Syntheses of Fluorescence-labeled Sphingosine 1-Phosphate Methylene and Sulfur Analogues as Possible Visible Ligands to the Receptor

Tetsuya Yamamoto, Hiroko Hasegawa, Toshikazu Hakogi, and Shigeo Katsumura*
Department of Chemistry and Open Research Center on Organic Tool Molecules, School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda, Hyogo 669-1337

(Received October 15, 2007; CL-071137; E-mail: katsumura@ksc.kwansei.ac.jp)

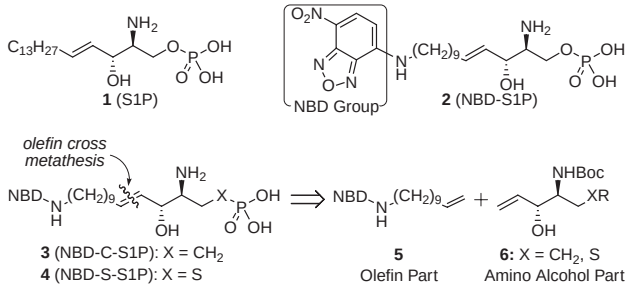
The methylene and sulfur analogues of sphingosine 1-phosphate labeled with a fluorescent group at the terminus of the backbone skeleton were stereoselectively synthesized as possible nonhydrolyzable visible ligands to the sphingosine 1-phosphate receptor, S1P1.

Sphingosine 1-phosphate (S1P) **1** is regarded as a remarkable phospholipid[1] because of its very attractive biological activities, such as the stimulation of DNA synthesis,[2] cell growth,[3] and platelet activation.[4] During the course of our project to develop effective tool molecules for the elucidation of the sphingolipid behavior,[5] we previously reported that the fluorescent-labeled sphingosine 1-phosphate (NBD-S1P) **2**, which was recognized as a ligand of its biological receptor (S1P1), could be visualized it in cells under a fluorescent microscope.[6] This fluorescent molecule was, however, hydrolyzed at the moiety of the phosphate ester function by S1P hydrolytic enzymes to be metabolized into NBD-sphingosine and NBD-ceramide.

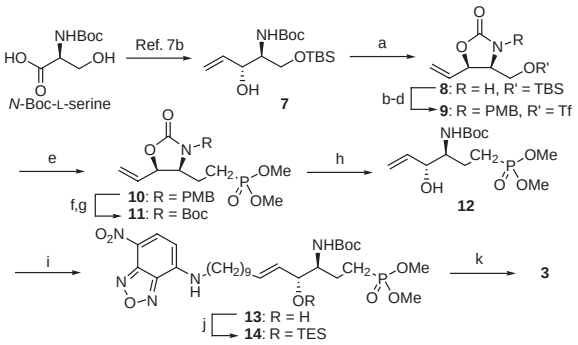
In this paper, we disclose the stereoselective syntheses of both the fluorescence-labeled sphingosine 1-phosphonate **3** (NBD-C-S1P), the so-called methylene analogue, and sphingosine 1-thiophosphate **4** (NBD-S-S1P), the so-called sulfur analogue, as new second generation nonhydrolyzable tool molecules to be useful for the elucidation of the S1P behavior in a cell.

In previous papers, we reported a versatile method for the stereocontrolled synthesis of various kinds of sphingolipids, such as natural sphingosine, ceramide, sphingosine 1-phosphate, and sphingomyelin,[7] by utilizing the olefin cross metathesis protocol.[8,9] As shown in Scheme 1, the syntheses of **3** and **4** were then achieved by applying the cross-coupling method between the fluorescence-labeled olefin **5** and amino alcohol parts **6**. The amino alcohol parts were stereoselectively synthesized starting from the L-serine and from L-cysteine derivatives, respectively.

The oxazolidinone derivative **8**, an intermediate of the methylene analogue, was prepared by the NaH treatment of **7**,

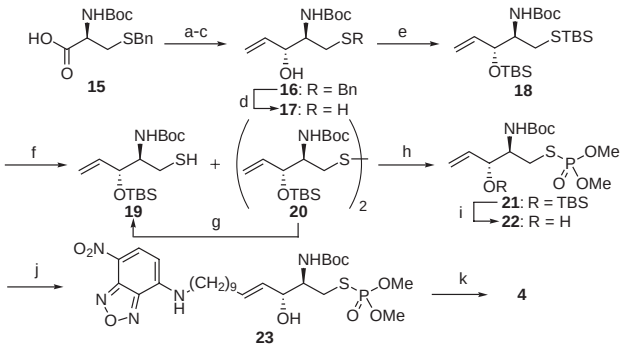


Scheme 1. Structure of sphingosine 1-phosphate and synthetic plan of NBD-labeled analogues.



