Enantioselective Synthesis of D-erythro-Sphingosine and of Ceramide

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43. Enantioselective Synthesis of D-*erythro*-Sphingosine and of Ceramide

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(9.I.86)

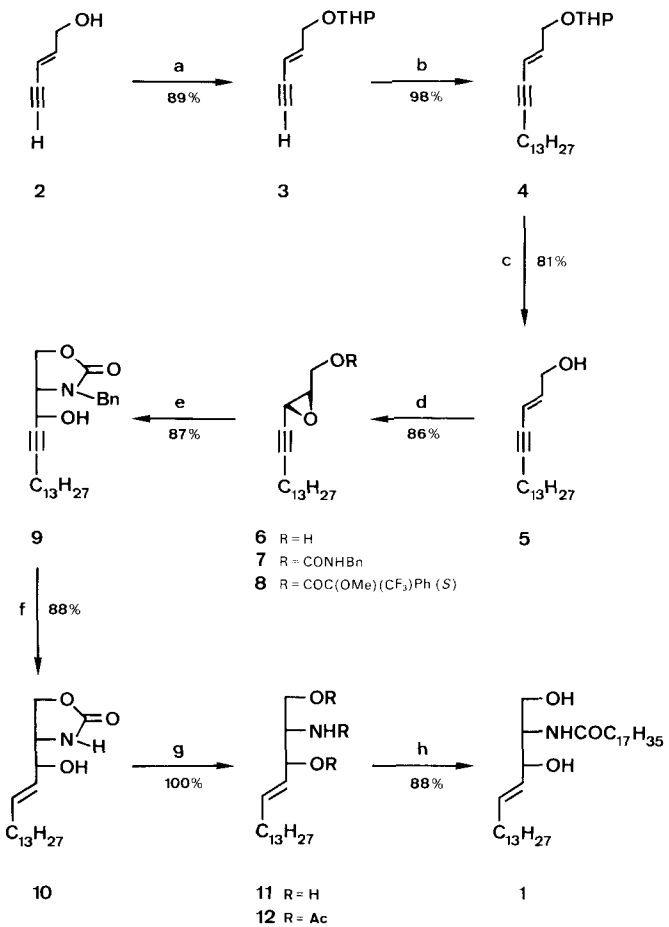
The enynol **2** was transformed into D-*erythro*-sphingosine **11** (7 steps, 46%) and into ceramide **1** (8 steps, 41% overall yield). The key steps were the mono-epoxidation of the enynol **5** (Ti(*t*-BuO)$_4$, (–)-D-diethyl tartrate, *t*-BuOOH) to **6** (86%, ≥ 98% ee), the regioselective intramolecular opening of the oxirane **6** *via* the benzylurethane **7**, and the reductive transformation of the acetylene **9** into the oxazolidinone **10** (Li, EtNH$_2$, 88%).

Introduction. – Glycosphingolipids are major constituents of cell membranes where they are assumed to play important roles as antigens and receptors [1] [2]. They are anchored in the outer cell membrane by their hydrophobic ceramide moiety. Recently, several syntheses of the enantiomerically pure ceramide **1** have been reported [3–6], since a convenient access to ceramides is still one of the limiting factors in the chemical syntheses of glycosphingolipids [7]. We have described an enantioselective synthesis of D-*erythro*-sphingosine **11** based on the *Sharpless* asymmetric epoxidation of the enynol **5** (→**6**) and the regioselective intramolecular opening of the oxirane ring of the *N*-benzylurethane **7** [8]. We now describe an improved modification of this reaction sequence which allows the synthesis of ceramide **1** in 8 steps and in 41% overall yield on a multigram scale *(Scheme)*.

Results. – We had originally [8] prepared the enynol **5** from pentadecyne and (*E*)-3-bromoprop-2-en-1-ol according to *Sonogashira et al.* [9]. A *C*-alkylation of the enynol **2**, however, appeared more straightforward *(Scheme)*. This enynol is available in one step from epichlorohydrin and sodium acetylide [10], but it could not be alkylated *via* its dianion due to the very different solubilities of the starting materials in common solvents under reaction conditions. However, the tetrahydropyranyl derivative **3** (89%) was conveniently alkylated to **4** with 1-bromotridecane (BuLi, THF/HMPA (hexamethylphosphoramide) 4:1, –80°, 98%). Deprotection of **4** gave the enynol **5** (81%), which was epoxidized according to *Katsuki* and *Sharpless* [11] using Ti(*t*-BuO)$_4$ [12] and (–)-D-diethyl tartrate ((–)-DET) as catalysts (CH_2Cl_2, –25°) to give the epoxide **6** (86%, ≥ 98% ee). The poor solubility of **5** in CH_2Cl_2 at –25° had originally been overcome by using 2,3-dimethyl-2-butene as cosolvent; we have found that a slow addition of **5** to the reaction mixture prevents the crystallization of **5** and gives equally good results *(cf. Exper. Part)*. The regioselective intramolecular opening of the oxirane **6** *via* the anion of **7**, formed according to the *Roush* procedure [13] (**6**, benzylisocyanate, NaH, THF), gave the oxazolidinone **9** (87%) in one step from **6**[1]). As reported earlier [8], *N*-debenzylation

