Regio- and Stereoselective Incorporation of a ^{13}C Nuclide into D-*ribo*-Phytosphingosine *via* SmI_2-Mediated C–C Formation with ^{13}C-Labeled Isocyanide

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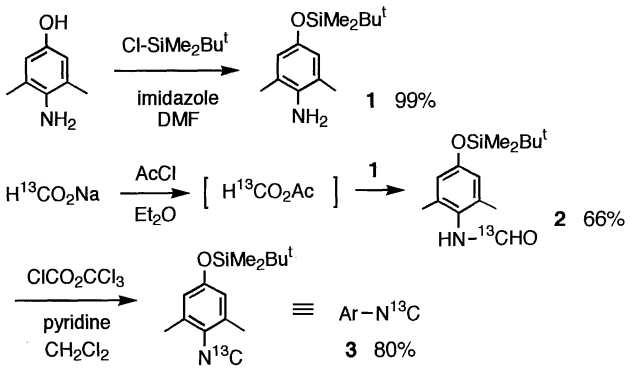
Preparation of a ^{13}C-labeled isocyanide and its application to the total synthesis of [2-^{13}C]D-*ribo*-C_{18}-phytosphingosine are described. The synthesis of the labeled phytosphingosine is based on the use of the isocyanide as a ^{13}CH–NH_2 precursor in the SmI_2-mediated three-component coupling reaction, wherein regio- and stereoselective incorporation of a ^{13}C nuclide in the carbon skeleton is achieved.

The insight in the interactions between biologically active compounds and their receptors are of crucial importance for the understanding of biological action. Recent advancement of isotope-assisted techniques in NMR spectroscopy[1] has made a ^{13}C-labeled compound a powerful tool for studying ligand/receptor complexes in solution.[2] This situation has created an increased need for synthetic methods of target organic molecules with incorporation of a ^{13}C nuclide at desired positions with defined configurations. On the other hand, investigations concerning the intriguing biological role of sphingolipids have prompted intensified interest in their binding at cellular surface.[3] We report here the preparation of a ^{13}C-labeled isocyanide and its application to the stereoselective total synthesis of [2-^{13}C]D-*ribo*-C_{18}-phytosphingosine, a lipophilic component broadly distributed in animal as well as plant sphingolipids. The synthesis of the labeled phytosphingosine is based on the use of the isocyanide as a ^{13}CH–NH_2 precursor in the SmI_2-mediated three-component coupling reaction,[4] wherein regio- and stereoselective incorporation of a ^{13}C nuclide is achieved.

First, a ^{13}C nuclide was installed in the starting aromatic isocyanide. Sodium formate with 99% ^{13}C enrichment, a commercially available compound, was treated with acetyl chloride. The resultant mixed anhydride was subsequently reacted with an aromatic amine **1** to give a labeled formamide **2**. Dehydration of **2** with trichloromethyl chloroformate furnished isocyanide (**3**) with the isocyano carbon labeled by a ^{13}C nuclide.[5]