Scheme 2. Reagents and conditions: (a) NaH, THF, 50 °C, 2 h. (b) PMBCl, TBAI, NaH, THF, rt, 16 h. (c) 2 M HCl, MeOH, rt, 16 h. (d) Tf_2O, 2,6-lutidine, CH_2Cl_2, −78 °C, 94% (three steps). (e) $MePO(OMe)_2$, *n*-BuLi, THF, −78 °C, 73%. (f) CAN, CH_3CN, H_2O, 0 °C, 80%. (g) Boc_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C, 94%. (h) K_2CO_3, MeOH, rt, 74%. (i) **5**, Grubbs cat. 2nd Generation, CH_2Cl_2, reflux, 2 h, 85%. (j) TESCl, imidazole, DMF, 0 °C. (k) TMSBr, CH_2Cl_2, rt, then MeOH, rt, 68% (two steps).

which was synthesized from *N*-Boc-L-serine in 84% yield over four steps as previously reported (Scheme 2).[7b] The introduction of the dimethylphosphonylmethyl group to **8** was one of the key steps of the synthesis of **3**. After several trials, we found that the electron-donating PMB group for protection of the oxazolidinone nitrogen and trifluoromethanesulfonyl group as a leaving one were very important for the successful substitution reaction. Thus, **8** was converted into **9** by the sequence of introducing a PMB group, acid treatment, and formation of triflate in 94% yield over three steps. The slow addition of the lithium anion of dimethylphosphonylmethyl to the triflate at −78 °C successfully produced the desired phosphonate **10** in 73% yield.[10,11] The substitution reaction of the corresponding mesylate, bromide, and iodide was unsuccessful. Attempts of a similar substitution in the linear systems were also unsuccessful. Next, we exchanged the PMB group with a Boc group at this stage, because the selective oxazolidinone ring opening of **10** without hydrolysis of the phosphonate ester moiety was not successful. Removal of the PMB group of **10** by treatment with CAN followed by the reaction with Boc_2O produced **11** in 94% yield, which was treated with potassium carbonate in MeOH to afford the functionalized **12** in 74% yield. With the amino alcohol part in hand, the olefin cross metathesis reaction was attempted under the previously established conditions.[7b] Thus, **12** was stirred with 4 equiv. of the NBD-labeled olefin **5** and 0.03 equiv. of the Grubbs catalyst 2nd generation in CH_2Cl_2 for 2 h at reflux to produce the desired cross coupling product **13** in 85% yield, whose geometry of the double bond was approximately 20:1 of the E:Z-based on the ^{1}H NMR.



Scheme 3. Reagents and conditions: (a) Me(MeO)NH·HCl, EDCI, NMM, CH_2Cl_2, −15 °C. (b) Vinyl bromide, Mg, THF, rt. (c) LiAl(O*t*-Bu)$_3$H, EtOH, −78 °C. (d) Li, liq. NH_3, THF, reflux, 70% (four steps). (e) TBSCl, DMAP, Et_3N, DMF, 0 °C. (f) TBAF, THF, −78 °C. (g) PBu_3, CH_3CN, H_2O, 52% (three steps). (h) Trimethylphosphite, CBr_4, 2,6-lutidine, CH_2Cl_2, 0 °C, 56%. (i) 2 M HCl, THF, rt, 75%. (j) **5**, Grubbs cat. 2nd Generation, CH_2Cl_2, reflux, 2 h, 85%. (k) TMSBr, CH_2Cl_2, rt, then MeOH, rt, 80%.

