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Novel fullerene derivatives as dual inhibitors of Hepatitis C virus NS5B polymerase and NS3/4A protease

Hiroki Kataoka [a], Tomoyuki Ohe [a,*], Kyoko Takahashi [a], Shigeo Nakamura [b], Tadahiko Mashino [a,*]

[a] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo, Japan
[b] Department of Chemistry, Nippon Medical School, 1-7-1 Kyonan-cho, Musashino-shi, Tokyo, Japan

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ABSTRACT

We evaluated the Hepatitis C virus (HCV) NS5B polymerase and HCV NS3/4A protease inhibition activities of a new set of proline-type fullerene derivatives. All of the compounds had the potential to inhibit both the enzymes, indicating that the fullerene derivatives may be dual inhibitors against NS5B and NS3/4A and could be novel lead compounds for the treatment of HCV infections.

Hepatitis C virus (HCV) was identified as non-A, and non-B hepatitis virus in 1989.[1] HCV belongs to the *Flaviviridae* family and has a 9.6 kb positive-sense single-strand RNA. HCV genome encodes a large precursor polyprotein, which is cleaved by viral or host proteases into three structural proteins and seven non-structural (NS) proteins. It is estimated that more than 150 million people are infected with HCV worldwide. HCV infection is one of the most serious problems in public health, leading to the development of chronic hepatitis, liver fibrosis, cirrhosis, and hepatocarcinoma. The traditional treatment for HCV infection was the combination therapy of interferon-α and ribavirin, but this treatment was discontinued due to serious side effects. Urgent work for the discovery of agents that prevent HCV NS protein for viral replication has been widely undertaken, and novel NS3/4A protease, NS5A protein and NS5B polymerase inhibitors have been recently developed and commercially used.

Fullerene (C_{60}), discovered by Kroto et al. in 1985,[2] was the third allotrope of the carbon atom after graphite and diamond. Fullerene has been attractive to many fields due to its unique structure and physical properties. However, insolubility of fullerenes in aqueous media is one of the major problems for medicinal applications. To overcome this issue, a number of fullerene derivatives with hydrophilic substituents have been synthesized. There have been reports that various water-soluble fullerene derivatives exhibit a range of biological activities, including antioxidant,[3] anticancer,[4] antibacterial,[4–6] and antiviral activities.[7–10] In our previous work, we screened the synthesized fullerene derivatives that included anionic, cationic and amino acid types for inhibitory activity of NS5B polymerase.[8,9] Among these derivatives, the proline-type fullerene derivative **1a** (Fig. 1) had strong NS5B inhibitory activity without potential cytotoxicity in the low micromolar range.[9] In the present work, to improve the inhibitory activities of NS5B, we introduced various substituents at the methine position of the derivative **1a**. Moreover, we also examined whether the fullerene derivatives inhibit NS3/4A protease, as these derivatives would be promising multi-target agents for the treatment of HCV infection if they exhibited inhibition activity toward both the enzymes.

The synthetic routes of derivatives **1a–e** are shown in Scheme 1. Di-*tert*-butyl L-tartrate (**2**) was synthesized from L-tartaric acid and *O*-*tert*-butyl-*N*,*N*'-diisopropylisourea,[11] which was readily prepared from the inexpensive reactants, *tert*-butyl alcohol and *N*,*N*'-diisopropylcarbodiimide. Oxidative carbon–carbon bond cleavage of 1,2-diol on the tartrate **2** by (diacetoxyiodo)benzene gave *tert*-butyl glyoxylate (**3**). The precursors **4a–e** of novel proline-type fullerene derivatives **1a–e** were synthesized via 1,3-dipolar cycloaddition of azomethine ylides, generated from various amino acid esters and the prepared glyoxylate **3**, onto C_{60} in a similar manner to previous work by Prato et al.[12] Diastereomers formed

Abbreviations: HCV, Hepatitis C virus; NS, non-structural.

* Corresponding authors. Tel./fax: +81 3 5400 2691 (T.O.); tel.: +81 3 5400 2694; fax +81 3 5400 2691 (T.M.).
E-mail addresses: ohe-tm@pha.keio.ac.jp (T. Ohe), mashino-td@pha.keio.ac.jp (T. Mashino).

