ORGANIC LETTERS

2000
Vol. 2, No. 25
3955−3958

Design and Synthesis of Novel [60]Fullerene Derivatives as Potential HIV Aspartic Protease Inhibitors

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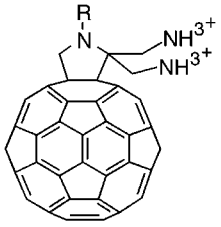
Received August 4, 2000

ABSTRACT



Two water-soluble fullerene derivatives have been computer-designed and synthesized. They may exhibit interesting anti-HIV activity owing to the presence of two ammonium groups strategically located on the spheroid surface.

The identification and characterization of novel inhibitors of HIV PR, a key enzyme of the human immunodeficiency virus 1, is a main goal in the development of new anti-AIDS drugs. HIV PR is an aspartic protease that cleaves viral polyproteins and is essential in the maturation of HIV particles.[1]

The active site of the HIV PR is an open-ended cylindrical hydrophobic cavity, with a diameter of about 10 Å for the central spherical extension. Inside the cavity, two amino acid residues, aspartate 25 and aspartate 125, catalyze the hydrolysis of the substrate. Inhibition of the aspartate activity may possibly lead to suppression of protein slicing and, as a consequence, to cessation of the entire replicative viral cycle.

In 1993, Wudl, Friedman, and co-workers proposed [60]fullerene as an HIV protease inhibitor.[2,3] On the basis of molecular modeling, they were the first to recognize that the [60]fullerene spheroid can be almost perfectly accommodated inside the active site. If the hydrophobic interactions are sufficiently strong, these compounds can serve as reversible inhibitors. In vitro studies, performed using a water-soluble fullerene derivative (Figure 1), confirmed that

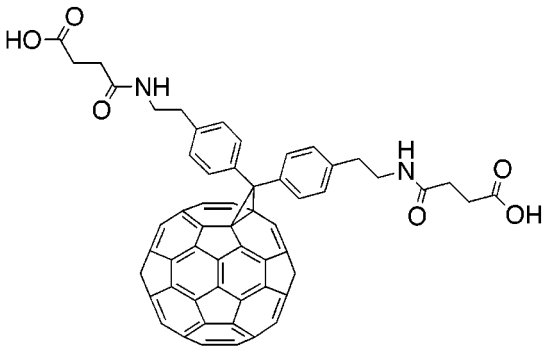


Figure 1. Molecular structure of the first water-soluble fullerene derivative employed for the inhibition of HIV PR.

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(1) Vacca, J. P. In *Retroviral Proteases*; Kuo, L. C., Shafer, J. A., Eds.; Academic Press: San Diego, 1994; Vol. 241, pp 311−334.
(2) Friedman, S. H.; DeCamp, D. L.; Sijbesma, R. P.; Srdanov, G.; Wudl, F.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6506−6509.

10.1021/ol000217y CCC: $19.00
Published on Web 11/17/2000

inhibition of acutely and chronically affected peripheral blood mononuclear cells (PBMC) indeed occurred with an EC_{50} of 7 μM.

The main mechanism of action of these derivatives is supposedly produced only by hydrophobic interactions. Since then, many groups have confirmed the validity of this approach, synthesizing different fullerene structures and obtaining analogous biological responses.[4,5]

To increase the anti-HIV PR activity of fullerenes, in their original contribution, Friedman et al. suggested that fullerene derivatization at specific positions with groups that may give electrostatic and/or hydrogen bond interactions with Asp 25 and Asp 125 should increase the binding constant by several orders of magnitude. The proposed molecule is the diamino compound **1**, whose PM3-minimized structure is reported in Figure 2 (nitrogen atoms are blue). As the same

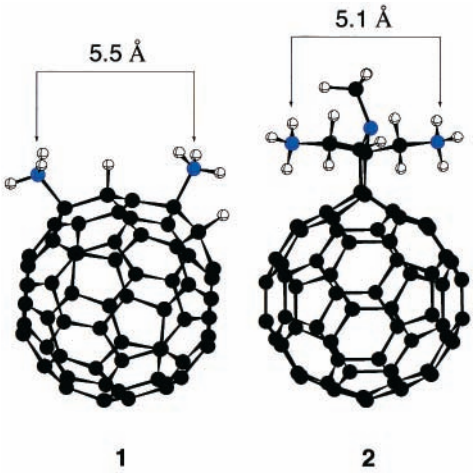


Figure 2. PM3-minimized structures of compounds **1** and **2**.

authors pointed out, the synthesis of compound **1** poses serious practical problems. In fact, even though the control of multiple additions to [60]fullerene has reached a high degree of sophistication,[6,7] the placement of two amino groups directly attached to the carbon cage in two well-defined spatial positions would still be an insurmountable task.

