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ORIGINAL ARTICLE

A practical route to long-chain non-natural α,ω-diamino acids

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Abstract An efficient method for the synthesis of long-chain α,ω-diamino acids, starting from natural α-amino acids, has been developed. The long-chain skeleton has been generated through condensation between a protected aldehyde, derived from L-aspartic acid, and an ylide obtained from an ω-hydroxy-alkyl phosphonium salt. After conversion of the ω-hydroxy group into an amine, catalytic hydrogenation produced the *N*,*N*′-protected α,ω-diamino acid. The present route to α,ω-diamino acids allows the modulation of the chain length depending on the length of the ylide used for the Wittig olefination reaction.

Keywords Lipidic α-amino acids (LAAs) · Unnatural fatty acids · Wittig olefination · α,ω-Diamino acids

Abbreviations

TEA	Triethylamine
LiHMDS	Lithium hexamethyldisilazide
TsCl	*p*-Toluenesulfonyl chloride
DMAP	4-Dimethylamino pyridine
DIPEA	*N*,*N*-Diisopropylethylamine
TBAI	Tetrabutylammonium iodide
PPh_3	Triphenylphosphine
Boc_2O	Di-*tert*-butyl dicarbonate

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Introduction

Lipidic α-amino acids (LAAs) are of great importance for the synthesis of drug delivery systems and bioactive lipid mimetics (Toth 1994; Constantinou-Kokotou and Kokotos 1999). They have been conjugated to a wide variety of different compounds in order to improve the absorption of drugs. Due to the structural feature of the long alkyl chain, amides or esters of saturated LAAs possess a high degree of membrane-like character, which may facilitate their crossing into the cell interior. Furthermore, the long alkyl side chains may have the additional effect of protecting a labile parent drug from enzymatic attack. LAAs have also received considerable attention for the development of self-adjuvanting peptide vaccines. They have been conjugated to peptide epitopes to change their physico-chemical and pharmacological characteristics in order to increase the delivery of potential agents for molecular therapy (Sarpietro et al. 2008; Li et al. 2012). Some LAAs exhibit a further amino group at the end of the long alkyl chain and the effect of their long chain in increasing drug efficiency has been widely reported. ω-Amino LAA derivatives have been used in the inhibition of metalloproteinases by *N*-carboxylalkyl peptides, in which a phenethyl group had been replaced with a long-chain ω-aminoalkyl group (Esser et al. 1995). A more selective and long-lasting response, induced by the presence of an ω-amino LAA, has been also observed for the angiotensin converting enzyme (ACE) inhibitor enalaprilat and some derivatives (Shirota et al. 1990; Saito et al. 1990), as well as in the case of benzothiazepine- and benzoxazepine-related compounds, where the duration of the ACE-inhibiting action is dependent on the length of the chain (Itoh et al. 1986). More recently, non-natural ω-amino LAAs have been incorporated into antimicrobial peptides (AMP) with the same

rationale to impart specific physico-chemical properties to the peptide and to develop compounds able to interact with membranes in novel ways. The antibacterial activity of these novel AMP peptides is affected by the length and charge distribution of LAA, and this could have important implications in the selectivity towards different bacterial strains. In fact, differences in the chemical composition of the membrane of bacteria reflect a different degree of complementarity between the membrane and the interacting AMP, which results in different activities (Russell et al. 2012). ω-Amino LAAs have also been used for the construction of capture agents for the detection of proteins, to be used in alternative to expensive antibodies (Agnew et al. 2009). On the other side, they can also be considered as valuable fatty acid analogues for the preparation of sphingolipids, modified in the sphingoid backbone. Sphingolipids are major components of eukaryotic plasma membranes, with ceramide, the sphingoid backbone, comprising a long-chain base attached to the fatty acid via an amide bond. The amino groups appended to the fatty acid alkyl chain could be used as derivatization points or anchors for the attachment of probes, e.g. photoactivable groups for the study of membrane microenvironments (Aureli et al. 2010).

