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Enantioselective Synthesis of Homosphingosine Derivatives from L-Aspartic Acid

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Abstract: The sterically demanding 9-phenylfluorenyl N-protection of a number of amino acids allows the formation of amino acid derived β -ketophosphonate reagents and their Horner–Wadsworth–Emmons olefination. In an attempt to develop a synthesis of D-erythro-homosphingosine in enantiopure form, we have shown that the reactivity of the intermediates is influenced by the distinctive conformational requirements of this large protecting group.

Key words: diastereoselectivity, enantioselectivity, sphingosine, ceramide

Sphingolipids constitute a major membrane component of eukaryotic cells, and important roles in cell recognition, differentiation, cell–cell contact and cell growth have been recognized.¹ The backbone of sphingolipids is D-erythro-sphingosine (**1**; Figure 1); ceramides (**2**) are formed by acylation of the nitrogen with a fatty acid (RCOOH). More complex sphingolipids are formed by further addition of a polar head-group to the primary hydroxy group of sphingosine or ceramide.²

Figure 1

We have previously developed a general and efficient synthesis of sphingosine based on a serine derived β -ketophosphonate.³ However, although this chemistry is reliable and robust enough to be used for sphingolipid synthesis and for several other targets, it still suffers from the fact that the original α -center of the amino acid remains vulnerable to epimerization.⁴ Although this epimerization can, in most cases, be suppressed to levels where it is not observed, in some highly sensitive cases these problems can be insurmountable.⁵

9-Phenyl-9-fluorenyl (Pf) protection of the amino group has been shown to efficiently protect the carbon atom next

to the nitrogen from epimerization, even in difficult cases.⁶ We therefore became interested in investigating whether the β -ketophosphonate chemistry we have developed for the Boc-protected amino acid derivatives⁷ can be translated to the corresponding Pf-protected amino acid derivatives. Towards this end, we chose a homosphingosine derivative as a first target molecule for testing the methodology.

Homosphingosine derivatives and homoceramides (Figure 2) have been the targets of only a few synthetic studies.⁸ A general synthetic approach to homoceramides could lead to the development of a new class of anti-cancer drugs,⁸ thus the synthesis of D-erythro-homosphingosine (**3**) was investigated in this work, with the aim of subsequently preparing a range of homoceramides.

Figure 2

We envisioned that D-erythro-homosphingosine (**3**) could be conveniently constructed from L-aspartic acid by internal asymmetric induction, whereby the two carboxy functionalities of L-aspartic acid could be transformed into the 2-amino-1,4-diol structure present in **3**.

Our general strategy (Scheme 1) was to convert a protected L-aspartic acid into homoserine **5**, and protect it as the *N,O*-acetal **6**. The C-5 carbon would then be added with the formation of a β -ketophosphonate **7**, and the *E* double bond as well as the aliphatic chain would be introduced through a modified Horner–Wadsworth–Emmons reaction⁷ to give enone **8**. Selective reduction⁹ of the carbonyl and removal of the protecting groups would lead to the desired compound homosphingosine (**3**). The diastereoselectivity of the reduction was expected to be enhanced by bulky protecting groups: *tert*-butyl ester of the α -amino acid and 9-phenyl-9-fluorenyl of the amino group.

Anticipating difficulties during the phosphonation/chain-elongation of **6b** because of steric hindrance from the *tert*-butyl and 9-phenyl-9-fluorenyl groups, we initially

Scheme 1

Scheme 2 Reagents and conditions: i) $\text{Cl}_3\text{C}(\text{NH})\text{OtBu}$, CH_2Cl_2 , 35% from L-Ala; ii) $\text{LiCH}_2\text{P}(\text{=O})(\text{OMe})_2$, THF, -78°C , 52%.

conducted a model study with the L-alanine derived compound **11** (Scheme 2).

Crude *N*-Pf-protected **9**^{6b,10} was directly esterified to give compound **10** in 42% yield as yellow crystals.¹¹ An analytical sample was recrystallized from hexanes, and the X-ray crystal structure was determined (Figure 3).¹²

Phosphonation¹³ of the model compound **10**, avoiding excess *n*-butyllithium [dimethyl methylphosphonate (5.5 equiv), *n*-BuLi (5.27 equiv)], gave **11** as a yellow oil in an acceptable, although low, yield of 52%. Encouraged by this finding, we embarked on the actual route with homoserine. *N*-Pf-homoserine *tert*-butyl ester (**5b**)¹⁴ was fully protected as the cyclic hemiaminal **6b** utilizing the

Scheme 3

Scheme 4 Reagents and conditions: i) TMSCl , Et_3N , $\text{Pb}(\text{NO}_3)_2$, PbBr , CH_2Cl_2 , r.t., 89%; ii) BH_3 ·THF, THF, 40°C , 52%; iii) CH_2O , *p*-TsOH, THF, r.t., 85%; iv) $\text{LiCH}_2\text{P}(\text{=O})(\text{OMe})_2$, THF, -78°C , 64%; v) tetradecanal, K_2CO_3 , MeCN, r.t., 67%; vi) DIBAL-H, toluene, -78°C , 55%.

method developed by Rapoport (Scheme 3).¹⁵ Encouraged by the successful phosphonation of model compound **10**, we submitted **6b** to the conditions developed by Lee [dimethyl methylphosphonate (6.5 equiv) and *n*-BuLi (9.0 equiv)].¹² However, **6b** failed to react under these conditions, and led to only extensive decomposition under more forcing reaction conditions. Apparently, the steric hindrance caused by the *tert*-butyl and 9-phenylfluorenyl groups is enhanced by the rigid acetal ring of **6b**.