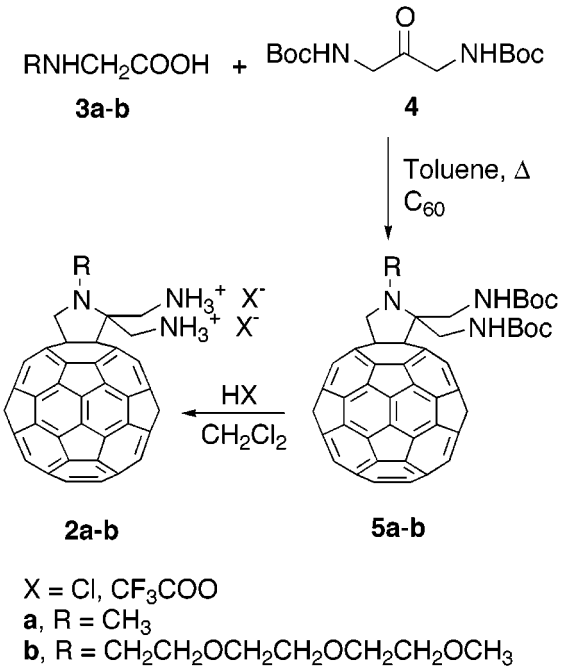
In this paper we present the synthesis of novel fullerene derivatives that possess ammonium groups strategically positioned on the fullerene spheroid that fulfill the requirement of potentially interacting with HIV PR aspartates but avoiding the direct regioselective bis-addition to the carbon cage. Figure 2 shows the PM3-minimized structure of the simplest compound of the series (**2a**), which confirms that the spatial arrangements of the two ammonium groups are very similar to those of compound **1**.

In fact, structure **2a** has a nitrogen−nitrogen distance between the two ammonium groups of 5.1 Å, whereas the same distance in compound **1** is 5.5 Å (Figure 2).

In addition, whereas the ammonium salts in **1** are rigidly locked on the surface of the fullerene spheroid, in **2a**,**b** the same groups have a certain degree of freedom, which should in principle allow them to efficiently interact with the two HIV PR aspartates through electrostatic and/or hydrogen bond interactions.

The general synthetic approach to compounds **2** is shown in Scheme 1.[8,9]

Scheme 1



1,3-Dipolar cycloadditions of azomethine ylides to [60]fullerene, starting from glycines **3a**,**b**[10] and bis-protected diamino ketone **4**,[11] led to reasonable yields (20−25%) of monoadducts **5a**,**b**, which were characterized by all standard spectroscopic means along with microanalysis.

It is interesting to note that in the ^{1}H NMR spectrum of **5a** the two methylene groups are identical, but each methylene has two magnetically nonequivalent protons, giving rise to an AB quartet (J = 14.5 Hz). This is probably due to hindered rotation of the two arms of the pyrrolidine ring, caused by the presence of the bulky *tert*-butyl groups.

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(7) Diederich, F.; Kessinger, R. *Acc. Chem. Res.* **1999**, *32*, 537−545.
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Treatment of **5a**,**b** with excess trifluoroacetic or hydrochloric acid gave rise to the corresponding bis-salts **2a**,**b**. Compounds **2a**,**b** were characterized by electrospray mass spectroscopy (ES-MS). The ES-MS spectrum of **2a**, taken in THF−MeOH, shows MH^+ at *m*/*z* 836 (relative intensity 100%) and MH_2^{2+} at 418.5 (relative intensity 5%). The ratio between these two peaks strongly depends on the medium used for the experiments. In neutral solutions, MH^+ peak (at 836) is preponderant, whereas the MH_2^{2+} peak (at 418.5) prevails in acidic solutions. This might imply that, in neutral physiological media, compounds **2** could exist as a singly positively charged species. On the other hand, the HIV PR ionization state of the two aspartates 25 and 125 is a critical issue in the design of protease inhibitors, but this important parameter is expected to vary depending on the nature of the inhibitor as well as the water content inside the cavity.[12−14]

In the solid, elemental analysis accounts for two trifluoroacetate groups in **2b**. Calcd for $C_{75}H_{17}O_7N_3F_6$: C, 75.3; H, 2.28; N, 3.51. Found: C, 75.6; H, 2.26; N, 3.57.

