Synthesis of D/L-*erythro*-Sphingosine Using a Tethered Aminohydroxylation Reaction as the Key Step

José Antonio Morales-Serna, Yolanda Díaz, M. Isabel Matheu, Sergio Castillón*

Departamento de Química Analítica y Química Orgánica, Universitat Rovira I Virgili, C/Marcel.li Domingo s/n, 43007 Tarragona, Spain
Fax +34(977)558446; E-mail: sergio.castillon@urv.net

Received 14 July 2008; revised 18 November 2008

Abstract: A diastereoselective synthesis of racemic D/L-*erythro*-sphingosine is described. The approach involves employing tethered aminohydroxylation (TA) to introduce the 2-amino and 3-hydroxy functions with required stereochemistry.

Key words: sphingolipids, sphingosine, ceramide, amino alcohols, diasteroselectivity

In 1881, when Johann Thudichum first described a compound that would later be fully characterized as sphingosine, he named it after the Greek mythological character, the Sphinx, 'in commemoration of the many enigmas which it has presented to the inquirer'.[1] Sphingolipids have emerged over the last decades as a family of key signaling molecules, which include sphingosine and ceramide.[2] These compounds, together with glycerophospholipids and cholesterol, are building blocks[3] that play essential roles as structural cell membrane components[4] and participate in higher order physiological processes including inflammation[5] and vasculogenesis.[6] Recent studies demonstrated the involvement of sphingolipids in many of the most common human diseases, including infection by microorganisms,[7] diabetes,[8] a range of cancers,[9] Alzheimer's,[10] and many others.[11]

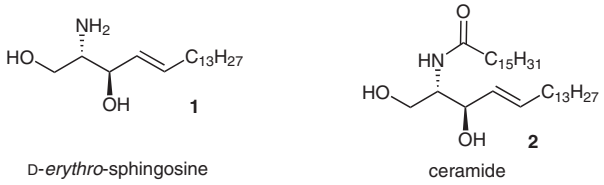
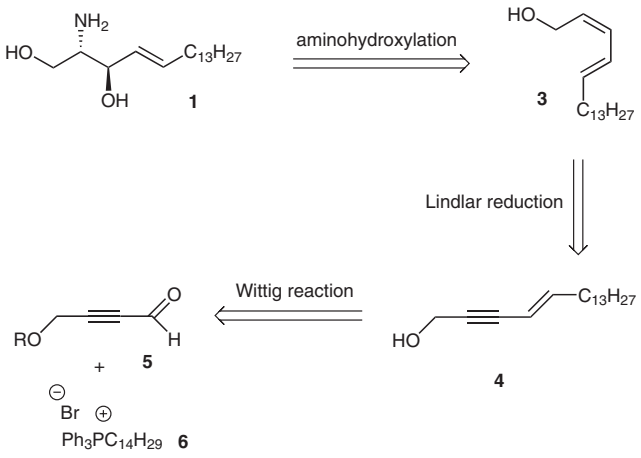


Figure 1 The prevalent backbone in sphingolipids

Structurally, the prevalent backbone in sphingolipids is sphingosine which, when bearing a long-chain fatty acid into the amino function, is called ceramide (Figure 1). There are four sphingosine stereoisomers with a wide range of biological activities.[12,13] The D-*erythro*-isomer is the most common metabolite and has been widely studied. Since, sphingosine and its derivatives are only available in limited amounts from natural sources, there is growing interest in developing efficient methods for their synthesis. There are many reported methods for synthesizing sphingosine,[14] which can be classified into four categories: (i) first, carbohydrates are used as the source of chirality; (ii) second, the Sharpless asymmetric epoxidation is used to generate the stereogenic centers; (iii) the third relies on the aldol reaction with a chiral auxiliary and finally (iv) the amino acid serine is used as the source of chirality.