[1]) In their synthesis of dihydrosphingosine, *Roush* and *Adam* [13] observed the formation of a 1:1 mixture of two isomeric oxazolidinones resulting from an intramolecular transacylation; under our conditions, however, only **9** was obtained. We thank Prof. Dr. *W. Roush* for a preliminary communication of his results.

Scheme



a) 3,4-Dihydro-2*H*-pyrane/TsOH. b) BuLi/THF/HMPA/$C_{13}H_{27}Br$, −78°. c) MeOH/THF/TsOH, r.t. d) Ti(*t*-BuO)$_4$/(−)-DET/*t*-BuOOH/CH_2Cl_2, −25°. e) Benzyl isocyanate/NaH/THF, 60°. f) Li/$EtNH_2$/*t*-BuOH, −78°. g) 2N NaOH/EtOH 1:1, 80°. h) *N*-succinimidyl octadecanoate/THF, r.t.

and selective reduction of the triple bond of the oxazolidinone **9** occurred under *Birch* conditions (Na or Li/NH_3), but although the *N*-benzyl group was rapidly cleaved, the reduction of the triple bond was incomplete. With Li in $EtNH_2$ at −80° (*Benkeser* conditions [14] [15]), however, the oxazolidinone **9** was cleanly reduced to **10** in one step (88%). An overreduction of the alkene **10** to the alkane was not observed[2]). Finally, base-catalyzed hydrolysis of the oxazolidinone **10** (2N NaOH/EtOH 1:1, 80°) afforded D-*erythro*-sphingosine **11** in nearly quantitative yields. The correct configuration of the synthetic sphingosine **11** was shown by its transformation into the crystalline triacetate **12**

[2]) The reduction is most conveniently performed in the apparatus depicted in the *Fig. (cf. Exper. Part)*.

with the correct melting point and optical rotation for the D-*erythro* compound *(cf. Exper. Part)* [16–18]. *N*-Acylation of **11** with *N*-succinimidyl octadecanoate (THF, 24 h at r.t.) [19] gave ceramide **1** (88%) [20]. All spectroscopic and analytical data of **1** are in accord with the literature [20].

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Experimental Part

General. All solvents were distilled before use. All reagents were obtained from *Fluka* (*purum* or *puriss. p.a.*). Solns. were evaporated at or below 40° in a *Büchi* rotary evaporator. TLC: *Merck* precoated silica gel *60 F-254* plates; detection by spraying with a 0.025M I_2 soln.in 10% aq. H_2SO_4 or by dipping the plates in 10% phosphomolybdic acid in EtOH followed by heating at *ca.* 200°. Column chromatography: silica gel *Merck* 60 (flash chromatography (FC): 40–63μ). M.p. (uncorrected): *Büchi-510* apparatus. Optical rotations: *Perkin-Elmer-241* polarimeter, 1-dm cell, at 365, 436, 546, 578, and 589 nm; the specific rotation at 589 nm was determined using a regression curve. IR: unless otherwise stated, 3% $CHCl_3$ solns.; *Perkin-Elmer-298* spectrometer. ^{1}H- and ^{13}C-NMR: *Varian-HA-100* (^{13}C (25 MHz)), *Varian-XL-200* (^{1}H (200 MHz), ^{13}C (50 MHz)), or *Bruker-AM-400* spectrometer (^{1}H (400 MHz), ^{13}C (100.6 MHz)); $CDCl_3$ solns. unless otherwise specified; δ values are indicated in ppm relative to TMS as internal standard. MS: *Varian-112S* apparatus (EI: 70 eV; CI: isobuten). Microanalysis: *FR-84* CHN analyser.

(E)-Pent-2-en-4-yn-1-ol (**2**). According to [10], **2** (67.7 g, 55%) was prepared from epichlorohydrin (138.7 g, 1.5 mol). B.p. 73°/20 Torr ([10]: b.p. 68°/12 Torr). IR (film): 3340*m* (br.), 3290*s*, 2920*w*, 2860*w*, 2100*w*, 1630*w*, 1090*m*, 1040*m*, 990*m*, 955*m*, 905*w*. ^{1}H-NMR (200 MHz, CD_3OD): 6.27 (*ddt*, *J* = 15.9, 0.6, 4.8, H–C(2)); 5.71 (*ddt*, *J* = 15.9, 2.1, 1.9, H–C(3)); 4.84 (*s*, exchangeable with D_2O, OH); 4.10 (*ddd*, *J* = 4.8, 1.9, 0.7, 2 H–C(1)); 3.19 (br. *d*, *J* = 2.1, H–C(5)). ^{13}C-NMR (50 MHz, CD_3OD): 145.3 (*d*, C(2)); 109.6 (*d*, C(3)); 82.6 (*s*, C(4)); 78.7 (*d*, C(5)); 62.6 (*t*, C(1)). EI-MS: 82 (7, M^+), 81 (88), 63 (23), 54 (65), 55 (100), 51 (41), 50 (39), 39 (97), 38 (20).