A variety of methods for the asymmetric synthesis of α-amino acids have been developed (for general reviews see for example: Constantinou-Kokotou and Kokotos 1999; Ma 2003; Nájera and Sansano 2007; Soloshonok and Sorochinsky 2010). The synthesis of ω-amino LAAs, i.e. non-natural long-chain α,ω-diamino acids, are commonly based on methods developed for the preparation of ornithine, lysine or higher homologues, which generally consist in the alkylation of acetamidomalonate derivatives (Gaudry 1953; Payne and Boger 1985). Despite the wide range of potential applications, there are very few examples of the preparation of ω-amino LAAs longer more than 10 carbon atoms (Markidis and Kokotos 2002; Agnew et al. 2009). Herein, we report a practical and general method to prepare α,ω-diamino long fatty acids, starting from readily available chiral building blocks, i.e. derivatized natural L-amino acids. We illustrate this procedure by the synthesis of the α,ω-diamino-octadecanoic acid **1**, which is appropriately protected for inclusion in more complex structures through its carboxylic group (Fig. 1).

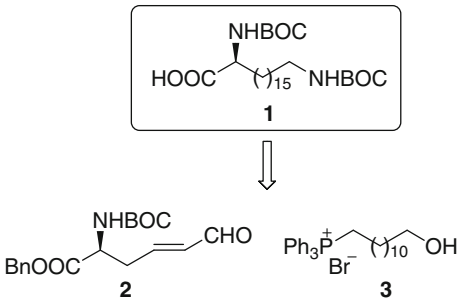


Fig. 1 Chemical structure of the α,ω-diamino-octadecanoic acid **1** and its precursors

Materials and methods

All reagents were bought at highest commercial quality and used without further purification except where noted. Air- and moisture-sensitive liquids and solutions were transferred via oven-dried syringe or stainless steel cannula through septa. Organic solutions were concentrated by rotary evaporation below 45 °C at approximately 20 mmHg. All reactions carried out under anhydrous conditions were performed using oven-dried glassware within an argon atmosphere. Dry solvents and liquid reagents were distilled prior to use. Tetrahydrofuran (THF) and toluene were distilled from sodium; dichloromethane (DCM) and pyridine (Py) were distilled from calcium hydride; dimethylformamide (DMF), acetonitrile (CH_3CN), and methanol (MeOH) were dried on activated 4 Å molecular sieves; TEA was distilled from KOH and DIPEA from calcium hydride. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded at 298 K with a Bruker FT-NMR AVANCE^{TM} DRX500 spectrometer using a 5-mm z-PFG (pulsed field gradient) broadband reverse probe. Chemical shifts are reported on the δ (ppm) scale and are relative to TMS as internal reference. The signals were unambiguously assigned by 2D COSY and HSQC experiments (standard Bruker pulse program). Scalar coupling constants are reported in Hertz. The mass spectrometric analyses were performed in positive or negative electrospray mode (ESI–MS). MS spectra were recorded on a Thermo Quest Finnigan LCQ^{TM}deca ion trap mass spectrometer; the mass spectrometer was equipped with a Finnigan ESI interface. Data were processed by Finnigan Xcalibur software system. All reactions were monitored by TLC on silica gel 60 F-254 plates (Merck), spots being developed with a phosphomolybdate-based reagent, anisaldehyde or ninidrine solutions and subsequently heated at 110 °C. Flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck). TsCl was crystallized from hexane.

Benzyl (2*S*)-2-(*tert*-butoxycarbonylamino)-4-oxo-butanoate (**5**)

Freshly distilled oxalyl chloride (1.49 mL, 17.63 mmol) was slowly added to a solution under argon of DMF (0.47 mL, 6.03 mmol) in DCM (15 mL) at 0 °C. A white solid was formed, which gradually dissolved to give a