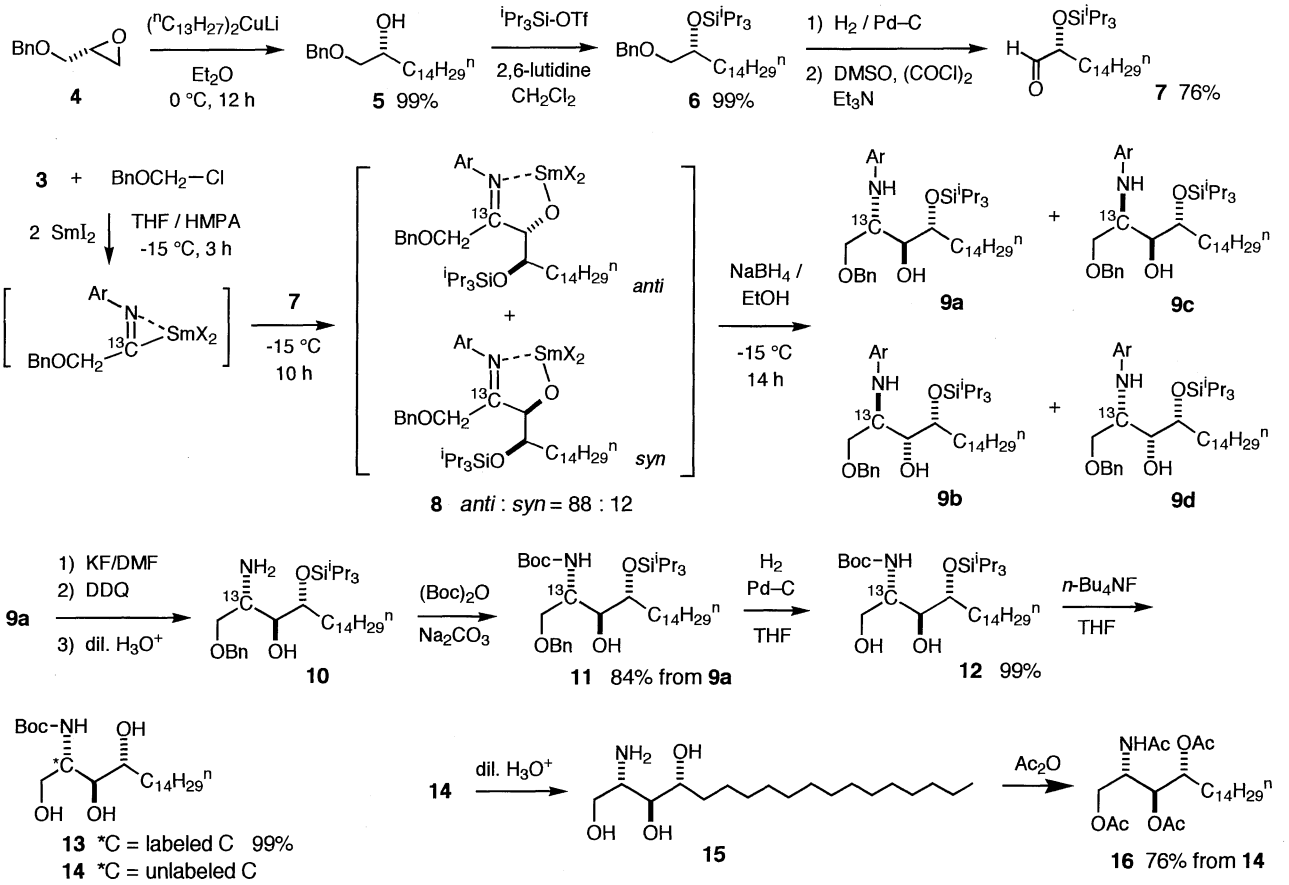
Next, [2-^{13}C]D-*ribo*-C_{18}-phytosphingosine (**13**) was synthesized on the basis of the synthetic route shown in Scheme 1. An optically active aliphatic aldehyde **7**, (*R*)-2-(triisopropylsiloxy)hexadecanal, was prepared from a chiral glycidol derivative **4**. A long aliphatic chain was introduced by alkylation of lithium dialkylcuprate with **4**. The secondary hydroxyl group of the ring-opened product **5** was protected as a triisopropylsilyl ether, giving **6**. Debenzylation by hydrogenolysis on Pd–C and the subsequent Swern oxidation of the resultant primary alcohol furnished the α-siloxy aldehyde **7**.[6] ^{13}C-Labeled (imidoyl)–Sm(III), generated by the SmI_2-mediated coupling of the isocyanide **3** with benzyl chloromethyl ether,[4] underwent the addition to the α-siloxy aldehyde **7** with fair 1,2-asymmetric induction (88:12). The stereoisomer expected based on Felkin's model,[7] designated as *anti*-**8** in Scheme 1, was produced preferentially. Addition of $NaBH_4$ to the resultant reaction mixture in the presence of ethanol[4c,8] led to stereoselective



reduction of the imino functionality to afford a mixture of stereoisomers of amino alcohol (**9**). The isomers ratio was estimated as **9a**:**9b**:**9c**:**9d** = 86:11:2:<1[9] on the basis of the HPLC analysis of the sample obtained from an analogous pilot experiment using an unlabeled correspondent to **3** (*vide infra*). The fine stereoselection during the hydride reduction (*ca.* 98:2) is formulated as arising from the attack of hydride from the less-hindered side of the 5-membered chelate ring of *anti*-**8**. After the major isomer **9a** was isolated in 85% by MPLC, the aromatic substituent of the nitrogen atom was removed through desilylation by KF, oxidation to quinone imine by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and acidic hydrolysis.[4c] Then, the primary amino group of **10** was protected with *t*-butoxycarbonyl (Boc) group for the ease of handling of the final product in the further manipulation.[10] Debenzylation of **11** by hydrogenolysis and the subsequent cleavage of triisopropylsilyl ether by *n*-Bu_4NF completed the synthesis of [2-^{13}C]D-*ribo*-C_{18}-phytosphingosine having the Boc-protected amino group (**13**).[11]

