FAB MS $(M+1)^+$ 907.1420, calcd for $C_{69}H_{19}N_2O_2$ 907.1447; **9c**: high-resolution FAB MS $(M+1)^+$ 921.1592, calcd for $C_{70}H_{21}N_2O_2$ 921.1603; **3a**: high-resolution FAB MS $(M+1)^+$ 993.1804, calcd for $C_{68}H_{17}N_2O_2$ 993.1814; **3b**: high-resolution FAB MS $(M+1)^+$ 1007.1962, calcd for $C_{74}H_{27}N_2O_4$ 1007.1971; **3c**: high-resolution FAB MS $(M+1)^+$ 1021.2093, calcd for $C_{75}H_{29}N_2O_4$ 1021.2127; **12**: ^{1}H NMR (500 MHz, $C_6D_4Cl_2$): δ 5.27 (d, 1H), 5.19–5.09 (m, 2H), 4.71–4.68 (m, 2H), 4.29–4.24 (m, 9H), 2.98–2.95 (m, 4H), 1.98–1.45 (m, 32H); MALDI TOF MS $(M+1)^+$ 2084.92, calcd for $C_{155}H_{57}N_5O_6$ 2085.14; **13**: ^{1}H NMR (500 MHz, $C_6D_4Cl_2$): δ 5.21–5.11 (m, 3H), 4.84–4.76 (m, 2H), 4.56–4.45 (m, 2H), 4.30–4.23 (m, 12H), 3.02–2.93 (m, 6H), 1.98–1.45 (m, 42H); MALDI TOF MS $(M+1)^+$ 2989.81, calcd for $C_{225}H_{76}N_7O_7$ 2989.05.
12. (a) Schick, G.; Kampe, K. D.; Hirsch, A. *J. Chem. Soc., Chem. Commun.* **1995**, 2023–2024; (b) Hirsch, A.; Li, Q.; Wudl, F. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1309–1310.
13. Nishimura, T.; Takatani, K.; Sakurai, S.; Maeda, K.; Yashima, E. *Angew. Chem., Int. Ed.* **2002**, *41*, 3602–3604.