white opalescent suspension. After the mixture was stirred for 1 h, the solvent was evaporated under reduced pressure maintaining an inert atmosphere, by connecting directly the flask under stirring to the vacuum pump (medium vacuum) and then drying the solid by flushing a stream of nitrogen. To the residual white solid, dissolved under an argon atmosphere in THF (15 mL) and CH_3CN (9 mL), a solution of BOC-L-Asp-OBzl **4** (1.5 g, 4.64 mmol) and pyridine (0.45 mL) in THF (15 mL) was slowly added at −30 °C, and the reaction was stirred at the same temperature for 1 h. Then, the solution was cooled to −78 °C and $LiAlH(O^tBu)_3$ (1 M THF sol., 5.10 mL) was added dropwise. After 0.5 h, the reaction was quenched by the addition of 1 N HCl solution (30 mL) followed by extraction with EtOAc (3 × 20 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by flash chromatography (hexane/EtOAc 7:3, v/v, 1 % Et_3N) to yield compound **5** (0.85 g, 60 %) as a colourless oil. $[\alpha]_D^{20}$ +15.5 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, –*t*Bu), 3.04 (dd, 1H, $J_{2,3a} = 4.5$, $J_{3a,3b} = 18.3$ Hz, H-3a); 3.13 (dd, 1H, $J_{2,3b} = 4.9$, $J_{3a,3b} = 18.3$ Hz, H-3b), 4.60–4.70 (m, 1H, H-2); 5.20 (s, 2H, CH_2Ph), 5.37–5.51 (m, 1H, –NH), 7.30–7.45 (m, 5H, arom), 9.74 (s, 1H, H-4) ppm. ^{13}C NMR ($CDCl_3$): δ 28.3 (3C, *t*-Bu), 46.0 (C-3), 48.8 (C-2), 67.6 (CH_2Ph), 80.3 (CMe_3), 128.3–128.6 (5C, arom), 135.1 (arom), 155.3 (CONH), 170.9 (C-1), 199.3 (C-4) ppm. ESI–MS (positive-ion mode, *m*/*z*): 361.9 (87) $[M+OMe+Na]^+$, 700.7 (100) $[2(M+OMe)+Na]^+$. Anal. calcd for $C_{16}H_{21}NO_5$ (307.14): C 62.53, H 6.89, N 4.56. Found: C 62.27, H 6.97, N 4.23.

Benzyl (2*S*,4*E*)-2-(*tert*-butoxycarbonylamino)-6-oxo-hex-4-enoate (**2**)

(Triphenylphosphoranylidene)acetaldehyde (1.25 g, 4.10 mmol) was added to a solution under argon of compound **5** (0.84 g, 2.73 mmol) in dry toluene (27 mL) and the resulting orange reaction mixture was warmed to 70 °C and stirred at this temperature for 3.5 h. After removal of the solvent under reduced pressure, the crude was purified by flash chromatography (petroleum ether/EtOAc, 8:2, v/v, 1 % Et_3N) to afford compound **2** (0.68 g, 75 %) as a pale yellow oil. $[\alpha]_D^{20}$ +10.0 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, *t*Bu), 2.66–2.77 (m, 1H, H-3a), 2.83–2.96 (m, 1H, H-3b), 4.52–4.63 (m, 1H, H-2); 5.10–5.30 (m, 3H, CH_2Ph and NH), 6.09 (dd, 1H, $J_{4,5} = 15.6$, $J_{5,6} = 7.8$ Hz, H-5); 6.67 (dd, 1H, $J_{4,5} = 15.6$, $J_{3a,4} = J_{3b,4} = 7.3$ Hz, H-4), 7.32–7.43 (m, 5H, arom), 9.40 (d, 1H, $J_{5,6} = 7.8$ Hz, H-6) ppm. ^{13}C NMR ($CDCl_3$): δ 28.2 (3C, *t*-Bu), 35.9 (C-3), 52.4 (C-2), 67.6 (CH_2Ph), 80.4 (CMe_3), 128.6–128.8 (5C, arom), 134.9 (arom), 135.6 (C-5), 151.1 (C-4), 155.0 (CONH), 171.0 (C-1), 193.2 (C-4) ppm. ESI–MS (positive-ion mode, *m*/*z*): 356.0 (100) $[M+Na]^+$, 387.9 (83) $[M+OMe+Na]^+$. Anal. calcd for $C_{18}H_{23}NO_5$ (333.16): C 64.85, H 6.95, N 4.20. Found: C 64.52, H 7.24, N 4.01.

