A Convenient Asymmetric Synthesis of a β-amino Ester with Additional Functionalization as a Precursor for Peptide Nucleic Acid (PNA) Monomers

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Abstract: We report the asymmetric synthesis of di-3-pentyl (3S,αS,7E)-3-N-benzyl-N-α-methylbenzylamino-dec-7-enedioate (9), which contains the correct functionalization to produce δ-amino acid derivatives to be used as monomers for Peptide Nucleic Acid (PNA) formation. With this aim, thymine-pentanoic acid 15 and some of its ester derivatives were obtained, their reactivity was studied and the noteworthy ethyl ester 12 was quantitatively produced by transesterification of methyl ester 11, thus paving the way for the synthesis of the thymine-containing amino ester 11, which has been designed as a building block for a Nucleic-Acid analog with a chiral, flexible peptide backbone.

Keywords: Peptide nucleic acids (PNA), thymine derivatives, transesterification, asymmetric synthesis, β-amino acids.

Introduction

Recently, the chemistry of nucleic-acid analogs (Figure 1, DNA/RNA I) has gained considerable attention due to their potential use as antisense or antigene agents [1]. Among the known oligonucleotide analogues [2,3], acyclic N-(2-aminoethyl)-glycyl peptide nucleic acids (Figure 1, aegPNA II), are found to be very good mimics of DNA/RNA hybrids, and their stability towards proteases and nucleases has generated interest in medicinal chemistry [3]. A great deal of work is
currently being undertaken in this field [4]. Leumann et al. [5] decided to investigate the influence of higher flexibility of the amide backbone (relative to PNA) of the base–pairing properties with complementary DNA and RNA (Figure 1, δaa PNA III). They performed the synthesis of the monomer building block IV (Figure 1), related to the repeating monomeric δ-amino acid in III, containing the nucleobase thymine.

**Figure 1.** Structure of DNA, PNA and modified PNAs.

We have demonstrated the asymmetric synthesis of the monoaddition products 5 and 6 by chiral lithium amide [(R)-1] Michael addition to diendioate esters 2 and 3 respectively (Scheme 1) [6]. While addition to (E,E)-octa-2,6-diendioate gave the cyclopentane adduct through a domino reaction initiated by an asymmetric Michael addition, followed by a 5-exo-trig intramolecular cyclization [7], addition to the (E,Z)-counterpart gave the monoaddition adduct 4, as it is known that lithium amide does not produce addition to (Z)-α,β-unsaturated esters [8].

**Scheme 1.**

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<th>2 (R)-1</th>
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<td>ROOC</td>
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<td>COOR</td>
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<td>Ph N Ph</td>
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<td>R = CH(CH2CH3)2</td>
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<th>3 (R)-1</th>
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n = 1; V: γ-aminoacid derivate
n = 3; VI: ε-aminoacid derivate
The monoaddition product 6 is obtained instead by treating the acceptor 3 with an equimolecular amount of the lithium amide, as an excess of amide produces the diaddition adduct [6]. We envisaged that degradation of the remaining alkenes in these compounds and subsequent transformation of the ester group would make them precursors of γ- and ε-amino acid derivatives, V and VI respectively. Davies et al. [9] has recently published a comprehensive review in this area of chemistry covering the scope, limitation and synthetic applications of the use of enantiomerically pure lithium amides as homochiral ammonia equivalents in conjugate addition reactions. We present here the synthesis of monoaddition adduct 9 precursor of IV, and the study of the reactivity of thymine-pentanoic acid and derivatives, with similar functionality, in order to apply the results to the synthesis of PNA monomers.

Results and Discussion

Whereas (E)-α,β-unsaturated esters are very susceptible to conjugate addition, their (Z)-counterparts are highly susceptible to γ-deprotonation [8,9]. This suggested a strategy towards the monoadduct 9 from di-3-pentyl (E,Z)-deca-2,8-dienoate 7. Addition of 7 to an excess (3 equiv.) of (S)-1 generated the (E)-β,γ-unsaturated monoadduct 9, [α]_D^{26}=−6.9 (c 0.93, CHCl_3), in 70% yield after work-up and > 95% de [6], as expected from α-protonation of the intermediate dienoate 8 (Scheme 2). The absolute configuration at C(3) within (3S,αS)-9 relative to the N-α-methylbenzyl stereocentre is assigned by analogy with previous authenticated models developed to explain the stereoselectivity observed during addition of lithium amide (S)-1 to α,β-unsaturated acceptors [10].

Scheme 2.