*(E)-3,4,5,6-Tetrahydro-2-(pent-2-en-4-yn-1-yloxy)-2*H*-pyrane* (**3**). According to [21], **2** (36.9 g, 0.45 mol) was converted to **3** (66.6 g, 89%). B.p. 78°/1 Torr ([21]: b.p. 78–80°/3 Torr).

*(E)-3,4,5,6-Tetrahydro-2-(octadec-2-en-4-yn-1-yloxy)-2*H*-pyrane* (**4**). BuLi (*Fluka*, 1.54M in hexane, 118.2 ml, 182 mmol) was added dropwise over 30 min to a soln. of **3** (29.9 g, 180.3 mmol) in abs. THF (720 ml) at −78° under Ar. After stirring at −78° for 15 min, 1-bromotridecane (57.0 g, 216.6 mmol) [22] in abs. HMPA (144 ml) was added slowly keeping the temp. below −65° (*ca.* 30 min). The heterogenous mixture was allowed to warm to r.t. overnight. Dilution with H_2O (5 l), extraction with Et_2O (5 × 300 ml), washing of the org. layer with H_2O (400 ml) and sat. NaCl soln. (400 ml), drying ($MgSO_4$), and evaporation *i.v.* afforded 74.3 g of crude **4**. For analysis, 4.96 g of crude **4** were purified by FC (hexane/AcOEt 20:1) to yield pure **4** (4.14 g, 98.7%) as a colorless oil. R_f (hexane/AcOEt 3:1) 0.69. ^{1}H-NMR (200 MHz): 6.10 (*dt*, *J* = 15.9, 5.5, H–C(2')); 5.73 (*quint. d*, *J* = 1.7, 15.9, H–C(3')); 4.64 (*t*, *J* = 3.0, H–C(2)); 4.25 (*ddd*, *J* = 13.7, 5.5, 1.7, H–C(1')); 4.00 (*ddd*, *J* = 13.7, 5.5, 1.7, H–C(1')); 3.89–3.79 (*m*, 1 H); 3.56–3.47 (*m*, 1 H); 2.29 (*dt*, *J* = 1.7, 7.0, 2 H–C(6')); 1.85–1.15 (*m*, 28 H); 0.88 (*t*, *J* = 6.7, 3 H–C(18')). Anal. calc. for $C_{23}H_{40}O_2$ (348.57): C 79.25, H 11.57; found: C 79.20, H 11.60.

(E)-Octadec-2-en-4-yn-1-ol (**5**). A soln. of crude **4** (74.3 g) and TsOH (2.0 g, 10.4 mmol) in MeOH (1.71 l) and THF (185 ml) was stirred at r.t. for 4 h. After addition of Na_2CO_3 (10 g, 94 mmol) and further stirring for 45 min, the mixture was filtered, the filtrate was treated with Et_3N (1 ml) and concentrated *i.v.* The red residue was dissolved in AcOEt (300 ml) and washed with H_2O (3 × 150 ml). Extraction of the aq. layer with Et_2O (3 × 150 ml), drying of the combined org. layers ($MgSO_4$), evaporation *i.v.*, FC (hexane/AcOEt 6:1) and crystallization (hexane, −10°) afforded **5** (38.6 g, 81%). M.p. 53–54°, R_f (hexane/AcOEt 4:1) 0.26. IR (KBr): 3380*m* (br.), 2960*m*, 2920*s*, 2850*s*, 2210*w*, 1635*w*, 1470*m*, 1090*m*, 1010*m*, 960*m*, 720*m*. ^{1}H-NMR (200 MHz): 6.17 (*dt*, *J* = 15.8, 5.5, H–C(2)); 5.73 (*quint. d*, *J* = 1.7, 15.8, H–C(3)); 4.19 (br. *d*, *J* = 5.5, 2 H–C(1)); 2.30 (*td*, *J* = 6.8, 1.7, 2 H–C(6)); 1.59 (*s*, exchangeable with D_2O, OH); 1.27 (*m*, 22 H); 0.89 (*t*, *J* = 6.8, 3 H–C(18)). ^{13}C-NMR (50 MHz): 140.0 (*d*, C(2)); 111.4 (*d*, C(3)); 91.5 (*s*, C(5)); 78.3 (*s*, C(4)); 63.0 (*t*, C(1)); 31.9 (*t*, C(16)); 29.6–28.7 (9*t*, C(7–15)); 22.7 (*t*, C(17)); 19.4 (*t*, C(6)); 14.0 (*q*, C(18)). EI-MS: 264 (2, M^+), 235 (≤ 1, M^+ − Et), 221 (1, M^+ − Pr), 207 (≤ 1), 165 (≤ 1), 151 (4), 137 (7), 123 (6), 109 (11), 95 (100), 81 (31), 67 (47), 57 (20), 55 (36), 43 (50). CI-MS: 265 ($[M + 1]^+$), 247 ($[M - H_2O]^+$), 135, 121. Anal. calc. for $C_{18}H_{32}O$ (264.46): C 81.75, H 12.20; found: C 81.78, H 12.30.