12-Hydroxydodecyltriphenylphosphonium bromide (**3**)

12-Bromododecanol (1.00 g, 3.77 mmol) was dissolved in EtOH (10 mL) and PPh_3 (1.28 g, 4.90 mmol) was added. The reaction was refluxed for 48 h, and then the solvent was removed under reduced pressure. Purification by flash chromatography (DCM/MeOH, 10:1, v/v) gave phosphonium salt **3** (1.79 g, 90 %) as a colourless oil, which became a white solid after treatment with dry THF followed by evaporation. Physical data of compound **3** were in agreement with those reported in Tran-Thi and Falk (1995).

Benzyl (2*S*)-2-(*tert*-butoxycarbonylamino)-18-hydroxy-octadeca-4,6-dienoate (**6**)

To a suspension of the phosphonium salt **3** (2.61 g, 4.95 mmol) in dry THF (30 mL) at −60 °C under argon, LiHMDS (1 M THF sol., 9.9 ml) was added dropwise. The orange ylide was stirred for 40 min, then a solution of aldehyde **2** (0.55 g, 1.65 mmol) in dry THF (15 mL) was added dropwise within 30 min. The reaction was stirred for 1.5 h at −60 °C, then quenched with HCl 1 N (30 mL) and extracted with EtOAc (3 × 70 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and the solvent evaporated under reduced pressure. Purification by flash chromatography (DCM/EtOAc, 9:1, v/v, 1 % Et_3N) gave compound **6** (0.54 g, 65 %), a pale yellow oil, as a mixture of isomers. ^{1}H NMR ($CDCl_3$): δ 1.24–1.52 (m, 25H, 8 CH_2 and *t*Bu), 1.54–1.62 (m, 2H, 2 H-17), 2.03–2.11 (m, 0.8H, 2 H-8_{MIN}), 2.11–2.19 (m, 1.2H, 2 H-8_{MAG}), 2.47–2.68 (m, 2H, 2 H-3), 3.66 (t, 2H, $J_{17,18} = 6.6$ Hz, 2 H-18); 4.38–4.50 (m, 1H, H-2), 5.00–5.27 (m, 3H, CH_2Ph and NH), 5.32–5.52 (m, 1.6H, H-4 and H-7_{MAG}), 5.58–5.67 (m, 0.4H, H-7_{MIN}), 5.87–6.08 (m, 1.4H, H-6 and H-5_{MIN}), 6.33–6.42 (m, 0.6H, H-5_{MAG}), 7.32–7.42 (m, 5H, arom) ppm. ESI–MS (positive-ion mode, *m*/*z*): 524.2 $[M+Na]^+$. Anal. calcd for $C_{30}H_{47}NO_5$ (501.35): C 71.82, H 9.44, N 2.79. Found: C 71.59, H 9.71, N 2.58.

(2*S*)-2-(*tert*-butoxycarbonylamino)-18-hydroxy-octadecanoic acid (**7**)

To a solution of compound **6** (0.04 g, 0.08 mmol) in $CHCl_3$/MeOH 1:1 (3 mL) a catalytic amount of 10 % Pd/C was added and the reaction was stirred under H_2 for 24 h. The reaction was diluted with $CHCl_3$/MeOH and filtered through a MILLIPORE filter. After evaporation of the

solvent purification by flash chromatography (DCM/MeOH 95:5, v/v) gave compound **7** (0.029 g, 87 %) as a colourless oil. $[\alpha]_D^{20}$ +5.9 (*c* 1, $CHCl_3$:MeOH 1:1); ^{1}H NMR ($CDCl_3$/MeOD 1:1, v/v): δ 1.20–1.40 (m, 26H, 13 CH_2), 1.44 (s, 9H, *t*-Bu), 1.48–1.56 (m, 2H, 2 H-17), 1.58–1.67 (m, 1H, H-3a), 1.73–1.86 (m, 1H, H-3b), 3.55 (t, 2H, $J_{17,18}$ = 6.8 Hz, 2 H-18), 4.05–4.14 (m, 1H, H-2) ppm. ^{13}C NMR ($CDCl_3$/MeOD 1:1, v/v): δ 25.4, 25.7, 27.9, 28.4–32.4 (12 C), 54.0 (C-2), 62.0 (C-18), 79.5 (CMe_3), 156.3 (CONH), 176.2 (C-1) ppm. ESI–MS (negative-ion mode, *m*/*z*): 414.2 $[M-H]^-$. Anal. calcd for $C_{23}H_{45}NO_5$ (415.33): C 66.47, H 10.91, N 3.37. Found: C 66.12, H 11.23, N 3.09.