Here, we wish to enrich this diverse range of strategies for sphingosine synthesis. In this paper, we report an efficient method for the synthesis of racemic D/L-*erythro*-sphingosine employing an aminohydroxylation reaction as the key step. Unfortunately, this reaction is not compatible with the use of cinchona alkaloid-derived chiral ligands, which therefore precludes an enantioselective version. Our choice of starting material was dictated by the type of reaction that we planned to employ to generate the asymmetric centers. The chiral aminohydroxylic functions were introduced in the last step by an aminohydroxylation of diene **3**, which has the appropriate *E*,*Z*-configuration in the aminohydroxylation reaction. This diene can be prepared by reduction of **4**, which in turn can be obtained from aldehydes **5** through a Wittig reaction (Scheme 1).

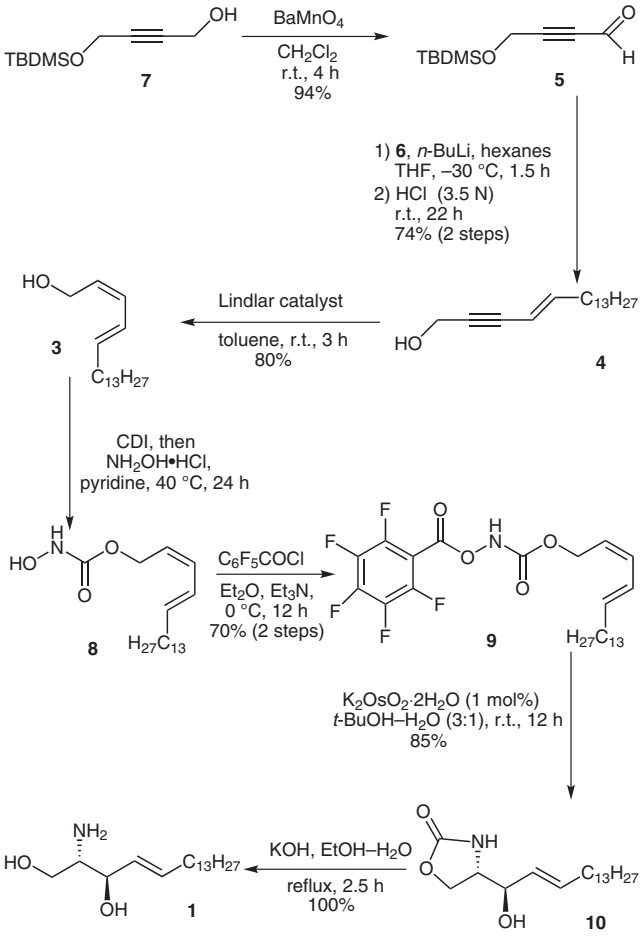


Scheme 1 Retrosynthetic analysis of D-*erythro*-sphingosine

The synthesis of the diene **3**[15] started from acetylenic alcohol **7** which, under oxidation conditions, gave aldehyde **5**. Reaction of **5** with the phosphonium salt **6**, followed by deprotection of the hydroxy group under acidic conditions, afforded the *E*-enyne **4**. The desired *E*,*Z*-diene **3** was obtained in 80% yield by Lindlar reduction of **4** (Scheme 2).

SYNTHESIS 2009, No. 5, pp 0710–0712
Advanced online publication: 11.02.2009
DOI: 10.1055/s-0028-1083367; Art ID: T11308SS

The Sharpless asymmetric aminohydroxylation[16] (AA) and tethered aminohydroxylation[17] (TA) allows the catalytic and diastereoselective synthesis of amino alcohols. However, in AA the drawbacks of regioselectivity persist during the oxidation of unsymmetrical alkenes. This inconvenience can be avoided through the use of TA. At this point of the synthesis, we were interested in extending the TA methodology[18] as a general strategy in order to aminohydroxylate the double bond. Thus, the *E*,*Z*-diene **3** was reacted with *N*,*N*′-carbonyldiimidazole (CDI) in the presence of pyridine, followed by the addition of hydroxylamine hydrochloride, to give the hydroxycarbamate **8**. The latter was treated with pentafluorobenzoyl chloride (C_6F_5COCl) to yield the hydroxycarbamate **9**. When **9** was treated under TA conditions, oxazolidinone **10** was obtained in 85% yield with complete control of the regio- and relative stereoselectivity. Alkaline hydrolysis of **10** afforded racemic sphingosine **1** in quantitative yield.