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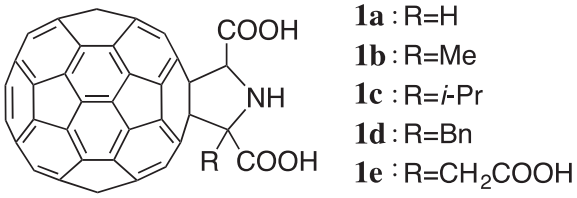


Figure 1. Structure of fullerene derivatives **1a**–**e**.

in this reaction were separated by silica gel column chromatography. To identify *cis*/*trans* isomerism, ^{1}H NMR analysis was performed. In the NOESY experiments for *cis*-**4d** and *cis*-**4e**, a cross-peak between the methine proton on C-5 position of the pyrrolidine ring and the proton on the substituent (R) introduced to C-2 position was observed. Conversely, in the case of *trans*-**4d** and *trans*-**4e**, the cross-peak between the corresponding protons in *cis* isomer was not observed. Furthermore, the observation that the methine proton on C-5 of the pyrrolidine ring in the *trans* isomer was downfield shifted compared to the *cis* isomer, which was consistent with the report Salvatore et al. previously described.[13] This downfield shift led us to identify the *cis*/*trans* isomerism of the other precursors more easily. In these reactions, the *cis* isomers of **4a**–**e** were generated more dominantly than the *trans* isomers, which would be due to the stability of azomethine ylides generated in situ. The synthesized precursors **4a**–**e** were subsequently treated with the strong Brønsted acid, trifluoromethanesulfonic acid, for the deprotection of *tert*-butyl esters to afford the target proline-type derivatives **1a**–**e**.

In our previous Letter, we evaluated various biological activities of **1a** as diastereomeric mixtures.[9] In the present study, we separately prepared *cis*-**4a** and *trans*-**4a** to compare the inhibition activities between both diastereomers. According to the synthetic route indicated in Scheme 1, the major isomer, *cis*-**4a** was purified by silica gel column chromatography; however, the minor isomer, *trans*-**4a** was obtained as an inseparable mixture with *cis*-**4a**. Therefore, as shown in Scheme 2, *trans*-**1a** was synthesized alternatively, using *N*-(*p*-methoxybenzyl)glycine *tert*-butyl ester (**5**).[14]

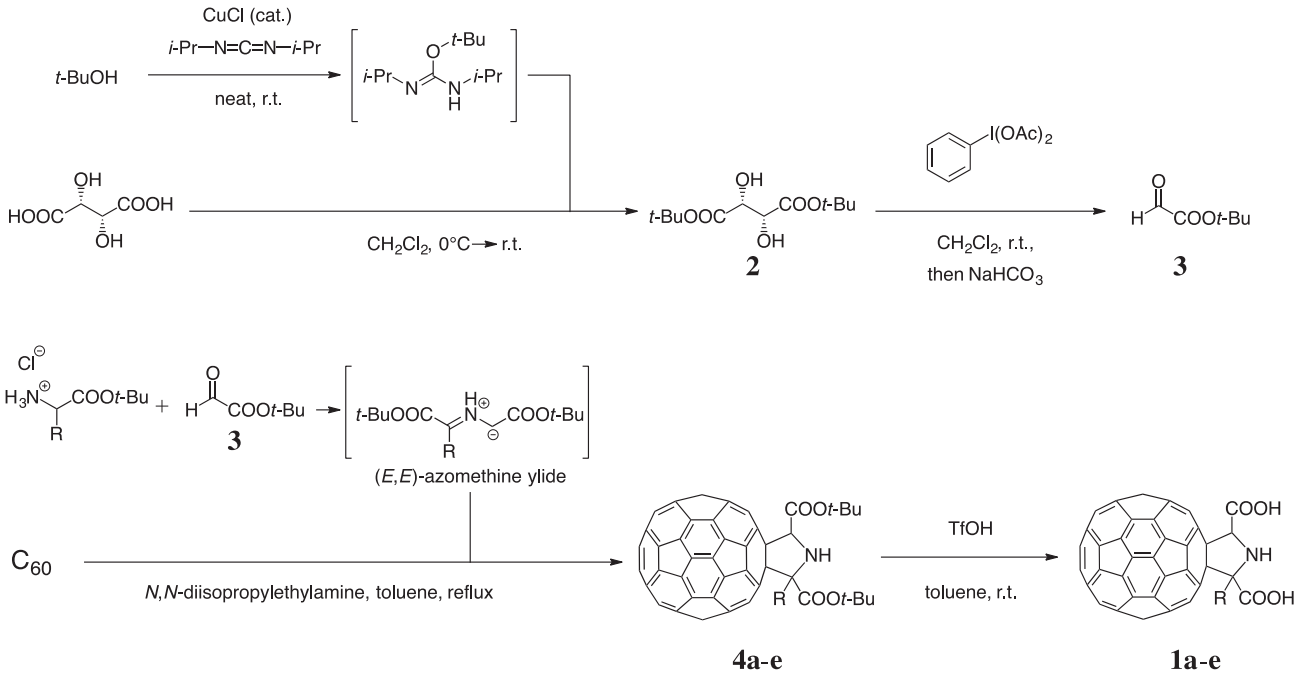
In the case of a primary amine such as an amino acid ester, (*E*,*E*)-azomethine ylide (W-shape) is generated in situ by the reaction with an aldehyde, which gives a *cis* isomer. In contrast, in the case of a secondary amine such as **5**, (*E*,*Z*) or (*Z*,*E*) azomethine ylide, which produces a *trans* isomer, would be generated dominantly due to the steric stability. According to the synthetic route indicated in Scheme 2, the dominancy between *cis*/*trans* conformation in the 1,3-dipolar cycloaddition was completely inversed, thus we obtained *trans*-**6** as the major product. Subsequent co-deprotection of *tert*-butyl esters and *p*-methoxybenzyl group with the acid afforded *trans*-**1a** as expected.