Benzyl (2*S*)-2-(*tert*-butoxycarbonylamino)-18-(*p*-toluensulfonyloxy)-octadeca-4,6-dienoate (**8**)

To a solution under argon of compound **6** (0.50 g, 1.0 mmol) in dry DCM (10 mL) DIPEA (0.70 mL, 2.0 mmol), TsCl (0.38 g, 2.0 mmol) and DMAP (0.12 g, 1.0 mmol) were added at 0 °C. The reaction was spontaneously warmed to room temperature and after 5 h, quenched by addition of methanol. The solvent was evaporated under reduced pressure and the crude purified by flash chromatography (hexane/EtOAc, 85:15, v/v, 1 % Et_3N) to afford compound **8** (0.41 g, 63 %), an oil, as a mixture of isomers. ^{1}H NMR ($CDCl_3$): δ 1.12–1.53 (m, 25H, 8 CH_2 and *t*Bu), 1.58–1.70 (m, 2H, 2 H-17), 2.02–2.10 (m, 0.8H, 2 H-8_{MIN}), 2.11–2.18 (m, 1.2H, 2 H-8_{MAG}), 2.47 (s, 3H, CH_3Ph), 2.50–2.68 (m, 2H, 2 H-3), 4.04 (t, 2H, $J_{17,18}$ = 6.5 Hz, 2 H-18); 4.38–4.49 (m, 1H, H-2), 5.00–5.10 (m, 1H, NH), 5.11–5.28 (m, 2H, CH_2Ph), 5.32–5.51 (m, 1.6H, H-4 and H-7_{MAG}), 5.58–5.67 (m, 0.4H, H-7_{MIN}), 5.87–6.09 (m, 1.4H, H-6 and H-5_{MIN}), 6.32–6.42 (m, 0.6H, H-5_{MAG}), 7.33–7.42 (m, 7H, arom), 7.78–7.84 (m, 2H, arom) ppm. ESI–MS (positive-ion mode, *m*/*z*): 678.1 $[M+Na]^+$. Anal. calcd for $C_{37}H_{53}NO_7S$ (655.35): C 67. 76, H 8.14, N 2.14, S 4.89. Found: C 67. 47, H 8.39, N 2.01, S 4.59.

Benzyl (2*S*)-18-azido-2-(*tert*-butoxycarbonylamino)-octadeca-4,6-dienoate (**9**)

Tosylate **8** (0.39 g, 0.59 mmol), NaN_3 (0.12 g, 1.77 mmol) and TBAI (0.015 g, 0.04 mmol) were dissolved in dry DMF (12 mL) and the reaction was stirred at 60 °C for 2 h. The mixture was then diluted with EtOAc (20 mL) and washed with water (20 mL). The organic layer was extracted with EtOAc (2 × 20 mL) and the combined organics dried over Na_2SO_4 and evaporated. Purification by flash chromatography (hexane/EtOAc, 9:1, v/v, 1 % Et_3N) gave compound **9** (0.29 g, 92 %), an oil, as a mixture of isomers. ^{1}H NMR ($CDCl_3$): δ 1.21–1.52 (m, 25H, 8 CH_2 and *t*Bu), 1.55–1.70 (m, 2H, 2 H-17), 2.02–2.11 (m, 0.8H, 2 H-8_{MIN}), 2.11–2.20 (m, 1.2H, 2 H-8_{MAG}), 2.48–2.68 (m, 2H, 2 H-3), 3.28 (t, 2H, $J_{17,18}$ = 6.9 Hz, 2 H-18); 4.38–4.51 (m, 1H, H-2), 4.99–5.28 (m, 3H, CH_2Ph and NH), 5.31–5.53 (m, 1.6H, H-4 and H-7_{MAG}), 5.57–5.68 (m, 0.4H, H-7_{MIN}), 5.86–6.09 (m, 1.4H, H-6 and H-5_{MIN}), 6.32–6.44 (m, 0.6H, H-5_{MAG}), 7.31–7.45 (m, 5H, arom) ppm. ESI–MS (positive-ion mode, *m*/*z*): 549.1 $[M+Na]^+$. Anal. calcd for $C_{30}H_{46}N_4O_4$ (526.35): C 68.41, H 8.80, N 10.64. Found: C 68.11, H 9.03, N 10.33.