![Scheme 2](image)

We propose the monoadduct 9 as a precursor of the the δ-aminoacid monomer IV, via transformation of the ester group and degradation of the remaining double bond in 9 to an ester, further work within our group haven proven that the double bond can easily be transformed into an aldehyde, the corresponding dioxolane derivative or the related methyl ester [11]. We envisaged performing some reactions once the thymine nucleobase is attached, so we decided to synthesize the methyl thymine-pentanoate 11 and undertake the reactions shown in Scheme 3. It is of great interest to achieve, with the nucleobase attached, the transesterification process from methyl or other alkyl ester to the ethyl one, which is the ester group in the compound IV described in the literature [5], in order to ascertain the optical rotation and thereby the enantiomeric excess produced over the whole process. Nevertheless, the reactions shown in scheme 3, and the preparation of thymine pentanoic acid 15 are
of importance on their own, as the homologous thymine acetic acid has already been used to form an amide bond with a diamino acid derivative to furnish a chiral peptide nucleic acid monomer [12].

Esterification of 5-bromovaleric acid (10) with diazomethane and subsequent treatment with thymine in basic media produces methyl ester 11 (62%), which upon transesterification by treatment with HCl(g) in EtOH yields ethyl ester 12 quantitatively.

Scheme 3.

Reaction of ester 11 with DIBAL-H produces aldehyde 13 (69%) which upon further treatment with ethane-1,2-diol and p-TsOH produces the acetal compound 14 in 72% yield. When aldehyde 13 was treated with different oxidant reagents such as aq. NaClO₂ only degradation products were obtained. However, PDC oxidation in MeOH [13] gave methyl ester 11 in good yield (73%).

Our attempts to obtain ester 17 by oxidation of dioxolane 14 were unsuccessful, since when compound 14 was treated with PDC and t-BuOOH in dichloromethane (DCM) [14] for long periods of time or with Oxone® and wet Al₂O₃ in CHCl₃ [15] only starting material was recovered, and treatment with O₃ [16] produced a complex mixture.

Hydrolysis of 11 with KOH/MeOH 2M gave a quantitative yield of 15, which upon treatment with diazomethane yielded a 1:1 mixture of esters 11 and 16 in 80% yield. Compound 16 is a methyl ester where the nitrogen of the thymine group has been alkylated. When acid 15 was subjected to treatment with EtI in NaOH and HMPA it did not produce the ethyl ester 12.
Conclusions

We have demonstrated an efficient strategy for the asymmetric synthesis of β-amino ester 9 by asymmetric Michael addition of (S)-1 to the (E) double bond and γ-deprotonation of the (Z) double bond in the (E,Z)-deca-2,8-diendioate ester 7. Reactivity assays on the nucleobase containing the model thymine-pentanoic acid and derivatives showed that they are prone to degradation upon treatment with oxidants such as O3 or NaClO2; the oxidation of dioxolane 14 to produce ester 17, needs further study. On the other hand, the aldehyde and acid functions in 13 and 11 can be easily interchanged; the diazomethane esterification of the acid function in compound 15 is competitive with nitrogen methylation of the thymine moiety, but interestingly, quantitative transesterification of methyl ester 11 to produce ethyl ester 12 was achieved, paving the way to obtain the required ester in the preparation of the previously synthesized δ-amino acid 11. The application of this strategy for the preparation of δ-amino acid monomer 11 is currently under investigation in our laboratory and will be published in due course.

Experimental Section

General

1H-NMR and 13C-NMR spectra were recorded in CDCl3 at 200 and 400 MHz (1H) or 50 and 100 MHz (13C) on Varian 200 VX and Bruker DRX 400 instruments, respectively. Multiplicities were determined by DEPT experiments. IR spectra were recorded using a BOMEM 100 FTIR spectrophotometer. Optical rotations were determined using a Perkin-Elmer 241 polarimeter in a 1 dm cell and are given in units of 10-1 deg cm2 g-1. Concentrations are quoted in g per 100mL. The electron impact (EI) mass spectra were run on a VG-TS 250 spectrometer using a 70 eV ionizing voltage. HRMS were recorded using a VG Platform (Fisons) spectrometer using Chemical Ionization (ammonia as gas) or Fast Atom Bombardment (FAB) techniques. Thin layer chromatography (tlc) was performed on aluminum sheets coated with 60 F254 silica. Sheets were visualized using iodine, UV light or 1% aqueous KMnO4 solution. Column chromatography (CC) was performed with Merck silica gel 60 (70-230 mesh). Solvents and reagents were generally distilled prior to use: DMF from CaH2 and dichloromethane (DCM) from KOH.