Having failed to form the β -ketophosphonate from acetal **6b**, we decided to opt for the less hindered methyl ester (Scheme 4). Thus, α -methyl aspartate¹⁶ was converted into the crystalline α -methyl *N*-Pf-aspartate **12** (Figure 4).

Borane reduction of **12** to the *N*-Pf-homoserine methyl ester (**5a**)¹⁷ was performed using the Alberg protocol.¹⁸ The starting material **12** needed to be heated and stirred for 2–3 days to yield 52% of **5a**. *N,O*-Acetal protection of **5a** proceeded smoothly, giving **6a** in 85% yield. Simple recrystallization from hexanes gave pure **6a**. The crystal structure of **6a** is shown in Figure 5. It is noteworthy that both the methyl ester group and the bulky nitrogen pro-

Figure 3 X-ray crystal structure of **10**

Figure 4 X-ray crystal structure of **12**

Figure 5 X-ray crystal structure of **6a**

protecting group occupy axial positions. This unusual steric disposition of the groups would surely affect the chemistry of such compounds, as we were soon to observe.

Phosphonation of **6a** with 3.3 equivalents of dimethyl methylphosphonate and 3.0 equivalents of *n*-BuLi gave **7** in 64% yield. Horner–Wadsworth–Emmons olefination gave the *Z*-olefin **8** in 67% yield.

DIBAL-H reduction⁹ of **8** in toluene was expected to give the desired *anti*-alcohol. However, presumably due to the conformational features of the ring substituents, the oxazinane underwent reductive ring opening, leaving a methyl group attached to the nitrogen (determined by HMBC 2D-NMR). The yield was modest, reaching only 55%, but only the *anti* diastereomer could be detected by ¹H NMR after HPLC.¹⁹

In conclusion, we have shown that the β-ketophosphonate/Horner–Wadsworth–Emmons olefination chemistry developed previously for Boc-protected serine derivatives can be successfully transferred to 9-phenylfluorenyl-protected amino acid derivatives for the synthesis of homosphingosine derivatives. Olefination followed by highly diastereoselective reduction of the enone were accompanied by the unwanted reductive cleavage of the hemiaminal moiety. The development of milder methods for the reduction of the sensitive enone will be reported in due course.

CH₂Cl₂ and MeCN were distilled over CaH₂, THF was distilled over sodium/benzophenone, MeOH over Mg(OMe)₂, toluene over sodium, EtOH over Mg(OEt)₂, and Et₃N over NaOH pellets. Pyridine was simply distilled. All other reagents were used as obtained from the supplier without further purification. Reactions were performed under an inert argon atmosphere, and anhydrous conditions were applied if dried solvents were used. Flash chromatography silica and TLC plates (silica gel 60 F₂₅₄) were obtained from Merck. TLC plates were visualized under UV irradiation (254 nm). Melting points were measured with a Gallenkamp melting point apparatus MFB 595 and are uncorrected. NMR spectra were recorded with a Bruker Avance 400 (¹H 400 MHz, ¹³C 100 MHz) instrument, and chemical shifts (δ) are reported in ppm relative to TMS (β = 0 ppm). ¹³C NMR spectra were calibrated with the solvent signal. Infrared (IR) spectra were measured with a Perkin–Elmer Spectrum One instrument. Optical rotations were measured with a Perkin–Elmer Polarimeter 343, and elemental analyses (CHN) were performed with a Perkin–Elmer Elemental Analyzer 2400 CHN. The high resolution mass spectra (HRMS) were measured at the University of Oulu with a Micromass LCT mass instrument. HPLC was performed with the following conditions: Shandon Hypersil (5 μm, 250 × 4.6 mm), Shandon Hyperprep (12 μm, 250 × 4.6 mm) and Shandon Hyperprep (12 μm, 250 × 10 mm); EtOAc–hexanes, 17–20%; flow rates: 1–5 mL/min.

(*S*)-tert-Butyl 3-(9-Phenyl-9H-fluorene-9-yl)-1,3-oxazinane-4-carboxylate (6b**)**

Alcohol **5b** (0.102 g, 0.25 mmol, 1.0 equiv) was dissolved in distilled THF (2 mL) under argon. Formaldehyde (0.3 mL, ~4 mmol, 16.0 equiv, 35–40% in H₂O) was added, followed by a catalytic amount of *p*-TsOH·H₂O. The mixture was stirred for 1 d, and quenched with sat. aq NaHCO₃ (2 mL). The mixture was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with brine (5 mL), dried over MgSO₄ and evaporated. Purification by flash chromatography (EtOAc–hexanes, 5%) yielded **6b**.