Scheme 2 Synthesis of sphingosine **1**

In conclusion, D/L-*erythro*-sphingosine (**1**) has been synthesized in eight steps and 33% overall yield from alcohol **7** using a tethered aminohydroxylation (TA) as key step. This approach allowed the introduction of the 2-amino and 3-hydroxy groups with complete regio- and stereoselectivity.

All reactions were conducted under a dried argon stream. Solvents (CH_2Cl_2, 99.9%; benzene, 99.9%) were purchased in capped Pure Solv System-4® bottles, stored under argon and used without further purification. All other solvents and reagents were used without further purification. All glassware utilized was flame-dried before use. Reactions were monitored by TLC carried out using silica gel plates (0.25 mm, E. Merck). Developed TLC plates were visualized under a short-wave UV lamp and by heating plates that were dipped in EtOH–H_2SO_4 (15:1). Flash column chromatography was performed using flash silica gel (32–63 μm) and employed a solvent polarity correlated with TLC mobility. NMR experiments were conducted on a Varian 400 MHz instrument using $CDCl_3$ (99.9% D) as solvent, with chemical shifts (δ) referenced to internal standards $CDCl_3$ (δ = 7.26 ppm for ^{1}H, 77.23 ppm for ^{13}C) or TMS (0.00 ppm). Chemical shifts are given in parts per million (ppm) relative to the deuterated solvent peak.

Hydroxycarbamate 9

CDI (182 mg, 1.12 mmol) was added to alcohol **3** (200 mg, 0.75 mmol) in pyridine (20 mL) at 40 °C. When the adduct between the alcohol and the CDI was totally formed, NH_2OH·HCl (130 mg, 1.87 mmol) was added. The resulting reaction mixture was stirred for 24 h at 40 °C, then quenched with HCl (1 M, 10 mL), and EtOAc (10 mL) was added. After separation of the organic phase, the aqueous phase was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed sequentially with H_2O (10 mL) and brine (3 × 10 mL), dried ($NaSO_4$), filtered and the solvent was azeotropically removed with toluene. The crude product was purified by flash column chromatography on silica gel (hexane–EtOAc, 85:15) to give **8**, which was used directly in the next step.

A solution of **8** (243 mg, 0.75 mmol) and Et_3N (83 mg, 0.82 mmol) in anhydrous Et_2O (20 mL) was cooled to 0 °C. The acid chloride C_6F_5COCl (172 mg, 0.75 mmol) in anhydrous Et_2O (10 mL) was added dropwise over 1 h at 0 °C, then the reaction was stirred under argon at r.t. for 12 h. The reaction was quenched with aq HCl (1 M, 10 mL) and the aqueous layer was extracted with Et_2O (3 × 10 mL). The combined organic layers were washed sequentially with aq HCl (10%, 10 mL), aq $NaHCO_3$ (7%, 10 mL), and brine (3 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash column chromatography on silica gel (hexane–EtOAc, 85:15) to give **9**.

Yield: 270 mg (70%); clear oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.36 (s, 1 H), 6.3 (dd, *J* = 13.8, 11.1 Hz, 1 H), 6.15 (dd, *J* = 11.1, 10.6 Hz, 1 H), 5.8 (dq, *J* = 13.8, 6.6 Hz, 1 H), 5.4 (dt, *J* = 10.6, 7.1 Hz, 1 H), 4.73 (d, *J* = 7.1 Hz, 2 H), 2.12 (q, *J* = 7.0 Hz, 2 H), 1.37–1.2 (m, 22 H), 0.85 (t, *J* = 6 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.5, 155.7, 148.9, 147.2, 145.6, 143.0, 138.4, 133.1, 124.5, 121.9, 61.1, 32.8, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 22.6, 14.0.

HRMS (FAB+): *m*/*z* [M + H] calcd for $C_{26}H_{35}F_5NO_4$: 520.2486; found: 520.2494.