(2R,3R)-2,3-Epoxyoctadec-4-yn-1-ol (**6**). The soln. of freshly distilled Ti(*t*-BuO)$_4$ (41.1 ml, 107.6 mmol) [12] in abs. CH_2Cl_2 (100 ml) was cooled to −25°. (−)-DET (35 ml, 3.14M in abs. CH_2Cl_2, 110 mmol) was added during 15 min. After 15 min t −25°, the soln. of **5** (15.0 g, 56.7 mmol) in abs. CH_2Cl_2 (200 ml) was added at such a rate (*ca.* 60 min) that the mixture remained homogenous[3]) followed by the addition of *t*-BuOOH (34 ml, 3.79M in abs. toluene, 129 mmol) [23]. After 4–5 h at −30° and the addition of 10% aq. DL-tartaric acid (500 ml), the mixture was warmed to r.t. Dilution with Et_2O (1 l), washing with 10% aq. DL-tartaric acid (2 × 500 ml) and sat. NaCl soln. (2 × 750 ml), drying ($MgSO_4$), concentration *i.v.*, and drying under high vacuum afforded a yellow oil. FC (hexane/AcOEt 4:1) gave **5** (1.05 g, 6.6%) and pure **6** (12.62 g, 86% related to recovered **5**, ⩾ 98% ee determined by anal. HPLC of the *Mosher* ester of **8** (see below)). Two crystallizations from hexane (−10°) gave pure **6** (100% ee). Elution of the column with AcOEt and distillation gave pure (−)-DET (11.3 g, 50% recovery). **6**: M.p. 55–56°, R_f (hexane/AcOEt 2:1) 0.38, $[\alpha]_D^{25} = -2.0°$ (*c* = 2.05, $CHCl_3$), $[\alpha]_{365}^{25} = -41.5°$ (*c* = 2.05, $CHCl_3$). IR (KBr): 3300*m* (br.), 3180*m* (br.), 3000*w*, 2960*m*, 2920*s*, 2850*s*, 2240*w*, 1460*m*, 1320*m*, 1070*m*, 1030*m*, 875*s*, 725*m*. ^{1}H-NMR (200 MHz): 3.94 (*ddd*, *J* = 12.9, 4.9, 2.2; with D_2O: *dd*, *J* = 12.9, 2.2, H–C(1)); 3.70 (*ddd*, *J* = 12.9, 7.9, 3.4; with D_2O: *dd*, *J* = 12.9, 3.4, H–C(1)); 3.43 (*q*, *J* = 1.7, H–C(3)); 3.27 (*ddd*, *J* = 3.4, 2.2, 1.7, H–C(2)); 2.20 (*td*, *J* = 7.0, 1.7, 2 H–C(6)); 1.55 (*m*, exchangeable with D_2O, OH); 1.26 (*m*, 22 H); 0.88 (*t*, *J* = 6.7, 3 H–C(18)). ^{13}C-NMR (50 MHz): 85.6, 75.8 (2*s*, C(4),C(5)); 60.4 (*t*, C(1)); 60.0 (*d*, C(3)); 43.1 (*d*, C(2)); 31.9 (*t*, C(16)); 29.6–28.3 (9*t*, C(7–15)); 22.6 (*t*, C(17)); 18.7 (*t*, C(6)); 14.0 (*q*, C(18)). EI-MS: 249 (1, $M^+ - CH_2OH$), 168 (2), 149 (5), 135 (9), 121 (17), 107 (22), 95 (39), 93 (46), 83 (25), 81 (50), 79 (66), 69 (32), 67 (57), 57 (35), 55 (68), 43 (85), 41 (100). CI-MS: 281 ($[M + 1]^+$). Anal. calc. for $C_{18}H_{32}O_2$ (280.45): C 77.09, H 11.50; found: C 76.81, H 11.44.