Yield: 0.106 g (99%); colorless oil; *R*_f = 0.56 (EtOAc–hexanes, 50%); [*α*]_D²⁰ +151 (*c* 0.43, CHCl₃).

IR (NaCl): 3060–2843, 1724 cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ = 7.82–7.80 (m, 1 H, ArH), 7.70–7.67 (m, 1 H, ArH), 7.61–7.59 (m, 1 H, ArH), 7.52–7.13 (m, 10 H, ArH), 4.96 (d, *J* = 11.7 Hz, 1 H, N-CHH-O), 4.84 (d, *J* = 11.7 Hz, 1 H, N-CHH-O), 3.77 (m, 1 H, C-CHH-O), 3.63 (m, 1 H, C-CHH-O), 3.29 (m, 1 H, ROOC-CHNR-C), 1.48 (s, 9 H, *t*-Bu), 1.44–1.31 (m, 2 H, CHN-CH₂-CH₂O).

¹³C NMR (CDCl₃, 100 MHz): δ = 172.2 (CO, *t*-Bu ester), 149.2, 147.9, 143.8, 140.7, 139.2, 128.7, 128.4, 128.3, 128.2, 127.6, 127.5, 127.3, 127.1, 125.6, 119.8, 119.6 (Ar), 80.7 (*t*-Bu quat.), 78.1 (two peaks, Pf quat. aliph. and NPfCH₂OCH₂), 64.4 (CHNCH₂CH₂OCH₂NPf), 54.5 (*t*-BuOOCCHNPFCH₂), 28.1 [(CH₃)₃CO], 25.4 (CHCH₂CH₂OCH₂NPf).

HRMS: *m/z* [M + Na] calcd. for C₂₈H₂₉NO₃Na: 450.2045; found: 450.2052.

(S)-tert-Butyl 2-[(9-Phenyl-9H-fluoren-9-yl)amino]alanate (10)

Crude (*S*)-*N*-[9-(9-phenylfluorenyl)]alanine (**9**; 2.1 g, max. 6 mmol) was dissolved in anhydrous CH₂Cl₂ (15 mL) under argon and the solution was transferred to the reaction flask using a syringe. *O*-tert-Butyl trichloroacetimidate (2.2 mL, 12.3 mmol) was added, and the solution was stirred for 3 d. The mixture was filtered and evaporated, then the residue was submitted to the same conditions as described above, and mixed for 3 d. The mixture was filtered, evaporated and purified by flash chromatography (EtOAc–hexanes, 5%), MPLC (EtOAc–hexanes, 5%) and MPLC (EtOAc–hexanes, 2%) to give **10**.

Yield: 1.03 g (35% from L-alanine); yellow crystalline solid; mp 114 °C; [α]_D²⁰ –153 (*c* 0.6, CHCl₃).

IR (KBr): 3063–2873, 1727 cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ = 7.68–7.65 (m, 2 H, ArH), 7.42–7.15 (m, 11 H, ArH), 3.08 (br s, 1 H, CNHPf), 2.67 (q, *J* = 7.1 Hz, 1 H, ROOCCNHPf-CH₃), 1.20 [s, 9 H, (CH₃)₃COC(=O)], 1.08 (d, *J* = 7.1 Hz, 3 H, ROOCCNHPf-CH₃).

¹³C NMR (CDCl₃, 100 MHz): δ = 175.8 (CO ester), 149.8, 149.6, 144.8, 140.6, 140.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.0, 126.1, 125.9, 125.2, 119.9, 119.6 (Ar), 80.3 (quat. aliph.), 73.1 (quat. aliph.), 52.0 (ROOCCNHPfCH₃), 27.8 [(CH₃)₃C], 22.1 (CHNPFCH₃).

Anal. Calcd for C₂₆H₂₇NO₂: C, 81.01; H, 7.06; N, 3.63. Found: C, 81.16; H, 7.01; N, 3.53.

(S)-Dimethyl [2-Oxo-2-[3-(9-phenyl-9H-fluoren-9-yl)-1,3-oxazin-4-yl]ethyl]phosphonate (11)

To a stirred solution of dimethyl methylphosphonate (0.153 mL, 1.43 mmol, 5.5 equiv) in anhydrous THF (4 mL) under argon at –78 °C, was added dropwise *n*-BuLi (2.5 M in hexanes, 0.55 mL, 1.37 mmol, 5.27 equiv). The mixture was stirred for 10 min then a solution of **10** (0.10 g, 0.26 mmol, 1.0 equiv) in anhydrous THF (1 mL) was added dropwise. The solution was stirred for 5.5 h while slowly warming to –6 °C. The reaction was quenched with sat. aq NH₄Cl (3 mL), which formed a white solid. A little water was added to dissolve the solid, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na₂SO₄ and evaporated. Purification by flash chromatography (EtOAc–hexanes, 50→75%) gave **11**.

Yield: 0.059 g (52%); yellow oil; *R*_f = 0.3 (EtOAc, 100%); [α]_D²⁰ –55 (*c* 0.4, CH₂Cl₂).