Preparation of di-3-pentyl (3S,αS,7E)-2-N-benzyl-N-α-methylbenzylamino-dec-7-enedioate (9)

n-BuLi (1.6 M, 0.41 mL, 0.66 mmol) was added to a stirred solution of (S)-N-benzyl-N-α-methylbenzylamine (145 mg, 0.69 mmol) in THF (3 mL) at −78°C and the mixture stirred for 30 minutes prior to the addition of a solution of 7 (76 mg, 0.22 mmol) in THF (1 mL) at −78°C. After 80 minutes, saturated aqueous NH4Cl solution was added and the resulting solution warmed to r.t., partitioned between DCM (3 x 50 mL) and brine and dried over Na2SO4. Concentration followed by flash chromatography on silica gel (95:5 Hex-EtOAc) gave 9 (85 mg, 70%); IR (film) ν (cm⁻¹): 2971, 2880, 1732, 1458, 1260, 1157, 1117, 968; 1H-NMR (400 MHz): 0.84 (6H, t, J= 7.5 Hz, CH(CH2C6H5)2), 0.89 (6H, t, J= 7.5 Hz, CH(CH2CH3)2), 1.34 (3H, d, J= 7.0 Hz, C(α)Me), 1.50 (4H, m, H-4 and H-5), 1.57 (8H, m, CH(CH2CH3)2), 1.98 (4H, m, H-2 and H-6), 3.01 (2H, d, J= 5.1 Hz, H-9), 3.36 (1H, m, H-3),
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3.49 (1H, d, J = 13.9 Hz, NCH₆HPhH), 3.81 (1H, d, J = 13.9 Hz, NCH₆HPh), 3.82 (1H, q, J = 7.0 Hz, C(a)H), 4.68 (1H, quin, J = 5.4 Hz, CH(CH₂CH₃)₂), 4.77 (1H, quin, J = 5.4 Hz, CH(CH₂CH₃)₂), 5.54 (2H, m, H-7 and H-8), 7.21-7.41 (10 H, m, Ar-H); 13C-NMR (50 MHz): 9.5 (4 CH₃, CH(CH₂C₆H₃)₂), 20.5 (CH₃, C(a)Me), 26.3 (2CH₂, CH(CH₂CH₃)₂), 26.5 (2CH₂, CH(CH₂CH₃)₂), 26.6 (CH₂, C-5), 32.4 (CH₂, C-4), 33.2 (CH₂, C-6), 36.7 (CH₂, C-2), 38.5 (CH₂, C-9), 50.1 (CH₂, NCH₂Ph), 53.7 (CH, C-3), 58.3 (CH, C(a)H), 76.6 (CH, CH(CH₂CH₃)₂), 77.0 (CH, CH(CH₂CH₃)₂), 122.0 (CH, C-7), 134.4 (CH, C-8), 126.6-128.2 (10CH, Ar-C), 141.8 (C, C₆H₅O₂), 172.6 (2C, COOR); EIMS m/z (%): 549 (M⁺, 10), 462 (8), 352 (40), 248 (15); HRMS (EI): C₃₅H₅₁NO₄, M⁺ requires 549.382; found 549.383; [α]₂⁶D = -6.9 (c= 0.93, CHCl₃).

Preparation of 1-(4'-methoxycarbonylbutyl)-thymine (11)