The enantiomeric excess of **6** was determined by its transformation into the *Mosher* ester **8** [24]: a soln. of **6** (4 mg), 4-(dimethylamino)pyridine (4 mg) and (−)-(*S*)-α-methoxy-α-phenyl-α-(trifluoromethyl)acetyl chloride (4 µl) in abs. CH_2Cl_2 (0.5 ml) was stirred at r.t. for 10 min. After filtration through silica, concentration *i.v.* and dilution with hexane (0.75 ml), this material was directly analyzed by anal. HPLC (*Zorbax-Sil* 4.6 × 250 mm; hexane/Et_2O 98:2, 1.5 ml/min; detection: UV (254 nm); *k'* (2*R*,3*R*) = 3.50, *k'* (2*S*,3*S*) = 3.28).

(2R,3R)-2,3-Epoxyoctadec-4-ynyl N-Benzylcarbamate (**7**). For analysis, **7** was prepared according to [8]. M.p. 61°, R_f (hexane/AcOEt 4:1) 0.34, $[\alpha]_D^{25} = +10.3°$ (*c* = 1.0, $CHCl_3$). IR: 3450*m*, 3090*w*, 3060*w*, 3020*w*, 3000*m*, 2930*s*, 2860*s*, 2240*w*, 1725*s*, 1510*s*, 1465*s*, 1455*s*, 1440*m*, 1400*w*, 1380*w*, 1360*m*, 1315*m*, 1140*m*, 1080*m*, 1045*m*, 1030*m*, 995*m*, 915*w*, 880*m*. ^{1}H-NMR (200 MHz): 7.28 (*s*, 5 H); 5.3–4.7 (br. *s*, NH); 4.36 (*dd*, *J* = 12, 3, H–C(1)); 4.35 (*d*, *J* = 5.5, 2 H); 4.00 (*dd*, *J* = 12, 5, H–C(1)); 3.4–3.2 (*m*, 2 H); 2.20 (*t*, *J* = 6, 2 H–C(6)); 1.26 (br. *s*, 22 H); 0.88 (*t*, *J* = 6, 3 H–C(18)). ^{13}C-NMR (50 MHz): 155.7 (*s*, C=O); 138.1 (*s*); 128.4 (2*d*); 127.2 (3*d*); 85.4, 75.5 (2*s*, C(4),C(5)); 63.7 (*t*, C(1)); 57.4 (*d*, C(3)); 45.0 (*t*, PhCH_2); 43.7 (*d*, C(2)); 31.8 (*t*, C(16)); 29.6 (4*t*); 29.4–28.3 (5*t*); 22.6 (*t*, C(17)); 18.6 (*t*, C(6)); 14.1 (*q*, C(18)). EI-MS: 413 (2, M^+), 91 (100). Anal. calc. for $C_{26}H_{39}NO_3$ (413.60): C 75.50, H 9.50, N 3.39; found: C 75.35, H 9.68, N 3.19.

(4S,1'R)-3-Benzyl-4-(1'-hydroxyhexadec-2'-ynyl)-1,3-oxazolidin-2-one (**9**). NaH (2.14 g, 89.15 mmol; commercial NaH suspension in oil was washed with dry hexane and dried *i.v.*) was added under N_2 to a soln. of **6** (10.0 g, 35.66 mmol) and benzyl isocyanate (5.70 g, 42.79 mmol) [25] in abs. THF (175 ml) [13]. After 1 h at r.t., the mixture was heated to 60° and kept at this temp. for 3 h. Excess NaH was destroyed carefully with AcOH at 5°. The remaining mixture was diluted with Et_2O (300 ml) and washed with H_2O (2 × 80 ml), sat. $NaHCO_3$ (1 × 80 ml), and sat. NaCl soln. (1 × 80 ml). Drying ($MgSO_4$) and evaporation *i.v.* afforded crude **9** (17.2 g). After FC (hexane/AcOEt 4:1→2:1) and crystallization (hexane/−2°) pure **9** (12.84 g, 87%) was obtained. M.p. 51–52°, R_f (hexane/AcOEt 2:1) 0.21, $[\alpha]_D^{25} = -28.9°$ (*c* = 1.0, $CHCl_3$). IR: 3610*m*, 3400*w* (br.), 3000*m*, 2980*m*, 2930*s*, 2850*s*, 2230*w*, 1745*s*, 1605*w*, 1420*m*, 1380*m*, 1355*m*, 1220*m* (br.), 1135*m*, 1110*m*, 1095*m*, 1070*m*, 1030*m*, 970*w*. ^{1}H-NMR (400 MHz): 7.34 (*m*, C_6H_5); 4.74 (*d*, *J* = 15.3, 1 H, PhCH_2); 4.45 (*ddt*, *J* = 4.0, 3.1, 1.9, with D_2O: *dd*, *J* = 3.1, 1.9, H–C(1')); 4.40 (*dd*, *J* = 9.2, 5.3, H–C(5)); 4.35 (*d*, *J* = 15.3, 1 H, PhCH_2); 4.29 (*t*, *J* = 9.1, H–C(5)); 3.74 (*ddd*, *J* = 9.1, 5.3, 3.1, H–C(4)); 2.16 (*dt*, *J* = 1.9, 7.2, 2 H–C(4')); 1.89 (*d*, *J* = 4.0, exchangeable with D_2O, OH); 1.47 (*quint.*, *J* = 7.2, 2 H–C(5')); 1.25 (*m*, 20 H); 0.88 (*t*, *J* = 6.8, 3 H–C(16')). ^{13}C-NMR (50 MHz): 158.8 (*s*, C(2)); 136.2 (*s*), 128.9 (*d*), 128.1 (*d*), 128.0 (*d*, C_6H_5); 88.9, 76.4 (2*s*, C(2'),C(3')); 63.4 (*t*, C(5)); 61.2 (*d*, C(1')); 58.9 (*d*, C(4)); 46.8 (*t*, CH_2N); 31.9 (*t*, C(14')); 29.6–28.3 (9*t*, C(5'–13')); 22.6 (*t*, C(15')); 18.6 (*t*, C(4')); 14.1 (*q*, C(16')). EI-MS: 384 (1, $M^+ - C_2H_5$), 245 (2), 176 (77), 91 (100). CI-MS: 414 ($[M + 1]^+$). Anal. calc. for $C_{26}H_{39}NO_3$ (413.60): C 75.50, H 9.50, N 3.39; found: C 75.75, H 9.51, N 3.38.