IR (NaCl): 3295, 2954, 1713, 1258 cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ = 7.70–7.66 (m, 2 H, ArH), 7.41–7.13 (m, 11 H, ArH), 3.62 [dd, *J* = 11.2, 15.19 Hz, 6 H, (CH₃)₂P(=O)-C₄], 3.12 (br s, 1 H, C-NHPf), 2.81 (q, *J* = 7.0 Hz, 1 H, C(=O)CHNHPfCH₃), 2.77 [dd, *J* = 14.7, 22.2 Hz, 1 H,

(CH₃)₂P(=O)CHHC(=O)-C₂], 2.60 [dd, *J* = 14.7, 21.2 Hz, 1 H, (CH₃)₂P(=O)CHHC(=O)-C₂], 1.02 (d, *J* = 7.0 Hz, 3 H, CH₃CHNHPf-C₂).

¹³C NMR (CDCl₃, 100 MHz): δ = 205.83 and 205.77 (C=O), 149.6, 149.3, 144.3, 140.8, 140.1, 128.4, 128.3, 128.2, 128.1, 127.8, 127.2, 126.1, 126.0, 125.1, 119.9, 119.8 (Ar), 73.0 (quat. aliph.), 58.05 and 58.02 (COCHNHPfCH₃), 52.81, 52.74, 52.68 and 52.62 [(CH₃)₂P(=O)], 38.3 and 37.0 [PCH₂C(=O)], 20.0 (CHNHPfCH₃).

HRMS: *m/z* [M + Na] calcd. for C₂₅H₂₆NO₄NaP: 458.1497; found: 458.1479.

(2S)-α-Methyl N-(9-Phenyl-9H-fluoren-9-yl)aspartate (12)

To a stirred solution of (*S*)-α-methyl aspartate (4.0 g, 27 mmol, 1.00 equiv) in anhydrous CH₂Cl₂ (50 mL) was added TMSCl (3.7 mL, 29 mmol, 1.07 equiv) under argon. After 2 h, Et₃N (8.0 mL, 58 mmol, 2.15 equiv) was added and, after another 15 min, Pb(NO₃)₂ (5.96 g, 18 mmol, 0.67 equiv) and a solution of 9-bromo-9-phenylfluorene (11.57 g, 36 mmol, 1.33 equiv) in anhydrous CH₂Cl₂ (50 mL) were added. After stirring for 3 d, MeOH (14 mL) was added and, after another 15 min, the mixture was filtered and evaporated. The viscous residue was partitioned between aq. citric acid (5%, 150 mL) and Et₂O (150 mL). The layers were separated and the aqueous layer was extracted with Et₂O (2 × 100 mL). The combined organic layers were washed with brine (60 mL), dried over MgSO₄ and evaporated. The residue was dissolved in Et₂O (70 mL) and extracted with sat. aq NaHCO₃ (5 × 30 mL). The aqueous layers were acidified with concd H₃PO₄ to pH 5 and extracted with Et₂O (2 × 50 mL). The combined organic layers were washed with brine (30 mL), dried over MgSO₄ and evaporated to give **12**. An analytical sample was recrystallised from CH₂Cl₂–hexanes and again from EtOAc–hexanes.¹²

Yield: 9.3 g (89%); yellow foam; mp 128–129 °C; [α]_D²⁰ –297 (*c* 1.0, CHCl₃).

IR (KBr): 3059–2956, 2636, 1736 cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ = 7.77–7.71 (m, 2 H, ArH), 7.46–7.22 (m, 11 H, ArH), 3.42 [s, 3 H, CH₃OC(=O)C], 3.05 (dd, *J* = 4.9, 7.9 Hz, 1 H, MeOOCCHN), 2.51 (dd, *J* = 7.9, 16.2 Hz, 1 H, CHNPF-CHH-COOH), 2.18 (dd, *J* = 4.9, 16.2 Hz, 1 H, CHNPF-CHHCOOH).

¹³C NMR (CDCl₃, 100 MHz): δ = 173.2 (CO), 171.7 (CO), 146.5, 146.4, 142.5, 141.4, 139.9, 129.3, 129.2, 128.8, 128.6, 128.0, 127.9, 126.0, 125.7, 125.6, 120.4, 120.2 (Ar), 72.7 (quat. aliph.), 52.7 [CH₃OC(=O)], 51.9 (ROOCCNHPfC), 36.6 (CHNPFCH₂COOH).

Anal. Calcd for C₂₄H₂₁NO₄: C, 74.40; H, 5.46; N, 3.62. Found: C, 74.39; H, 5.38; N, 3.54.