Benzyl (2*S*)-2,18-di-(*tert*-butoxycarbonylamino)-octadeca-4,6-dienoate (**10**)

Compound **9** (0.27 g, 0.51 mmol) and PPh_3 (0.32 g, 1.23 mmol) were dissolved in THF (10 mL) and, after 1 h, water (1 mL) was added. The reaction was stirred overnight, then diluted with water (15 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. To a solution of the residue in dry DCM (10 mL), TEA (0.28 mL, 2.04 mmol) was added, and, after 10 min, a solution of BOC_2O (0.28 g, 1.28 mmol) in DCM (2 ml). The reaction was stirred at 40 °C for 6 h, then quenched with MeOH, diluted with water and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by flash chromatography (hexane:EtOAc, 85:15, v/v, 1 % Et_3N) to yield compound **10** (0.20 g, 65 %), an oil, as a mixture of isomers. ^{1}H NMR ($CDCl_3$): δ 1.19–1.57 (m, 36H, 9 CH_2 and 2 *t*Bu), 2.01–2.10 (m, 0.8H, 2 H-8_{MIN}), 2.10–2.19 (m, 1.2H, 2 H-8_{MAG}), 2.46–2.69 (m, 2H, 2 H-3), 3.03–3.19 (m, 2H, 2 H-18), 4.36–4.60 (m, 2H, H-2 and NH), 4.99–5.28 (m, 3H, CH_2Ph and NH), 5.31–5.51 (m, 1.6H, H-4 and H-7_{MAG}), 5.56–5.67 (m, 0.4H, H-7_{MIN}), 5.86–6.08 (m, 1.4H, H-6 and H-5_{MIN}), 6.32–6.43 (m, 0.6H, H-5_{MAG}), 7.31–7.44 (m, 5H, arom) ppm. ESI–MS (positive-ion mode, *m*/*z*): 623.2 $[M+Na]^+$ (78), 1222.7 $[2M+Na]^+$ (100). Anal. calcd for $C_{35}H_{56}N_2O_6$ (600.41): C 69.97, H 9.39, N 4.66. Found: C 69.62, H 9.73, N 4.45.

(2*S*)-2,18-di-(*tert*-butoxycarbonylamino)-octadecanoic acid (**1**)

To a solution of compound **10** (0.18 g, 0.30 mmol) in MeOH (10 mL) a catalytic amount of 10 % Pd/C was added. The reaction was stirred under hydrogen atmosphere for 15 h, and then diluted with MeOH and filtered through a MILLIPORE filter, washing the filter with $CHCl_3$/MeOH. After evaporation of the solvent, the crude was purified by flash chromatography (DCM:MeOH 95:5, v/v) to give compound **1** (0.13 g, 85 %) as a colourless oil.