Anal. Calcd for $C_{26}H_{34}F_5NO_4$: C, 60.11; H, 6.60; N, 2.70. Found: C, 60.13; H, 6.59; N, 2.72.

Oxazolidinone 10

To a solution of **9** (250 mg, 0.48 mmol) in *t*-BuOH–H_2O (3:1, 10 mL/mmol), was added dropwise a solution of K_2OsO_2·$2H_2O$ (1.8 mg, 1 mol%) in H_2O (0.25 mL). The resulting reaction mixture was then stirred at r.t. for 12 h. The reaction was quenched by addition of Na_2SO_3 (100 mg, mmol), allowed to stir for 30 min, then the solvent was azeotropically removed with toluene. The crude product was purified by flash column chromatography on silica gel (hexane–EtOAc, 85:15) to give **10**.

Yield: 132 mg (85%); white solid.[19]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.10 (s, 1 H), 5.83 (ddt, *J* = 15.4, 6.8, 0.8 Hz, 1 H), 5.37 (ddt, *J* = 15.4, 6.8, 1.2 Hz, 1 H), 4.39 (dd, *J* = 8.8, 8.5 Hz, 1 H), 4.33 (dd, *J* = 8.8, 5.2 Hz, 1 H), 4.12 (m, 1 H), 3.86 (m, 1 H), 2.92 (br s, 1 H), 2.04 (q, *J* = 7.0 Hz, 2 H), 1.37–1.2 (m, 22 H), 0.81 (t, *J* = 6.8 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.5, 136.6, 126.6, 73.3, 66.4, 56.4, 32.5, 32.1, 29.81, 29.79, 29.74, 29.6, 29.5, 29.4, 29.1, 22.8, 14.2.

HRMS (FAB+): *m/z* [M + H] calcd for $C_{19}H_{36}NO_3$: 326.2695; found: 326.2699.

Anal. Calcd for $C_{19}H_{35}NO_3$: C, 70.11; H, 10.84; N, 4.30. Found: C, 70.19; H, 10.80; N, 4.32.

Sphingosine (1)

Oxazolidinone **10** (100 mg, 0.30 mmol) in 1 M KOH (H_2O–EtOH, 1:1; 5 mL) was heated to reflux for 2.5 h, then cooled to r.t. and aq HCl (2 M, 2.5 mL) was added.[19] The mixture was extracted with EtOAc (3 × 10 mL) and the combined organic layers were dried over $MgSO_4$ and concentrated under vacuum to give **1**.

Yield: 89 mg (100%); white solid.[20]

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.76 (dt, *J* = 15.4, 6.7 Hz, 1 H), 5.47 (dd, *J* = 15.4, 7 Hz, 1 H), 4.11 (m, 1 H), 3.70 (dd, *J* = 11, 3 Hz, 1 H), 3.65 (dd, *J* = 11, 5.8 Hz, 1 H), 2.92 (m, 1 H), 2.05 (dt, *J* = 6.7, 7.3 Hz, 2 H), 1.37 (m, 2 H), 1.20–1.40 (m, 20 H), 0.88 (t, *J* = 6.9 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 134.7, 128.8, 74.7, 63.3, 56.3, 32.5, 31.9, 29.7–29.2, 22.7, 14.1.

HRMS (FAB+): *m/z* [M + H] calcd for $C_{18}H_{38}NO_2$: 300.2903; found: 300.2909.

Anal. Calcd for $C_{18}H_{37}NO_2$: C, 72.19; H, 12.45; N, 4.68. Found: C, 72.21; H, 12.41; N, 4.70.

Acknowledgment

Financial support from DGESIC CTQ-2005-03124/BQU (Ministerio de Educación y Ciencia, Spain) is acknowledged. We are also grateful to the Servei de Recursos Cientifics (URV) for its technical assistance. Fellowship from DURSI (Generalitat de Catalunya to J.A.M.S. is gratefully acknowledged).