(S)-Methyl 4-Hydroxy-2-[(9-phenyl-9H-fluoren-9-yl)amino]butanoate (5a)

To a stirred solution of **12** (2.0 g, 5 mmol, 1.00 equiv) in anhydrous THF (10 mL) at –5 °C under argon, borane–THF (1.5 M in THF–Et₂O, 6.7 mL, 10 mmol, 2.00 equiv) was added dropwise. After stirring for 4 h, further borane (3.3 mL, 5 mmol, 1.00 equiv) was added and the cooling bath was removed. After 22 h, the mixture was heated to 40 °C for 45 h and the reaction was quenched by adding aq citric acid (10%, 30 mL). Et₂O (50 mL) was added, the layers were separated, and the aqueous layer was extracted with Et₂O (3 × 25 mL). The combined organic layers were washed with brine (30 mL), dried over Na₂SO₄ and evaporated. Purification twice with flash chromatography (EtOAc–hexanes, 10→20→50→75→100% then EtOAc–hexanes, 40%) gave **14**. Partial recrystallisation from benzene–hexanes yielded colorless crystals for analytical purposes.

Yield: 0.97 g (52%); yellow oil; mp 96–97 °C (Lit.¹⁶ 96–97 °C).

(S)-Methyl 3-(9-Phenyl-9H-fluoren-9-yl)-1,3-oxazinane-4-carboxylate (6a)

To a stirred solution of **5a** (3.55 g, 9.5 mmol, 1.00 equiv) in distilled THF (80 mL), formaldehyde (11.5 mL, 152 mmol, 16.00 equiv, 35–40 wt% in H₂O) and *p*-TsOH·H₂O (0.133 g, 0.95 mmol, 10 equiv) were added under argon. The solution was stirred for 2 d then sat. aq NaHCO₃ (20 mL) was added, which formed a white solid, and the mixture was extracted with EtOAc (3 × 30 mL). A little water was added to the aqueous layer to dissolve the white solid. The aqueous layer was extracted with EtOAc (2 × 30 mL) and the combined organic layers were washed with brine (30 mL), dried over Na₂SO₄ and evaporated. Recrystallisation of the mother liquor three times with EtOAc–cyclohexane, cyclohexane, and hexanes yielded three crops of **6a**.

Yield: 3.109 g (85%); colorless to pale-yellow crystalline solid; mp 141 °C; [α]_D²⁰ +253 (*c* 0.74, CHCl₃).

IR (KBr): 3437, 2961–2850, 1732 cm^{−1}.

¹H NMR (CDCl₃, 400 MHz): δ = 7.80–7.77 (m, 1 H, ArH), 7.70–7.68 (m, 1 H, ArH), 7.62–7.60 (m, 1 H, ArH), 7.48–7.15 (m, 10 H, ArH), 4.97 (d, *J* = 11.5 Hz, 1 H, CNPFC^{HH}OC), 4.86 (d, *J* = 11.5 Hz, CNPFC^{HH}OC), 3.79–3.74 (m, 1 H, CHNPfCH₂CHHO), 3.64 [s, 3 H, CH₃OC(=O)], 3.65–3.58 (m, 2 H, CHNPfCH₂CHHO, overlapping with the 3.64 singlet), 3.41–3.40 (m, 1 H, ROOCCHNPfCH₂CH₂O), 1.50–1.42 (m, 2 H, CHNPfCH₂CH₂O).

¹³C NMR (CDCl₃, 100 MHz): δ = 173.5 (CO), 148.7, 147.7, 143.5, 140.7, 139.3, 128.6, 128.5, 128.4, 128.3, 127.8, 127.6, 127.4, 127.1, 125.6, 119.8, 119.7 (Ar), 78.0 and 77.9 (quat. aliph. and NCH₂O), 64.4 (CCH₂O), 54.0 (ROOCCHNPfC), 51.8 [CH₃OC(=O)], 25.7 (CHNPfCH₂CH₂O).

Anal. Calcd for C₂₅H₂₃NO₃: C, 77.90; H, 6.01; N, 3.63. Found: C, 77.73; H, 5.93; N, 3.60.

(S)-Dimethyl {2-Oxo-2-[3-(9-phenyl-9H-fluoren-9-yl)-1,3-oxazin-4-yl]ethyl}phosphonate (7)

To a stirred solution of dimethyl methylphosphonate (2.65 mL, 24.75 mmol, 3.30 equiv) in anhydrous THF (50 mL) at −78 °C, *n*-BuLi (2.5 M in hexanes, 9.0 mL, 22.5 mmol, 3.00 equiv) was added over 15 min under argon. The solution was stirred for 10 min then **6a** (2.874 g, 7.5 mmol, 1.00 equiv) in anhydrous THF (20 mL) was added over 35 min at −78 °C (the reaction mixture turned slowly orange). The mixture was allowed to warm slowly to 0 °C over 3.6 h. The reaction was quenched with sat. aq NH₄Cl (30 mL) and a little H₂O was added to dissolve the formed solid. The mixture was extracted with EtOAc (3 × 30 mL) and the combined organic layers were dried over Na₂SO₄ and evaporated. Purification by flash chromatography (EtOAc–hexanes, 50→75%) gave **7** as a pale-yellow oil, which solidified on standing. The solid was recrystallised from EtOAc–hexanes for analytical purposes.

Yield: 2.29 g (64%); white needles; mp 127 °C; [α]_D²⁰ +168 (*c* 0.47, CHCl₃).

IR (KBr): 3409, 3051–2847, 1713, 1271 cm^{−1}.