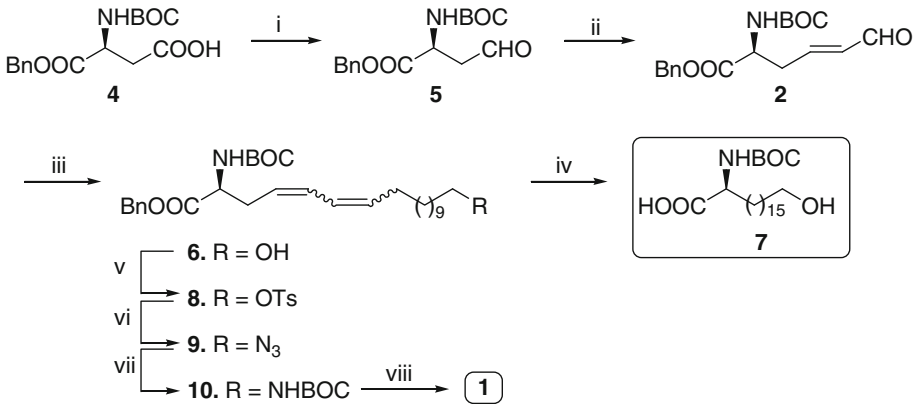
$[\alpha]_D^{20}$ +4.6 (*c* 0.5, $CHCl_3$/MeOH 1:1). ^1H NMR ($CDCl_3$/MeOD 1:1, v/v): δ 1.14–1.53 (m, 46H, 2 *t*Bu and 14 CH_2), 1.53–1.66 (m, 1H, H-3a), 1.72–1.86 (m, 1H, H-3b), 3.04 (t, 2H, $J_{17,18} = 7.1$ Hz, H-18), 3.92–4.05 (m, 1H, H-2) ppm. ^{13}C NMR ($CDCl_3$:MeOD 1:1, v/v): δ 25.5, 26.4, 27.9 (3C, *t*Bu), 29.2–29.7 (15C), 32.7 (C-3), 40.3 (C-18), 55.3 (C-2), 78.8 and 79.2 (2 CMe_3), 156.4 and 157.0 (2 CONH), 179.0 (C-1) ppm. ESI–MS (negative-ion mode, *m*/*z*): 513.3 $[M-H]^-$ (100); 1027 $[2\,M-H]^-$ (30). Anal. calcd for $C_{28}H_{54}N_2O_6$ (514.40): C 65.33, H 10.57, N 5.44. Found: C 65.20, H 10.89, N 5.22.

Results and discussion

The length of the alkyl skeleton of compound **1**, combined with the functionalization of the unnatural fatty acid, immediately oriented the synthetic strategy towards the use of a series of Wittig reactions for the convergent preparation of the C-18 fatty acid, by condensations of readily available and suitably functionalized building blocks. In a Wittig reaction the elongation of the alkyl chain occurs through a condensation between an aldehyde and a phosphonium salt, via the formation of an intermediate ylide, which evolves to an alkene. It is possible to take advantage of properly derivatized L-amino acids as the aldehyde counterpart, since they already contain the α-aminoacid group, while, the phosphonium salt could be derived from ω-functionalized alkyl bromides. It is unquestionable that many different disconnections are possible for the preparation of compound **1**, but our choice was oriented towards the reduction of critical synthetic steps and by the commercial availability of the building blocks, easily obtainable from common suppliers. Due to this, the use of *N*-protected ω-amino-alkyl bromide was discarded for two main reasons. First, due to the availability of only short homologues those are expensive. Second, because of problems relating to the commonly used amino protecting groups which are incompatible with the reaction conditions for the Wittig condensation. In fact, the *N*-FMOC base labile protecting group does not resist to the basic conditions of the Wittig reaction, while, with *N*-BOC, there are problems of instability connected with the preparation of the phosphonium salt at high temperature, as already reported in the literature and confirmed by our preliminary experiments (Wiktelius and Luthman 2007). On the contrary, the use of ω-bromoalkanol is highly encouraged for the commercial availability of different homologues and because the conversion of an ω-hydroxy group into an amine can be easily accomplished by well-established chemical transformations. Consequently, the choice of the reaction partners entailed into aldehyde **2**, derived from L-aspartic acid, and the phosphonium salt **3**, which can efficiently be obtained from 12-bromo-dodecanol (Fig. 1).