The secondary hydroxy group in **13** was first protected with a TES group to avoid the side reaction such as lactonization resulting from nucleophilic attack of the hydroxy group to the phosphonate moiety during the following demethylation. The obtained TES derivative **14** was then treated with trimethylsilyl bromide[12] in CH_2Cl_2 followed by treatment with MeOH. The desired **3** (NBD-C-S1P) was obtained in a pure form in 68% yield over two steps.[13]

The synthesis of NBD-S-S1P **4** was also achieved from *N*-Boc-*S*-benzyl-L-cysteine **15** by a procedure similar to that of NBD-C-S1P **3** (Scheme 3). Thus, the sequence of the Weinreb amide formation, introduction of a vinyl group, the highly anti-selective reduction of the resulting ketone with lithium tri- *tert*-butoxyaluminohydride, and then treatment with lithium in liquid ammonia produced **17** in 70% yield over four steps through **16**. In order to introduce a phosphate group into the primary mercapto group, the protection of the secondary hydroxy group was necessary. The regioselective desilylation with 0.95 equiv. of $Bu_4NF{\cdot}nH_2O$ at the sulfur of **18**, which was prepared from **17** by the usual silylation, gave O-silylated thiol **19** as a mixture of disulfide **20**, which was converted into **19** by a tributylphosphine treatment. The phosphorylation of **19** with trimethylphosphite and carbon tetrabromide successfully produced dimethylthiophosphate **21** in 56% yield with the aid of 2,6-lutidine. The desired phosphorylated amino alcohol **22** was obtained in 75% yield by the hydrochloric acid treatment of **21** in THF, although the treatment in MeOH gave a complex mixture. With three types of thiol derivatives, benzyl thioether **16**, thiol **17**, and thiophosphate **22** in hand, the olefin cross metathesis reactions between these thiol derivatives and olefin **5** were examined. In the case of **16**, the reaction poorly proceeded to afford the corresponding coupling product in only 22% yield. The reaction with thiol **17** did not proceed at all. On the other hand, the reaction of **22** smoothly proceeded to produce the desired coupling product **23** in 85% yield. The objective NBD-S-S1P **4** was successfully obtained by removal of both the methyl and Boc groups using trimethylsilyl bromide and then MeOH.[14]

In conclusion, we achieved the syntheses of new fluorescence-labeled methylene and sulfur analogues, **3** and **4**, by our olefin cross metathesis protocol as the key step. These analogues are expected to be useful nonhydrolyzable tool molecules to investigate the behavior of sphingosine 1-phosphate in a cell.[15]

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan. This work was also supported by the Maching Fund Subsidy for Private University.

This paper is dedicated to the late Professor Yoshihiko Ito for his outstanding contribution to synthetic organic chemistry.

References and Notes

1 For recent reviews on signal transduction mediated by sphingolipids, see: a) J. Liao, J. Tao, G. Lin, D. Liu, *Tetrahedron* **2005**, *61*, 4715. b) A. Delgado, J. Casas, A. Llebaria, J. L. Abad, *ChemMedChem* **2007**, *2*, 580. c) T. Baumruker, A. Billich, *Curr. Immun. Rev.* **2006**, *2*, 101, and references cited therein.

2 H. Zhang, N. N. Desai, A. Olivera, T. Seki, G. Brooker, S. Spiegel, *J. Cell Biol.* **1991**, *114*, 155.

3 M. J. Lee, S. Thangada, K. P. Claffey, N. Ancellin, C. H. Liu, M. Kluk, M. Volpi, R. I. Sha'afi, T. Hla, *Cell* **1999**, *99*, 301.

4 Y. Yatomi, F. Ruan, S. Hakomori, Y. Igarashi, *Blood* **1995**, *86*, 193.

5 We reported the syntheses of the following various sphingomyelin analogues: a) Carbon analogues: T. Hakogi, Y. Monden, M. Taichi, S. Iwama, S. Fujii, K. Ikeda, S. Katsumura, *J. Org. Chem.* **2002**, *67*, 4839. b) Nitrogen analogue: T. Hakogi, M. Taichi, S. Katsumura, *Org. Lett.* **2003**, *5*, 2801. c) Sulfur analogue: T. Hakogi, S. Fujii, M. Morita, K. Ikeda, S. Katsumura, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2141. d) Difluoromethylene analogue: T. Hakogi, T. Yamamoto, S. Fujii, K. Ikeda, S. Katsumura, *Tetrahedron Lett.* **2006**, *47*, 2627.

6 T. Hakogi, T. Shigenari, S. Katsumura, T. Sano, T. Kohno, Y. Igarashi, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 661.