¹H NMR (CDCl₃, 400 MHz): δ = 7.89–7.87 (m, 1 H, ArH), 7.72–7.69 (m, 1 H, ArH), 7.64–7.61 (m, 1 H, ArH), 7.49–7.18 (m, 10 H, ArH), 5.09 (dd, *J* = 1.65, 12.1 Hz, 1 H, NCHHO), 4.54 (d, *J* = 12.1 Hz, 1 H, NCHHO), 3.76 [dd, *J* = 11.2, 18.6 Hz, 6 H, (CH₃O)₂P(=O)C], 3.65–3.50 (m, 2 H, CHNPfCH₂CH₂O), 3.54 (dd, *J* = 15.3, 21.0 Hz, 2 H, PCHHC(=O)C, overlapping with 3.65–3.50 multiplet), 3.37 [dd, *J* = 15.3, 21.0 Hz, 1 H, PCHHC(=O)C], 3.16 [br d, *J* = 5.9 Hz, CC(=O)CHNPfC], 1.51–1.47 (m, 1 H, CHNPfCH₂CH₂O), 1.11–1.01 (m, 1 H, CHNPfCH₂CH₂O).

¹³C NMR (CDCl₃, 100 MHz): δ = 203.5 and 203.4 (CO), 148.6, 147.2, 142.3, 140.9, 139.6, 130.1, 128.7, 128.7, 128.6, 128.0, 127.7, 127.5, 127.1, 125.5, 120.0, 119.6 (Ar), 78.4 and 78.3 (quat. aliph. and NCH₂O), 64.1 (CHNPfCH₂CH₂O), 60.5 [two peaks,

CC(=O)CHNPfC], 52.94, 52.88, 52.85 and 52.79 [(CH₃O)₂P(=O)C], 37.5 and 36.2 [PCH₂C(=O)C], 22.2 (CHNPfCH₂CH₂O).

Anal. Calcd for C₂₇H₂₈NO₅P: C, 67.92; H, 5.91; N, 2.93. Found: C, 67.87; H, 5.91; N, 2.88.

(S,E)-1-[3-(9-Phenyl-9H-fluoren-9-yl)-1,3-oxazinan-4-yl]hexadec-2-en-1-one (8)

Compound **7** (0.50 g, 1.0 mmol, 1.00 equiv) was dissolved in anhydrous MeCN (10 mL) in a Morton-flask under argon, and K₂CO₃ (0.42 g, 3.0 mmol, 3.00 equiv) was added. After 20 min, tetradecanal (0.40 g, 1.1 mmol, 1.10 equiv, 58 wt% mixture) was added. The white suspension was stirred for 48 h, then tetradecanal (0.21 g, 0.57 mmol, 0.57 equiv, 58 wt% mixture) was added. After stirring for a further 2 d, the reaction was quenched with aq citric acid (5%, 40 mL) and diluted with Et₂O (40 mL). The layers were separated, and the aqueous layer was extracted with Et₂O (3 × 20 mL). The combined organic layers were dried over Na₂SO₄ and evaporated. Purification by flash chromatography (EtOAc–hexanes, 0→1→2→5%) gave **8**. An analytical sample was recrystallised from hexanes.

Yield: 0.38 g (67%); white solid; mp 97 °C; [α]_D²⁰ −191 (*c* 0.43, CHCl₃).

IR (KBr): 3436, 2923, 2852 cm^{−1}.

¹H NMR (CDCl₃, 400 MHz): δ = 7.73–7.70 (m, 1 H, ArH), 7.65–7.62 (m, 1 H, ArH), 7.57–7.54 (m, 1 H, ArH), 7.47–7.16 (m, 10 H, ArH), 5.50 [td, *J* = 6.6, 15.4 Hz, 1 H, CCH=CHC(=O)C], 5.06 (d, *J* = 7.2 Hz, 1 H, NCHHO), 5.01 (d, *J* = 7.2 Hz, 1 H, NCHHO), 4.62 [td, *J* = 1.51, 15.4 Hz, 1 H, CCH=CHC(=O)C], 4.06 (td, *J* = 6.0, 8.8 Hz, 1 H, CHNPfCH₂CHHO), 3.77 (td, *J* = 3.9, 8.1 Hz, 1 H, CHNPfCH₂CHHO), 2.78 [dd, *J* = 2.2, 7.1 Hz, 1 H, C(=O)CHNPfC], 1.89 (dddd, *J* = 2.5, 3.8, 6.2, 12.7 Hz, 1 H, CHNPfCH₂CH₂O), 1.78–1.69 [m, 3 H, overlapping CHNPfCH₂CH₂O and CCH₂CH=CHC(=O)], 1.35–1.10 [m, 22 H, CH₃(CH₂)₁₁CH₂CH=CHC(=O)], 0.88 (t, *J* = 6.9 Hz, 3 H, CH₃(CH₂)₁₁C).