To ascertain the binding ability of these new derivatives to the cavity of HIV PR, modeling studies have been performed, using the Discover (Biosym/MSI) program with *cvff* force field. Figure 3 shows the accommodation of

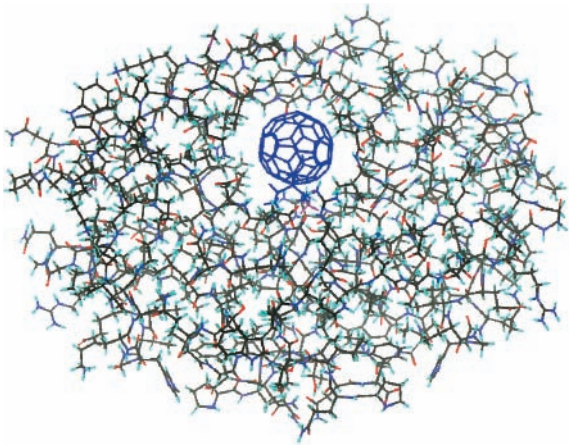

Figure 3. Computer-designed accommodation of potential inhibitor **2a** in the cavity of HIV PR.

compound **2a** inside the cavity of HIV PR. The complexation energies of HIV PR with derivatives **2a**,**b** were evaluated as differences between the energy of the optimized complexes (HIV PR)-**2a** or (HIV PR)-**2b** or (HIV PR)-C_{60} minus the energies of the free components (isolated enzyme plus isolated inhibitor).[15] Both mono- and diprotonated states in **2a**,**b**, i.e., (NH_3^+, NH_2) and (NH_3^+, NH_3^+) were modeled. The results, reported in Table 1, show that substrate **2b** has

Table 1. Stabilization Energies of HIV PR Inhibitor ($E_{complex}$) As Compared with HIVP-C_{60} (ΔE) in kcal/mol

	HIVP-C_{60}	HIVP-**2a** (2+)	HIVP-**2a** (1+)	HIVP-**2b** (2+)	HIVP-**2b** (1+)
$E_{complex}$	−102.5	−121.2	−121.5	−127.9	−134.6
ΔE	0.0	−18.7	−19.0	−25.4	−32.1

markedly improved binding characteristics as compared to that of unmodified fullerene C_{60}. The relative complexation energy is approximately −32 kcal/mol (in the case of monoprotonation) and about −25 kcal/mol if both the amine groups are protonated. Greater stabilization in the former case is due to better H-bond interactions between the neutral COOH aspartic group with neutral NH_2 rather than the positively charged NH_3^+ group. Increased stabilization of **2b** as compared to **2a** is probably due to stabilizing interactions of the oligoethylene oxide chain in **2b** with nearby moieties of HIV PR.

Figure 4 focuses on the active site of the complex, emphasizing the presence of hydrogen bonds between

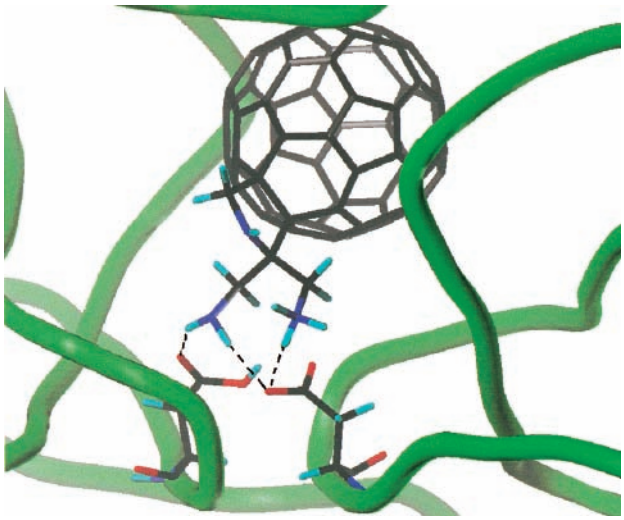

Figure 4. Closer view of the (HIV PR)-**2a** complex, showing the H-bond between NH_2 or NH_3^+ groups with Asp 25 and 125.

aspartatic/aspartate and amine/ammonium groups. Of course, also strong Coulombic attractions are at work here. The interatomic distance between each of the amine/ammonium nitrogens and the carboxylic/carboxylate oxygens corresponds to about 2.8 Å, offering stabilization interactions.

The solubility in aqueous solvents was checked by dissolving **2a**,**b** in DMSO and then diluting 1:9 with water. In this solvent mixture, suitable for most biological tests,

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(14) We thank Prof. F. Diederich (ETH Zurich) for drawing our attention to this issue.

(15) For details on modeling of HIV PR with various inhibitors, see: Nair A. C.; Miertus S.; Tossi A.; Romeo D. *Biochem. Biophys. Res. Commun.* **1998**, *242*, 545−551.

2a,**b** have a maximum concentration on the order of 10^{-5} M. The solubility did not change much with pH. A slight increase of solubility was observed in acidic solutions, but not as much as expected for a fully diprotonated species, probably due to salt effects. Biological tests are currently underway and will be reported in due course.

Acknowledgment. This work was supported in part by Regione Friuli-Venezia Giulia (Fondo 1998) and by MURST (Italy, cofin prot. n. 9803194198). We thank Mr. Paolo Braiuca for help in editing Figures 3 and 4. Dedicated to Prof. Fred Wudl, on the occasion of his 60th birthday and in recognition of his fundamental contributions to this research area.

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