References

(1) Thudichum, J. L. W. *A Treatise on the Chemical Constitution of the Brain*; Bailliere, Tindall and Cox: London, **1884**.
(2) Tani, M.; Ito, M.; Igarashi, Y. *Cell. Signal.* **2007**, *19*, 229.
(3) Riethmüller, J.; Riehle, A.; Grassmé, H.; Gulbins, E. *Biochim. Biophys. Acta* **2006**, *1758*, 2139.
(4) Snook, C. F.; Jones, J. A.; Hannun, Y. A. *Biochim. Biophys. Acta* **2006**, *1761*, 927.
(5) El Alwani, M.; Wu, B. X.; Obeid, L. M.; Hannun, Y. A. *Pharmacol. Ther.* **2006**, *112*, 171.
(6) Argraves, K. M.; Wilkerson, B. A.; Argraves, W. S.; Fleming, P. A.; Obeid, L. M.; Drake, C. J. *J. Biol. Chem.* **2004**, *279*, 50580.
(7) Heung, L. J.; Luberto, C. h.; Del Poeta, M. *Infect. Immun.* **2006**, *74*, 28.
(8) Summers, S. A.; Nelson, D. H. *Diabetes* **2005**, *54*, 591.
(9) Modrak, D. E.; Gold, D. V.; Goldenberg, D. M. *Mol. Cancer. Ther.* **2006**, *5*, 200.
(10) Zhou, S.; Zhou, H.; Walian, P. J.; Jap, B. K. *Biochemistry* **2007**, *46*, 2553.
(11) Kolter, T.; Sandhoff, K. *Biochim. Biophys. Acta* **2006**, *1758*, 2057.
(12) Merril, A. H. Jr.; Nimkar, S.; Menaldino, D.; Hannun, Y. A.; Loomis, C.; Bell, R. M.; Tyahi, S. R.; Lambeth, J. D.; Stevens, V. L.; Hunter, R.; Liotta, D. C. *Biochemistry* **1989**, *28*, 3138.
(13) Sachs, C. W.; Ballas, L. M.; Mascarella, S. W.; Safa, A. R.; Lewin, A. H.; Loomis, C.; Carroll, F. I.; Bell, R. M.; Fine, R. L. *Biochem. Pharmacol.* **1996**, *52*, 603.
(14) For reviews, see: (a) Merrill, A. H. Jr.; Hannun, Y. A. *Methods Enzymol.* **2000**, *311*, 91. (b) Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998**, 1075. (c) Liao, J.; Tao, J.; Lin, G.; Liu, D. *Tetrahedron* **2005**, *61*, 4715.
(15) Garigipati, R. S.; Freyer, A. J.; Whittle, R. R.; Weinreb, S. M. *J. Am. Chem. Soc.* **1984**, *106*, 7861.
(16) (a) Li, G.; Chang, H.-T.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 451. (b) For a review, see: Bodkin, J. A.; McLeod, M. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2733.
(17) (a) Donohoe, T. J.; Helliwell, M.; Johnson, P. D.; Keenan, M. *Chem. Commun.* **2001**, 2078. (b) Donohoe, T. J.; Cowley, A.; Johnson, P. D.; Keenan, M. *J. Am. Chem. Soc.* **2002**, *124*, 12934. (c) Donohoe, T. J.; Johnson, P. D.; Pye, R. J. *Org. Biomol. Chem.* **2003**, *1*, 2025. (d) Donohoe, T. J.; Johnson, P. D.; Keenan, M.; Pye, R. J. *Org. Lett.* **2004**, *6*, 2583. (e) Donohoe, T. J.; Chughtai, M. J.; Klauber, D. J.; Griffin, D.; Campbell, A. D. *J. Am. Chem. Soc.* **2006**, *128*, 2514.
(18) Donohoe, T. J.; Bataille, C. J. R.; Gattrell, W.; Kloesges, J.; Rossignol, E. *Org. Lett.* **2007**, *9*, 1725.
(19) Torssell, S.; Somfai, P. *Org. Biomol. Chem.* **2004**, *2*, 1643.
(20) Duclos, R. I. *Chem. Phys. Lipids* **2001**, *111*, 111.