¹³C NMR (CDCl₃, 100 MHz): δ = 149.4, 147.0, 144.3, 141.7, 139.1 (Ar), 131.9 [C₁₃H₂₇CH=CHC(=O)], 128.8, 128.5, 128.2, 128.0, 127.8, 127.4, 127.3, 127.0 (Ar), 126.9 [C₁₃H₂₇CH=CHC(=O)], 125.6, 119.8, 119.5 (Ar), 113.7, 84.9 (NCH₂O), 77.2 (quat. aliph.), 68.8 (CHNPfCH₂CH₂O), 67.3 [C(=O)CHNPfC], 34.4 (CHNPfCH₂CH₂O), 31.9, 31.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 28.6, 22.7 (aliphatic chain), 14.1 [CH₃(CH₂)₁₁C].

HRMS: *m/z* [M + H]⁺ calcd for C₃₉H₅₀NO₂: 564.3842; found: 564.3860.

Anal. Calcd for C₃₉H₄₉NO₂: C, 83.08; H, 8.76; N, 2.48. Found: C, 83.19; H, 8.84; N, 2.44.

(3S,4S,E)-3-[Methyl(9-phenyl-9H-fluoren-9-yl)amino]nonadec-5-ene-1,4-diol (13)

Compound **8** (0.04 g, 0.07 mmol, 1.00 equiv) was dissolved in anhydrous toluene (3 mL) and cooled to −78 °C under argon. DIBAL-H (1.0 M in toluene) was added in three portions: First portion (0.21 mL, 0.21 mmol, 3.00 equiv), after 22 min second portion (0.1 mL, 0.1 mmol, 1.40 equiv) and, after another 15 min, third portion (0.04 mL, 0.04 mmol, 0.57 equiv). 14 min after the last addition the reaction was quenched with acetone (1 mL) and the mixture was allowed to warm to r.t. A little H₂O was added, followed by sat aq Na₂SO₄ (1.5 mL). After 15 min stirring, the mixture was filtered through Celite, and the Celite was washed with toluene. The filtered solution was concentrated under reduced pressure, H₂O (2 mL) was added and the layers separated. The aqueous layer was extracted with Et₂O (3 × 5 mL) and the combined organic layers were dried over Na₂SO₄ and evaporated. Purification by flash chromatography (EtOAc–hexanes, 25→75%) gave **13**.

Yield: 0.022 g (55%); colorless oil; R_f = 0.45 (EtOAc–hexanes, 50%); $[\alpha]_D^{20}$ +246 (c 0.95, CHCl_3).

IR (NaCl): 3369, 2924, 2853 cm^{-1} .

^1H NMR (CDCl_3 , 400 MHz): δ = 7.80–7.78 (m, 1 H, ArH), 7.65–7.62 (m, 1 H, ArH), 7.56–7.19 (m, 11 H, ArH), 5.65 (td, J = 6.8, 15.2 Hz, 1 H, $\text{CCH}=\text{CHCHOHC}$), 4.99 (dd, J = 8.2, 15.2 Hz, 1 H, $\text{CCH}=\text{CHCHOHC}$), 3.73 (t, J = 8.2 Hz, 1 H, $\text{CH}=\text{CHCHOHCHNPF}_3$), 2.98–2.91 (m, 1 H, CCHHOH), 2.70–2.63 (m, 1 H, CCHHOH), 2.57 (s, 3 H, $\text{CHNPF}_3\text{CH}_3$), 2.25 (ddd, J = 3.7, 5.7, 9.1 Hz, 1 H, $\text{CHOHCHNPF}_3\text{CH}_3$), 1.95–1.89 (m, 2 H, $\text{C}_{12}\text{H}_{25}\text{CH}_2\text{CH}=\text{CHC}$), 1.35–1.15 [m, 22 H, $\text{CH}_3(\text{CH}_2)_{11}\text{C}$], 1.12–1.05 (m, 2 H, $\text{CHNPF}_3\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$), 0.90 [t, J = 6.9 Hz, 3 H, $\text{CH}_3(\text{CH}_2)_{11}\text{C}$].

^{13}C NMR (CDCl_3 , 100 MHz): δ = 148.0, 146.6, 142.7, 141.3, 139.1 (Ar), 135.7 ($\text{CCH}=\text{CHCHOH}$), 129.8 ($\text{CCH}=\text{CHCHOH}$), 128.9, 128.6, 128.5, 128.4, 127.4, 127.3, 126.9, 126.6, 126.1, 120.4, 119.8 (Ar), 77.8 (quat. aliph.), 73.9 ($\text{CCH}=\text{CHCHOHCHNPF}_3\text{CH}_3$), 61.5 (CCH_2OH), 58.2 ($\text{CHOHCHNPF}_3\text{CH}_3$), 32.3 ($\text{CCH}_2\text{CH}=\text{CHC}$), 31.9 (aliphatic chain), 31.4 ($\text{CHNPF}_3\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$), 30.4 (NCH_3), 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 22.7 (aliphatic chain), 14.1 (CH_3).

HRMS: m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{39}\text{H}_{54}\text{NO}_2$: 568.4155; found: 568.4159.