7 a) H. Hasegawa, T. Yamamoto, S. Hatano, T. Hakogi, S. Katsumura, *Chem. Lett.* **2004**, *33*, 1592. b) T. Yamamoto, H. Hasegawa, T. Hakogi, S. Katsumura, *Org. Lett.* **2006**, *8*, 5569.

8 a) R. H. Grubbs, *Handbook of Metathesis*, Wiley-VCH, Germany, **2003**. b) S. Connon, S. Blechert, *Angew. Chem., Int. Ed.* **2003**, *42*, 1900, and references cited therein.

9 For other reports on functionalized sphingolipids: a) P. Nussbaumer, P. Ettmayer, C. Peters, D. Rosenbeiger, K. Hoegenauer, *Chem. Commun.* **2005**, 5086. b) C. Peters, A. Billich, M. Ghobrial, K. Hoegenauer, T. Ullrich, P. Nussbaumer, *J. Org. Chem.* **2007**, *72*, 1842, and references cited therein.

10 D. B. Berkowitz, G. Maiti, B. D. Charette, C. D. Dreis, R. G. MacDonald, *Org. Lett.* **2004**, *6*, 4921.

11 The alkylation of the triflate **9** with a dimethyl phosphonate anion resulted in a low yield when **9** was added to the lithium anion of a dimethyl methylphosphonate solution at −78 °C in Ref. 10.

12 C. E. McKenna, J. Schmidhauser, *J. Chem. Soc., Chem. Commun.* **1979**, 739.

13 Spectra data of **3**: $[\alpha]_D^{20.5}$ +2.8 (c = 0.10, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz), δ 8.42 (d, J = 8.5 Hz, 1H), 6.26 (d, J = 8.9 Hz, 1H), 5.86 (td, J = 6.6, 15.3 Hz, 1H), 5.48 (dd, J = 6.6, 15.4 Hz, 1H), 4.30 (dd, J = 4.3, 5.7 Hz, 1H), 3.48 (m, 2H), 3.30 (m, 1H), 2.07 (td, J = 6.8, 6.8 Hz, 2H), 2.06–1.81 (m, 4H), 1.75 (tt, J = 7.3, 7.3 Hz, 2H), 1.46–1.28 (m, 12H); ^{13}C NMR (CD_3OD, 100 MHz), δ 146.5, 145.6, 145.3, 138.5, 136.9, 122.5, 99.6, 72.3, 57.5 (d, J_{C-P} = 15.3 Hz), 44.8, 33.4, 30.52, 30.45, 30.33, 30.30, 30.1, 29.2, 28.0, 24.7 (d, J_{C-P} = 13.9 Hz), 23.2.

14 Spectra data of **4**: $[\alpha]_D^{25.5}$ −3.9 (c = 1.06, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz), δ 8.44 (d, J = 8.9 Hz, 1H), 6.28 (d, J = 8.9 Hz, 1H), 5.83 (td, J = 6.9, 15.6 Hz, 1H), 5.44 (dd, J = 6.6, 15.3 Hz, 1H), 4.28 (dd, J = 5.7, 5.7 Hz, 2H), 3.48 (m, 2H) 3.29 (m, 1H), 3.01 (ddd, J = 3.9, 15.9, 15.9 Hz, 1H), 2.85 (ddd, J = 8.9, 14.9, 16.2 Hz, 1H), 2.15 (td, J = 6.9, 7.1 Hz, 2H), 17.3 (tt, J = 7.1, 7.1 Hz, 2H), 1.45–1.23 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 146.6, 145.8, 145.4, 138.6, 137.0, 127.9, 122.7, 99.6, 72.2, 58.8, 44.9, 33.4, 30.54, 30.45, 30.35, 30.26, 30.08, 29.3, 29.0 (d, J_{C-P} = 11.4 Hz), 28.0.

15 In the preliminary qualitative tests, the synthesized **3** and **4** showed a moderate ability as ligands toward the sphingosine 1-phosphate receptor, S1P1, based on the results that the synthesized **3** and **4** reasonably expelled the radiolabeled S1P similarly to NBD-S1P in S1P1-expressing Chinese hamster ovary cells. We are grateful to Prof. Igarashi and co-workers of Hokkaido University for their biological testing.

Published on the web (Advance View) January 19, 2008; doi:10.1246/cl.2008.188