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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.

Keywords: 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. 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. We have previously developed a general and efficient synthesis of sphingosine based on a serine derived β-ketophosphonate. 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. 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.

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. We therefore became interested in investigating whether the β-ketophosphonate chemistry we have developed for the Boc-protected amino acid derivatives 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. A general synthetic approach to homoceramides could lead to the development of a new class of anticancer drugs, thus the synthesis of D-erythro-homosphingosine (3) was investigated in this work, with the aim of subsequently preparing a range of homoceramides.

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 reaction to give enone 8. Selective reduction 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 and 9-phenyl-9-fluorenyl of the amino group.

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

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

Crude N-Pf-protected \(9_{\text{b,10}}\) was directly esterified to give compound 10 in 42% yield as yellow crystals. 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 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 \(\beta\)-ketophosphonate from acetal 6b, we decided to opt for the less hindered methyl ester (Scheme 4). Thus, \(\alpha\)-methyl aspartate16 was converted into the crystalline \(\alpha\)-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-
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 reduction 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 were obtained from Merck. TLC plates were visualized under UV irradiation (254 nm).

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) 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, 17–20%) gave 6b.

Yield: 0.106 g (99%); colorless oil; Rp = 0.56 (EtOAc–hexanes, 50%); [α]23D +151 (c 0.43, CHCl3).

Figure 3 X-ray crystal structure of 10

Figure 4 X-ray crystal structure of 12

Figure 5 X-ray crystal structure of 6a

tecting 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.
IR (NaCl): 3060–2834, 1723 cm⁻¹.

1H NMR (CDCl₃, 400 MHz): δ = 7.82–7.80 (m, 1 H, ArH), 7.70–7.67 (m, 1 H, ArH), 7.51–7.13 (m, 11 H, ArH), 3.94 (dd, J = 11.2, 15.9 Hz, 6 H, (CH₃)₃P=O-(O)-C), 3.21 (br s, 1 H, C-NHPf), 2.81 (q, J = 7.0 Hz, 1 H, C-(O)CH₂NHPf), 2.77 (dd, J = 14.7, 22.2 Hz, 1 H, (CH₂)₃P=(O)(CHHC=(O)-C)), 2.60 [dd, J = 14.7, 21.2 Hz, 1 H, (CH₂)₃P=(O)(CHHC=(O)-C)], 1.02 (d, J = 7.0 Hz, 3 H, CH₂NHPf₂-C).

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 (CO(NH₂)PCH₂), 52.81, 52.74, 52.68 and 52.62 [(CH₃)₃P=O], 38.3 and 37.0 [(CH₂=CH=O)], 20.0 (CH₂NHPf). HRMS: m/z [M + Na] calcd. for C₂₅H₃₂NO₄NaP: 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) was added. The reaction 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 with sat. aq NaHCO₃ (5 × 30 mL). The aqueous layers were acidified with concd H₂PO₄ to pH 5 and extracted with Et₂O (2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over MgSO₄ and evaporated to give 12. An analytical sample was recrystallised from CH₂Cl₂–hexanes and again from EtOAc–hexanes.

Yield: 9.3 g (89%); yellow foam; mp 128–129 °C; [α]₁₀⁻²⁹ –297 (c 1.0, CHCl₃).

IR (KBr): 3059–2956, 2636, 1736 cm⁻¹.

1H NMR (CDCl₃, 100 MHz): δ = 7.77–7.71 (m, 2 H, ArH), 7.46–7.22 (m, 11 H, ArH), 3.42 [s, 3 H, CH₂O(C=O)⁻], 3.05 (dd, J = 4.9, 7.9 Hz, 1 H, MeOCONHCH₂), 2.51 (dd, J = 7.9, 16.2 Hz, 1 H, 1H(=N(CH₂=O))), 2.18 (dd, J = 4.9, 16.2 Hz, 1 H, CHF₂NHPfCOOH).

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 [CH₂O(C=O)], 51.9 (ROOCNHPf(C), 36.6 (CH₂NHPf₂CO₂H).