(2'E,4S,1'R)-4-(1'-Hydroxyhexadec-2'-enyl)-1,3-oxazolidin-2-one (**10**). The reduction was run in the apparatus described in the *Fig.* Under a dry Ar atmosphere **9** (5.0 g, 12.1 mmol) was added at −30° to a mixture of abs. *t*-BuOH (50 ml) and $EtNH_2$ (250 ml, distilled through a filter of glass wool). When **9** had dissolved, the soln. was

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[3]) Uncontrolled addition caused crystallization of **5**.

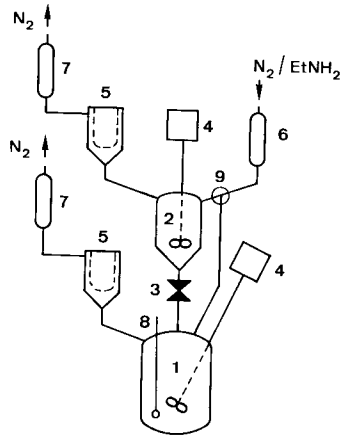

Figure
1) Reactor (*t*-BuOH and **9** in $EtNH_2$)
2) Reactor with bottom outlet (Li in $EtNH_2$)
3) Dosage valve
4) Stirrer (glass)
5) CO_2-Condenser
6) Gas filter (glass wool)
7) Drying tubes (KOH)
8) Thermometer
9) 2-Way cock

cooled to −80°. At this temp., a conc. soln. of Li metal (*Merck, ca.* 10 g)[4]) in $EtNH_2$ (450 ml, prepared in reactor 2, *cf. Fig.*) was added at such a rate that the blue color of the Li/$EtNH_2$ soln. continuously disappeared. At the end of the reduction, such an excess of the Li/$EtNH_2$ soln. was added that the blue color persisted for 2 h. After 2 h at −80°, and after addition of NH_4Cl (30 g) and CH_2Cl_2 (1 l), the mixture was slowly warmed to r.t. Dilution with H_2O (1 l), extraction of the aq. layer with CH_2Cl_2 (3 × 500 ml), washing of the org. layer with H_2O (3 × 800 ml) and sat. NaCl soln. (300 ml), drying ($MgSO_4$), concentration *i.v.* and recrystallization (hexane, +4°) afforded **10** (3.32 g, 84%). FC (hexane/AcOEt 1:4) of the mother liq. afforded further **10** (155 mg, 4%). M.p. 73–74°, R_f (AcOEt) 0.43, $[\alpha]_D^{25} = -0.8°$ (*c* = 2, $CHCl_3$). IR: 3600*w*, 3450*m*, 3330*m* (br.), 2920*s*, 2850*s*, 1750*s*, 1665*w*, 1465*m*, 1400*m*, 1375*w*, 1220*m*, 1090*m*, 1035*m*, 975*m*, 935*m*. ^{1}H-NMR (400 MHz): 5.82 (*dt*, *J* = 15.5, 7.0, H–C(3′)); 5.46 (br. *s*, exchangeable with D_2O, NH); 5.37 (*dd*, *J* = 15,5, 6.6, H–C(2′)); 4.41 (*t*, *J* = 8.8, H–C(5)); 4.31 (*dd*, *J* = 8.8, 4.9, H–C(5)); 4.06 (*m*, with D_2O: *dd*, *J* = 6.6, 5.0, H–C(1′)); 3.82 (*dd*, *J* = 8.8, 4.9, H–C(4)); 2.18 (br. *s*, exchangeable with D_2O, OH); 2.04 (*q*, *J* = 7.0, 2 H–C(4′)); 1.24 (*m*, 22 H); 0.86 (*t*, *J* = 6.7, 3 H–C(16′)). ^{13}C-NMR (50 MHz): 160.5 (*s*, C(2)); 136.0 (*d*, C(2′)); 126.4 (*d*, C(3′)); 72.7 (*d*, C(1′)); 66.1 (*t*, C(5)); 56.4 (*d*, C(4)); 32.4 (*t*, C(4′)); 31.9 (*t*, C(14′)); 29.6–28.9 (9*t*, C(5′–13′)); 22.6 (*t*, C(15′)); 14.1 (*q*, C(16′)). EI-MS: 294 (1, $M^+ - CH_3O$), 250 (5), 239 (15), 123 (8), 109 (20), 95 (38), 87 (57), 86 (27), 57 (65), 43 (100). CI-MS: 326 ($[M + 1]^+$). Anal. calc. for $C_{19}H_{35}NO_3$ (325.49): C 70.11, H 10.84, N 4.30; found: C 69.95, H 11.04, N 4.25.