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References

- (1) For reviews on sphingolipids, see: (a) Brunner, M.; Koskinen, A. M. P. *Curr. Org. Chem.* **2004**, *8*, 1629. (b) Koskinen, P. M.; Koskinen, A. M. P. *Methods Enzymol.* **1999**, *311*, 458. (c) Koskinen, P. M.; Koskinen, A. M. P. *Synthesis* **1998**, 1075.
- (2) (a) Pruett, S. T.; Bushnev, A. A.; Hagedorn, K.; Adiga, M.; Haynes, C. A.; Sullards, M. C.; Liotta, D. C.; Merrill, A. H. Jr. *J. Lipid Res.* **2008**, *49*, 1621. (b) Smith, W. L.; Merrill, A. H. Jr. *J. Biol. Chem.* **2002**, *277*, 25841.
- (3) (a) Koskinen, A. M. P.; Krische, M. J. *Synlett* **1990**, 665. (b) Koskinen, P. M.; Koskinen, A. M. P. *Methods Enzymol.* **1999**, *311*, 458.
- (4) Koskinen, A. M. P.; Kallatsa, O. A. *Tetrahedron* **2003**, *59*, 6947.
- (5) Pelšs, A.; Kumpulainen, E. T. T.; Koskinen, A. M. P. *J. Org. Chem.* **2009**, *74*, 7598.
- (6) (a) Christie, B. D.; Rapoport, H. *J. Org. Chem.* (b) Lubell, W. D.; Rapoport, H. *J. Am. Chem. Soc.* **1987**, *109*, 236. (c) Koskinen, A. M. P.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1859. (d) Koskinen, A. M. P.; Schwerdtfeger, J.; Edmonds, M. *Tetrahedron Lett.* **1997**, *38*, 5399.
- (7) Koskinen, A. M. P.; Koskinen, P. M. *Synlett* **1993**, 501.
- (8) (a) Shikata, K.; Niiro, H.; Azuma, H.; Tachibana, T.; Ogino, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 613. (b) Shikata, K.; Niiro, H.; Azuma, H.; Ogino, K.; Tachibana, T. *Bioorg. Med. Chem.* **2003**, *11*, 2723. (c) De Jonghe, S.; Lamote, I.; Venkataraman, K.; Boldin, S. A.; Hillaert, U.; Rozenski, J.; Hendrix, C.; Busson, R.; De Keukeleire, D.; Van Calenbergh, S.; Futerma, A. H.; Herdewijn, P. *J. Org. Chem.* **2002**, *67*, 988. (d) Tarnowski, A.; Retz, O.; Bär, T.; Schmidt, R. R. *Eur. J. Org. Chem.* **2005**, 1129.
- (9) Koskinen, A. M. P.; Koskinen, P. M. *Tetrahedron Lett.* **1993**, *34*, 6765.
- (10) Jamison, T. F.; Rapoport, H. *Org. Synth.* **1993**, *71*, 226.
- (11) Compound **10** has been reported in the literature, however, without data, see: Kaul, R.; Brouillette, Y.; Sajjadi, Z.; Hansford, K. A.; Lubell, W. D. *J. Org. Chem.* **2004**, *69*, 6131.
- (12) X-ray crystallography was performed at the University of Jyväskylä with a Nonius Kappa CCD diffractometer. Crystal data for **10**: Orthorhombic; $P2_12_12_1$ (No. 19); a = 9.0028 (3) Å, b = 9.4284 (3) Å, c = 25.0491 (8) Å; $R1$ = 0.0453, $wR2$ = 0.0847 ($I > 2\sigma$). **12**: Monoclinic; $P2_1$ (No. 4); a = 9.8177 (7) Å, b = 8.8352 (5) Å, c = 11.3458 (8) Å, β = 92.366 (3)°; $R1$ = 0.0490, $wR2$ = 0.0955 ($I > 2\sigma$). **6a**: Orthorhombic; $P2_12_12_1$ (No. 19); a = 14.9329 (6) Å, b = 15.1590 (5) Å, c = 17.2261 (5) Å; $R1$ = 0.0573, $wR2$ = 0.0936 ($I > 2\sigma$). CCDC 255363–255365 contain the supplementary crystallographic data (excluding structure factors) for this paper. These data can be obtained online free of charge or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ UK [fax: (+44)1223336033; or deposit@ccdc.cam.ac.uk].
- (13) Lee, J.-M.; Lim, H.-S.; Chung, S.-K. *Tetrahedron: Asymmetry* **2002**, *13*, 343.
- (14) Gosselin, F.; Lubell, W. D. *J. Org. Chem.* **1998**, *63*, 7463.
- (15) Lubell, W. D.; Jamison, T. F.; Rapoport, H. *J. Org. Chem.* **1990**, *55*, 3511.
- (16) Kovacs, J.; Kovacs, H. N.; Ballina, R. *J. Am. Chem. Soc.* **1963**, *85*, 1839.
- (17) Itaya, T.; Shimizu, S.; Nakagawa, S.; Morisue, M. *Chem. Pharm. Bull.* **1994**, *42*, 1927.
- (18) Garrard, E. A.; Borman, E. C.; Cook, B. N.; Pike, E. J.; Alberg, D. G. *Org. Lett.* **2000**, *2*, 3639.
- (19) The stereochemistry was assigned based on analogy with previous reductions, as well as on the coupling constants for similar compounds; see, for example, ref. 8d.