5-Bromovaleric acid 10 (300 mg, 1.6 mmol) was treated with a solution of gaseous CH₂N₂ in ether and stirred for 3 hours. Evaporation of the solvent gave methyl-5-bromo-pentanoate (300 mg, 96%); IR (film) ν (cm⁻¹): 2953, 1740, 1437, 1364, 1260, 1206, 1173, 1125, 1035; 1H-NMR (200 MHz): 1.60-1.95 (4H, m, H-3 and H-4), 2.27 (2H, m, H-2), 3.33 (2H, m, H-5), 3.59 (3H, s, COOCH₃); 13C-NMR (50 MHz): 23.6 (CH₂, C-3), 32.1 (CH₂, C-2), 32.2 (2CH₂, C-4 and C-5), 51.7 (CH₃, COOCH₃), 173.6 (C, COOCH₃); EIMS m/z (%): 196 (M⁺), 167 (15), 165 (15), 137 (12), 115 (100), 101 (45), 89 (40). To a solution of thymine (479 mg, 4.6 mmol) in DMF (400 mL), K₂CO₃ (317 mg) and Bu₄NI (124 mg) were added and the reaction mixture was stirred for 30 minutes at 0ºC and at 65ºC for another 30 minutes. To this solution the previously synthesized methyl-5-bromopentanoate (1.53 mmol) was added and the mixture was stirred at 65ºC overnight. The reaction mixture was then cooled to r.t. and extracted with EtOAc. The organic layer were washed with water and dried over Na₂SO₄. Concentration followed by flash chromatography on silica gel (95:5 DCM-MeOH) gave 11 (228 mg, 62%); IR (film) ν (cm⁻¹): 3190, 3057, 2955, 1720, 1684, 1458, 1362, 1204, 1082; 1H-NMR (200 MHz): 1.61-1.80 (4H, m, H-2’ and H-3’), 1.91 (3H, s, Me), 2.36 (2H, t, J= 8.0 Hz, H-4’), 3.67 (3H, s, Me), 3.70 (2H, m, H-1’), 6.98 (1H, s, H-6), 9.10 (1H, m, NH); 13C-NMR (50 MHz): 12.1 (CH₃, Me), 21.5 (CH₂, C-3’), 28.3 (CH₂, C-2’), 33.2 (CH₂, C-4’), 47.8 (CH₂, C-1’), 51.4 (CH₃, COOCH₃), 110.4 (C, C-5), 140.6 (CH, C-6), 151.2 (C, C-2), 164.8 (C, C-4), 173.4 (C, COOCH₃); EIMS m/z (%): 240 (M⁺, 52), 209 (42), 180 (25), 140 (39), 126 (62), 110 (38), 96 (100); HRMS (EI): C₃₅H₅₁NO₄, M⁺ requires 240.111; found 240.113.

Preparation of 1-(4'-ethoxycarbonylbutyl)-thymine (12)

A solution of 11 (17 mg, 0.07 mmol) in EtOH (5 mL) at 0ºC was treated with a stream of gaseous HCl till the mixture was saturated. The solution was stirred overnight at r.t., and the solvent was then removed under vacuum to give 12 (19 mg, 99%); IR (film) ν (cm⁻¹): 3185, 3057, 2955, 1724, 1684; 1H-NMR (200 MHz): 1.24 (3H, t, J= 6.8 Hz, CO₂CH₂CH₃), 1.60-1.80 (4H, m, H-3’ and H-2’), 1.91 (3H, s, Me), 2.35 (2H, m, H-4’), 3.70 (2H, m, H-1’), 4.12 (2H, q, J= 6.8 Hz, CO₂CH₂CH₃), 6.98 (1H, s, H-6), 9.22 (1H, s, NH); 13C-NMR (50 MHz): 12.3 (CH₃, Me), 14.2 (CH₃, CO₂CH₂CH₃), 21.7 (CH₂, C-3’), 28.4 (CH₂, C-2’), 33.2 (CH₂, C-4’), 48.2 (CH₂, C-1’), 60.2 (CH₂, CO₂CH₂CH₃), 110.7 (C, C-5),
140.4 (CH, C-6), 150.8 (C, C-2), 164.3 (C, C-4), 173.0 (C, CO2CH2CH3); HRMS (EI): C12H18O4N2 requires 254.127; found 254.127.

**Preparation of 1-(5'-oxopentyl)-thymine (13)**

To a solution of 11 (99 mg, 0.41 mmol) in DCM (4 mL) at –78°C, DIBAL-H (0.9 mL, 1.0 M) was added. The reaction mixture was stirred for 1 hour at -40°C and then H2O was added. The resulting solution was warmed to r.t and added to a mixture of NaHCO3 and Na2SO4 in ether. It was then filtered through Celite® and concentrated in vacuo to give 13 (60 mg, 69%); IR (film) ν (cm⁻¹): 3187, 3048, 2947, 1684, 1472, 1362, 1258, 1221, 1094; ¹H-NMR (200 MHz): 1.62-1.81 (4H, m, H-2’ and H-3’), 1.90 (3H, s, Me), 2.52 (2H, t, J= 8.0 Hz, H-4’), 3.70 (2H, t, J= 8.0 Hz, H-1’), 6.98 (1H, s, H-6), 9.35 (1H, s, NH), 9.77 (1H, s, CHO).