The compounds *cis*-**1a**–**e** and *trans*-**1a** were identified by ^{1}H NMR and HRMS. The purity was verified by ^{1}H NMR. All the products except *cis*-**1a** were synthesized as racemic mixtures.

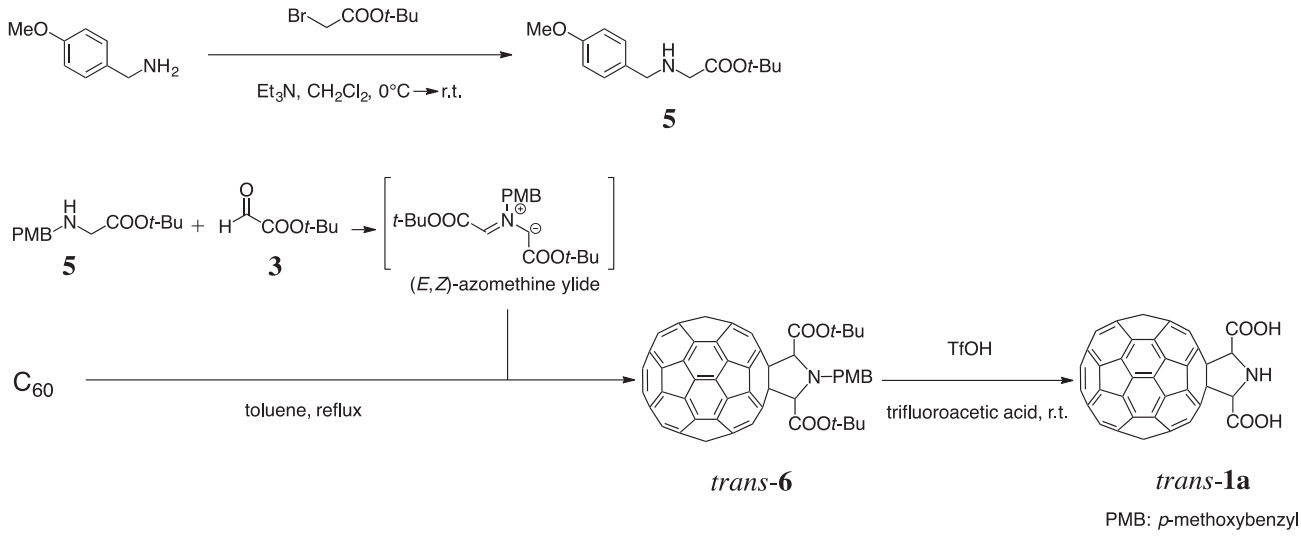
NS5B inhibitory activities of the synthesized derivatives were examined in a manner similar to our previous Letter with several modifications.[8] The examined fullerene derivatives **1a**–**e** significantly inhibited NS5B with IC_{50} values in the submicromolar range (Table 1). The introduction of various substituents on the pyrrolidine ring of **1a**, or *cis*/*trans* isomerism of **1a** appears to have no impact on NS5B inhibitory activity, although further investigation is required to determine the structure–activity relationships in detail. In our previous Letter, IC_{50} for the diastereomeric mixture of **1a** was determined to be 2.0 μM.[9] The lower IC_{50} values observed for each isomer of **1a** in the present study may be due to different experimental conditions.