Protected aspartic acid **4** was the starting material of our synthetic strategy (Scheme 1). A benzyl ester protection of the carboxylic group was decided, as it can be easily removed at the end of the synthesis by catalytic hydrogenolysis with the concomitant reduction of the double bonds formed during the Wittig reactions. Compound **4** was subjected to a regioselective reduction mediated by *N*,*N*-dimethylchloromethylenammonium chloride and lithium tri(*tert*-butoxy)aluminium hydride, according to a synthetic protocol already reported for a corresponding glutamic derivative (Bycroft et al. 2003), which gave aldehyde **5** in good yield after flash chromatography. A C-2 homologation was then performed at this stage, by reacting compound **5** with (triphenylphosphoranylidene)-acetaldehyde at high temperature to give compound **2** in high yield. Compound **2**, which is a homoglutamic acid δ-aldehyde, has the advantage to be already installed with the terminal aldehyde necessary for the main Wittig olefination for the construction of the long alkyl skeleton. The reaction of aldehyde **2** with the ylide generated from phosphonium salt of 12-bromododecanol was thus tested. The yield of the reaction is highly dependent on the temperature and the base used for the generation of the ylide. Also literature data on similar substrates indicate that there are not general rules, and the reaction conditions, i.e. the base to promote the generation of the ylide as well the temperature for the deprotonation or the addition of the aldehyde, are finely tuned on the particular reaction partners. We obtained our best results by performing both the formation of the ylide and the addition of the aldehyde at −60 °C, using lithium hexamethyldisilazide (LiHMDS) as the base. The quality of the hygroscopic phosphonium salt **3** also resulted very important. This was obtained from 12-bromo-dodecanol using standard protocols (Lei and Atkinson 2000), and, after chromatographic purification, treatment with anhydrous tetrahydrofuran makes it a white solid. Finally, after optimization of the reaction conditions alkenol **6** was obtained in satisfactory yield as a mixture of isomers. This is not a problem for our purposes, since we planned to remove the double bond at the end of the synthesis together with the carboxylic protecting group. Nevertheless, the identity of the condensation product was unequivocally confirmed after hydrogenation of compound **6**, under the catalysis of palladium, which gave the fully characterized compound **7**. The ω-hydroxy long α-amino acid **7** is another interesting unnatural LAA derivative, which can be used for the construction of modified peptides.

Compound **6** was finally converted into the ω-amino acid by replacing the hydroxyl group with an amine. This was accomplished by initial conversion of **6** into tosylate **8**, in order to have a good leaving group which was efficiently



Scheme 1 *i* $(COCl)_2$, DMF, Py, MeCN, THF, −30 °C, then $LiAlH(O^tBu)_3$, −78 °C, 60 %; *ii* Ph_3P=CH–CHO, toluene, 70 °C, 75 %; *iii* **3**, LiHMDS, THF, −60 °C, 65 %; *iv* H_2, Pd/C, $CHCl_3$/MeOH, 87 %; *v* TsCl, DMAP, DIPEA, DCM, 63 %; *vi* NaN_3, TBAI, DMF, 60 °C, 92 %; *vii* Ph_3P, THF, H_2O, then BOC_2O, DCM, 40 °C, 65 %; *viii* H_2, Pd/C, MeOH, 85 %

displaced by sodium azide in dimethylformamide at high temperature. The azido group of compound **9** was then selectively reduced by means of a Staudinger reaction to give the crude amine, which was in turn protected as a BOC derivative. Finally, catalytic hydrogenation of **10** produced the saturated α,ω-diamino octadecanoic acid derivative **1**, by simultaneous reduction of the double bonds and deprotection of the carboxylic group.

Conclusions

We have described a practical methodology for the synthesis of α,ω-diamino acids, which has been applied to the preparation of an octadecanoic derivative. The preparation of compound **1** provides a general route to α,ω-diamino analogues of long fatty acids, and resulted in the longest compound of the family ever synthesized. Furthermore, this approach permits the modulation of the alkyl chain length depending on the chain length of the ylide used for the Wittig olefination reaction. In order to allow the insertion in a peptide sequence, it would be also possible to differentiate the two amino groups at the end of the synthesis, by protecting the amine obtained after Staudinger reduction with a different protecting group. During the synthetic sequence another class of unnatural LAAs, i.e. α-amino-ω-hydroxy long fatty acids, is recovered. The synthetic protocol herein reported is of general applicability and is a useful sequence to obtain LAA derivatives for the synthesis of drug delivery system or for the study of biological membranes.

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