**Preparation of 1-(4'-[1,3]dioxolan-2-yl-butyl)-thymine (14)**

p-TsOH and ethane-1,2-diol were added to a solution of 13 (69 mg, 0.33 mmol) in dry benzene. The reaction mixture was refluxed in a Dean Stark apparatus for 12 hours. The solution was cooled and then H2O (1 mL) was added, extracted with Et2O and the organic layer washed with NaHCO3 and brine, dried and concentrated in vacuo to give 14 (60 mg, 72%); IR (film) ν (cm⁻¹): 3196, 3059, 2953, 1688, 1472, 1362, 1221, 1177, 1130; ¹H-NMR (200 MHz): 1.62-1.85 (4H, m, H-2’, H-3’ and H-4’), 1.91 (3H, s, Me), 3.69 (2H, t, J= 8.0 Hz, H-1’), 3.83 (2H, m, OCH2CH2O), 3.95 (2H, m, OCH2CH2O), 4.85 (1H, t, J= 8.0 Hz, H-5’), 6.97 (1H, s, H-6), 8.82 (1H, s, NH); ¹³C-NMR (50 MHz): 12.5 (CH3, Me), 21.0 (CH2, C-3’), 29.1 (CH2, C-2’), 33.7 (CH2, C-4’), 48.6 (CH2, C-1’), 65.0 (CH2, OCH2CH2O), 65.1 (CH2, OCH2CH2O), 104.3 (CH, C-5’), 110.8 (C, C-5), 140.7 (CH, C-6), 150.9 (C, C-2), 164.4 (C, C-4); EIMS m/z (%): 254 (M⁺, 5), 182 (12), 153 (10), 126 (14), 96 (19), 73 (100); HRMS (EI): C12H18O4N2 requires 254.1266; found 254.1298.

**Preparation of 1-(4’-methoxycarbonyl-butyl)-3-methylthymine (11)**

To a solution of 11 (50 mg, 0.21 mmol) in DMF (1 mL) and MeOH (0.05 mL) was added PDC (474 mg, 126 mmol) at r.t. and the mixture was stirred for 20 hours, then H2O (1 mL) was added and the reaction mixture was extracted with Et2O. The organic layer was washed with H2O and brine. It was then dried over Na2SO4 and filtered. Evaporation of the solvent followed by flash chromatography on silica gel (95:5 DCM-MeOH) gave 11 (36 mg, 73%).

**Preparation of 1-(4’-carboxybutyl)-thymine (15)**

Compound 11 (30 mg, 0.12 mmol) was added to the solution of KOH (0.1 mL) and MeOH (0.05 mL) and was stirred at r.t. for 8 hours, then H2O was added to the reaction mixture and the solution was treated with 2M HCl to pH = 2 and extracted with Et2O. The organic extracts were washed with water to give, after drying and concentration, compound 15 (28 mg, 99%); IR (film) ν (cm⁻¹): 3600, 3185, 3057, 2928, 1684, 1474, 1418, 1362, 1260; ¹H-NMR (200 MHz): 1.61-1.82 (4H, m, H-3’ and H-4’),
1.90 (3H, s, Me), 2.40 (2H, \( J = 6.8 \text{ Hz, } H-2' \)), 3.72 (2H, \( J = 6.8 \text{ Hz, } H-1' \)), 7.04 (1H, s, \( H-6 \)); 13C-NMR (50 MHz): 14.6 (CH3, Me), 25.4 (CH2, C-3'), 30.8 (CH2, C-2'), 31.9 (CH2, C-4'), 49 (CH2, C-1'), 113.8 (C, C-5), 145.6 (CH, C-6), 150.9 (C, C-2), 169.4 (C, C-4), 179.5 (C, COOH); EIMS m/z (%): 244 ((M+NH4)\(^+\), 38), 226 (M +, 8), 209 (35), 126 (70), 110 (40), 96 (100). Compound 15 (10 mg, 0.05 mmol) was dissolved in a solution of gaseous CH\(_2\)N\(_2\) in ether and stirred for 30 minutes at r.t. Concentration followed by flash chromatography on silica gel (95:5 DCM-MeOH) gave 16 (5 mg, 40%) and 11 (4 mg, 40%); Compound 16: \(^1\)H-NMR (200 MHz): 1.67-1.69 (4H, m, \( H-3' \) and \( H-4' \)), 1.93 (3H, s, Me), 2.36 (2H, \( J = 7.8 \text{ Hz, } H-2' \)), 3.34 (3H, s, Me-N), 3.65 (3H, s, COO\(_{\text{Me}}\)), 3.70 (2H, \( J = 8.0 \text{ Hz, } H-1' \)).

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References


*Sample Availability:* Contact the authors.