A reaction sequence identical with that for the labeled **13**, starting from an unlabeled isocyanide corresponding to **3**, led to the synthesis of the unlabeled counterpart **14**. Removal of the Boc-group under acidic conditions gave rise to D-*ribo*-C_{18}-phytosphingosine (**15**). Comparison of the physical data (^1H NMR, ^{13}C NMR, and optical rotation) of the tetraacetate (**16**), formed by acetylation of **15** with Ac_2O, with literature values[12] confirmed the identity of **15**, and hence, of [2-^{13}C]D-*ribo*-C_{18}-phytosphingosine (**13**).

In summary, an optically active labeled phytosphingosine was stereoselectively synthesized by the use of the ^{13}C-labeled isocyanide **3** as a ^{13}CH–NH_2 precursor. Although ^{13}C-labeled carbon monoxide is an important source of a ^{13}C nuclide in chemical syntheses, it is not easy to control the stoichiometry of the precious gaseous material. Moreover, many carbonylation reactions require a high CO pressure. The availability of ^{13}C-labeled isocyanide and its efficient use in the stereocontrolled construction of a carbon skeleton, as demonstrated here, promise to open a new synthetic pathway to a wide range of biologically interesting compounds with specific labeling, which will provide the means of detailed structural investigation.



Scheme 1.

Further details of experimental procedures for the reactions described, and of product characterization by ^1H and ^{13}C NMR are available from the corresponding authors.

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References and Notes

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5 **3**: Oil. ^1H NMR ($CDCl_3$) δ 0.19 (s, 6H), 0.97 (s, 9H), 2.35 (s, 6H), 6.54 (s, 2H); ^{13}C NMR ($CDCl_3$) δ -4.4, 18.2, 19.0, 25.6, 119.1, 136.4, 155.5, 166.2 (^{13}C); HRMS *m/z* calcd for $^{13}C^{12}C_{14}H_{23}NOSi$: 262.1583, found 262.1582.

6 **7**: Oil, $[\alpha]_D^{24}$ +9.4 ° (*c* 2.1, $CDCl_3$). ^1H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.2 Hz, 3H), 1.01–1.15 (m, 21H), 1.21–1.50 (br, 24H), 1.54–1.69 (m, 2H), 4.07 (dt, *J* = 5.9, 2.2 Hz, 1H), 9.63 (d, *J* = 2.2 Hz, 1H); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 12.2. 14.1, 17.9, 22.7, 23.9, 29.36, 29.40, 29.5, 29.7, 31.9, 33.5, 77.6, 204.9. Anal. Found: C, 72.62; H, 12.95%. Calcd for $C_{25}H_{52}O_2Si$: C, 72.75; H, 12.70%.

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8 The same stereoselectivity of the reduction was observed when 2-propanol was used instead of ethanol.

9 The assignment of the stereochemistries for the minor isomers is tentative.

10 Introduction of a long-chain fatty acid on the primary amino group instead of Boc-protection at this stage would lead to the synthesis of a ceramid.

11 **13**: Oil, $[\alpha]_D^{23}$ +7.7 ° (*c* 1.2, $CDCl_3$). ^1H NMR ($CDCl_3$) δ 0.87 (t, *J* = 6.7 Hz, 3H), 1.24 (br s, 24H), 1.40–1.60 (m, 2H), 1.44 (s, 9H), 3.40–4.20 (m, 7H), 3.81 (d, *J* = 137 Hz, 1H), 5.43 (d, *J* = 7.2 Hz, 1H); $^{13}C\{^1H\}$ NMR (CD_3OD: $CDCl_3$ = 5:1) δ 12.7, 21.7, 25.0, 27.0, 28.5, 28.8, 31.1, 31.4, 52.2(^{13}C), 60.5 (*J* = 39.4 Hz), 71.4, 74.6 (*J* = 40.4 Hz), 78.4, 155.8; HRMS *m/z* calcd for $^{13}C^{12}C_{22}H_{48}NO_5$ (M+H): 419.3568, found 419.3558.

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