Anal. Calcd. for C₁₃H₁₉NO₄: C, 54.41; H, 5.64; N, 3.46. Found: C, 54.29; H, 5.78; N, 3.44.

(5)-Methyl 4-Hydroxy-2-[[9-Phenyl-9H-fluoren-9-yl]aminobutanate (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%, 50 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.10 96–97 °C).
To a stirred solution of 5a (3.55 g, 9.5 mmol, 1.00 equiv) in anhydrous THF (80 mL), formaldehyde (11.5 mL, 152 mmol, 16.00 equiv, 35–40 wt% in H2O) and 1/4-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) and the mixture was extracted with EtOAc (3 × 30 mL). A little water was added to the aqueous layer to dissolve the formed solid. 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 D2O was added to dissolve the white solid. The mixture was extracted with EtOAc (3 × 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), 7.65–7.62 (m, 1 H, ArH), 7.54–7.52 (m, 1 H, ArH), 7.51–7.48 (m, 10 H, ArH), 5.50 [td, J = 11.5, 7.1 Hz, 1 H, NCH2CH2CH(O)] , 5.45 (dd, J = 12.1 Hz, 1 H, NCH2CH2O) , 3.77 [dd, J = 11.2, 18.6 Hz, 1 H, (CH2O)2P=NCH(CH2)2] , 3.65–3.50 (m, 2 H, 2 H, CHNCH2CH(O)) , 3.54 (dd, J = 15.3, 21.0 Hz, 2 H, PCH2C(=O)O) , 3.37 [dd, J = 15.3, 21.0 Hz, 1 H, PCH2C(=O)O] , 3.16 [br d, J = 5.9 Hz, C(=O)CH2=CH2O] , 1.78–1.69 [m, 3 H, overlapping with the 3.64 singlet], 3.41–3.40 (m, 1 H, CHNPfCH2CH2O) , 1.50–1.42 (m, 2 H, CHNPfC).
Yield: 0.022 g (55%); colorless oil; \( R_f = 0.45 \) (EtOAc–hexanes, 50%); \([\alpha]_D^{20} +246 (c 0.95, \text{CHCl}_3)\).

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

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta = 7.80–7.78 \) (m, 1 H, ArH), 5.78–5.76 (m, 1 H, ArH), 5.75–5.71 (m, 11 H, ArH), 5.65 (dd, \( J = 6.8, 15.2 \) 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, \( \text{CH} = \text{CHCHOHC} = \text{NPf} \)), 2.98–2.91 (m, 1 H, \( \text{CCOHC} \)), 2.70–2.63 (m, 1 H, \( \text{CCHOH} \)), 2.57 (s, 3 H, \( \text{CHNPfCH}_3 \)), 2.25 (dd, \( J = 3.7, 5.7, 9.1 \) Hz, 1 H, \( \text{CHOCHNPfCH}_3 \)).

\(^13\)C NMR (CDCl\(_3\), 100 MHz): \( \delta = 150.8, 145.2, 144.6, 142.7, 141.3, 139.1 \) (Ar), 135.7 (C=CHCHOH), 129.8 (C=CHCHOH), 128.9, 128.6, 128.5, 128.4, 127.4, 127.3, 126.9, 126.6, 126.1, 126.0, 120.4, 119.8 (Atr), 77.8 (quat. aliph.), 73.9 (C=CHCHOHCHNPfCH\(_3\)), 61.5 (CH\(_3\)OH), 58.2 (CHOCHNPfCH\(_3\)), 32.3 (C\(_6\)H\(_3\)(CH\(_3\))\(_3\)), 31.9 (aliphatic chain), 31.4 (CHNPfCH\(_3\)(CH\(_3\)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/e [M + H]\(^+\) calcld for C\(_{39}\)H\(_{54}\)NO\(_2\): 568.4155; found: 568.4159.

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(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.

(22) shrimp (H. azumi, 311, 633).