D-erythro-*Sphingosine* (= *(4E,2S,3R)-2-aminooctadec-4-en-1,3-diol;* **11**). A mixture of **10** (500 mg, 1.54 mmol), 2N NaOH (12 ml), and EtOH (12 ml) was stirred at 80° for 2.5 h. Cooling to r.t., dilution with Et_2O (100 ml), extraction of the org. layer with 2N NaOH (3 × 30 ml) and sat. NaCl soln., followed by drying ($MgSO_4$) and concentration *i.v.* afforded crude **11** (489 mg, *ca.* 100%).

1-O,2-N,3-O-Tri-acetyl-D-erythro-*sphingosine* (**12**). Crude **11** (92 mg, 0.28 mmol) in abs. CH_2Cl_2 (3.6 ml) was acetylated with Ac_2O (180 µl, 1.92 mmol), Et_3N (720 µl), and 4-(dimethylamino)pyridine (1 mg) during 1.5 h at r.t. Addition of MeOH (1 ml), stirring for 10 min, dilution with Et_2O, washing of the org. layer with sat. NaCl soln. (3 × 30 ml), drying ($MgSO_4$), and evaporation *i.v.* afforded crude **12** (130 mg), which was recrystallized twice from hexane (+4°). Yield: 107 mg. M.p. 101–102°, R_f (hexane/AcOEt 1:1) 0.15, $[\alpha]_D^{25} = -12.8°$ (*c* = 1, $CHCl_3$). Reported values for synthetic **12** [16]: m.p. 103.5–104°, $[\alpha]_D^{24} = -12.8°$; data for natural **12** [17]: m.p. 101–102°, $[\alpha]_D^{25} = -11.7°$.

N-*Octadecanoyl*-D-erythro-*sphingosine* (**1**). A mixture of crude **11** (489 mg, 1.54 mmol) and *N*-succinimidyl octadecanoate (601 mg, 1.57 mmol) [19] in abs. THF (50 ml) was stirred at r.t. for 24 h. Concentration *i.v.*, FC ($CHCl_3$/MeOH 100:0→95:5) and crystallization (EtOH) afforded **1** (767 mg, 88%). M.p. 97–98° ([26]: 97–98°), R_f ($CHCl_3$/MeOH 95:5) 0.25, $[\alpha]_D^{25} = -3.1°$ (*c* = 1.1, $CHCl_3$). IR (KBr): 3350*m* (br.), 3290*s*, 2960*m*, 2920*s*, 2850*s*, 1635*s*, 1545*m*, 1465*m*, 1375*w*, 1285*w*, 1130*w*, 1095*w*, 1065*w*, 1040*w*, 970*w*, 720*w*. ^{1}H-NMR (400 MHz): 6.21 (*d*, *J* = 7.3, exchangeable with D_2O, NH); 5.76 (*ddt*, *J* = 15.4, 1.1, 6.8, H–C(5)); 5.51 (*ddt*, *J* = 15.4, 6.5, 1.3, H–C(4)); 4.31 (*m*, H–C(3)); 3.93 (*dt*, *J* = 11.2, 3.6, H–C(1)); 3.88 (*dq*, *J* = 7.4, 3.6, with D_2O: *q*, *J* = 3.6, H–C(2)); 3.68 (*ddd*, *J* = 11.2, 7.5, 3.4, H–C(1)); 2.70 (*m*, 2 H, exchangeable with D_2O, OH); 2.21 (*t*, *J* = 7.6, 2 H–C(2′));

[4]) Small pieces of Li were dipped into EtOH and then into hexane before addition to $EtNH_2$.