In addition, the NS3/4A inhibition assay was performed in a manner reported by Sudo et al. previously with some modifications.[15] All fullerene derivatives we examined inhibited NS3/4A significantly in the submicromolar range (Table 1). Similar to the results of NS5B inhibition assay, there is no remarkable difference in the inhibitory activities of **1a**–**e**, while *cis*-**1a** has slightly more potent than other derivatives.

In conclusion, the present study demonstrates, for the first time, that the fullerene derivatives inhibit HCV NS3/4A protease in addition to NS5B polymerase. The inhibition activities against the enzymes were weaker than a known selective NS5B inhibitor, VX-222, and a clinically used NS3/4A inhibitor, telaprevir,



Scheme 1. Synthesis of novel proline-type fullerene derivatives.



Scheme 2. Synthesis of *trans*-**1a**.

Table 1
NS5B and NS3/4A inhibition activities of fullerene derivatives

Compound	R	NS5B inhibitory activity (IC_{50}, μM)	NS3/4A inhibitory activity (IC_{50}, μM)
cis-**1a**	H	0.29	0.15
trans-**1a**	H	0.23	0.85
cis-**1b**	Me	0.48	0.37
cis-**1c**	*i*-Pr	0.29	0.87
cis-**1d**	Bn	0.26	0.43
cis-**1e**	CH_2COOH	0.28	0.87
VX-222	—	0.052	—
Telaprevir	—	—	0.024

respectively (Table 1), but we propose that the fullerene derivatives synthesized in this work are dual inhibitors acting against both NS5B and NS3/4A that may strongly inhibit HCV replication by the synergistic effect and novel compounds for the treatment of HCV infections.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.08.086.

References and notes

1. Choo, Q. L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.
2. Kroto, H. W.; Heath, J. R.; O'Brien, S. C.; Curl, R. F.; Smalley, R. E. *Nature* **1985**, *318*, 162.
3. Okuda, K.; Mashino, T.; Hirobe, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 539.
4. Mashino, T.; Nishikawa, D.; Takahashi, K.; Usui, N.; Yamori, T.; Seki, M.; Endo, T.; Mochizuki, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4395.
5. Bosi, S.; Da Ros, T.; Castellano, S.; Banfi, E.; Prato, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1043.
6. Tsao, N.; Luh, T.; Chou, C.; Chang, T.; Wu, J.; Liu, C.; Lei, H. *J. Antimicrob. Chemother.* **2002**, *49*, 641.
7. Friedman, S. H.; Ganapathi, P. S.; Rubin, Y.; Kenyon, G. L. *J. Med. Chem.* **1998**, *41*, 2424.
8. Mashino, T.; Shimotohno, K.; Ikegami, N.; Nishikawa, D.; Okuda, K.; Takahashi, K.; Nakamura, S.; Mochizuki, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1107.
9. Nakamura, S.; Mashino, T. *J. Nippon Med. Sch.* **2012**, *79*, 248.
10. Yasuno, T.; Ohe, T.; Takahashi, K.; Nakamura, S.; Mashino, T. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3226.
11. Mathias, L. J. *Synthesis* **1979**, 561.
12. Maggini, M.; Scorrano, G.; Prato, M. *J. Am. Chem. Soc.* **1993**, *115*, 9798.
13. Filippone, S.; Maroto, E. E.; Martín-Domenech, Á.; Suarez, M.; Martín, N. *Nat. Chem.* **2009**, *1*, 578.
14. Arcelli, A.; Balducci, D.; Grandi, A.; Porzi, G.; Sandri, M.; Sandri, S. *Monatsh. Chem.* **2004**, *135*, 951.
15. Sudo, K.; Inoue, H.; Shimizu, Y.; Yamaji, K.; Konno, K.; Shigeta, S.; Kaneko, T.; Yokota, T.; Shimotohno, K. *Antiviral Res.* **1996**, *32*, 9.