2.03 (*dt*, *J* = 7.0, 2 H–C(6)); 1.23 (*m*, 52 H); 0.85 (*t*, *J* = 7.0, CH_3(18),CH_3(18')). ^{13}C-NMR (50 MHz, $CDCl_3$/CD_3OD 4:1 (v/v) [20]): 174.5 (*s*, C(1')); 133.7 (*d*, C(4)); 128.7 (*d*, C(5)); 73.1 (*d*, C(3)); 61.4 (*t*, C(1)); 54.7 (*d*, C(2)); 36.4 (*t*, C(2')); 36.1 (*t*, C(6)); 31.7 (2*t*, C(16),C(16')); 29.4–28.9 (21*t*, C(7–15), C(4'–15')); 25.5 (*t*, C(3')); 22.4 (2*t*, C(17),C(17')); 13.7 (2*q*, C(18),C(18')). CI-MS: 566 ([*M* + 1]$^+$), 548 ([*M* + 1 – H_2O]$^+$), 309, 281. Anal. calc. for $C_{36}H_{71}NO_3$ (565.97): C 76.40, H 12.64, N 2.47; found: C 76.20, H 12.50, N 2.25.

REFERENCES

[1] S. Hakomori, *Ann. Rev. Biochem.* **1981,** *50,* 733.
[2] S. Hakomori, in 'Handbook of Lipid Research: Sphingolipid Biochemistry', Ed. J. N. Kanfer and S. Hakomori, Plenum Press, New York, 1983, Vol. 3, pp. 1–150.
[3] E. J. Reist, P. H. Christie, *J. Org. Chem.* **1970,** *35,* 4127.
[4] R. R. Schmidt, R. Kläger, *Angew. Chem.* **1982,** *94,* 215.
[5] R. S. Garigipati, S. M. Weinreb, *J. Am. Chem. Soc.* **1983,** *105,* 4499.
[6] K. Koike, Y. Nakahara, T. Ogawa, *Glycoconjugate J.* **1984,** *1,* 107.
[7] M. Sugimoto, T. Ogawa, *Glycoconjugate J.* **1985,** *2,* 5.
[8] B. Bernet, A. Vasella, *Tetrahedron Lett.* **1983,** *24,* 5491.
[9] K. Sonogashira, Y. Tohda, N. Hagihara, *Tetrahedron Lett.* **1975,** 4467.
[10] L. Brandsma, in 'Preparative Acetylenic Chemistry', Elsevier Publishing Company, Amsterdam, 1971, pp. 63–64.
[11] T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.* **1980,** *102,* 5974.
[12] R. C. Mehrotra, *J. Am. Chem. Soc.* **1954,** *76,* 2266.
[13] W. R. Roush, M. A. Adam, *J. Org. Chem.* **1985,** *50,* 3752.
[14] R. A. Benkeser, G. Schroll, D. M. Sauve, *J. Am. Chem. Soc.* **1955,** *77,* 3378.
[15] E. M. Kaiser, *Synthesis* **1972,** 391.
[16] D. Shapiro, H. Segal, H. M. Flowers, *J. Am. Chem. Soc.* **1958,** *80,* 1194.
[17] H. E. Carter, W. P. Norris, F. J. Glick, G. E. Philips, R. Harris, *J. Biol. Chem.* **1947,** *170,* 269.
[18] P. Tkaczuk, E. R. Thornton, *J. Org. Chem.* **1981,** *46,* 4393.
[19] Y. Lapidot, S. Rappoport, Y. Wolman, *J. Lipid Res.* **1967,** *8,* 142.
[20] F. Sarmientos, G. Schwarzmann, K. Sandhoff, *Eur. J. Biochem.* **1985,** *146,* 59.
[21] J. S. Cowie, P. D. Landor, S. R. Landor, N. Punja, *J. Chem. Soc., Perkin Trans. 1* **1972,** 2197.
[22] J. Cason, D. M. Walba, *J. Org. Chem.* **1972,** *37,* 669.
[23] J. G. Hill, B. E. Rossiter, K. B. Sharpless, *J. Org. Chem.* **1983,** *48,* 3607.
[24] J. A. Dale, D. L. Dull, H. S. Mosher, *J. Org. Chem.* **1969,** *34,* 2543.
[25] J. W. Van Reijendam, F. Baardman, *Synthesis* **1973,** 413.
[26] E. Klenk, R. T. C. Huang, *Hoppe-Seylers Z. Physiol. Chem.* **1970,** *351,* 335.