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Synthesis and polymerisation of lipophilic peptide nucleic acids derived from stearic acid and pentacosa-10,12-diynoic acid

Nicola M. Howarth,* W. Edward Lindsell, Euan Murray and Peter N. Preston*

Chemistry, School of Engineering & Physical Sciences, William H. Perkin Building, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK

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Abstract—The adenine, cytosine and thymine peptide nucleic acid (PNA) monomers and PNA T_{10} oligomers bearing either a diacetylenic or stearoyl moiety at the N- or C-terminus have been successfully prepared. The resulting thymine monomeric and T_{10}-mer derivatives have been subsequently incorporated into polydiacetylene-containing liposomes.
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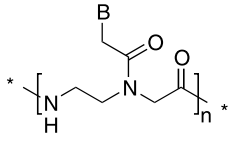
Polydiacetylenes (PDAs) can be prepared by topochemical polymerisation of conjugated 1,3-diynes by thermal treatment or by UV- and γ-irradiation (Scheme 1);[1] they can also be formed in thin films[2] or as liposomes.[3] An important feature of PDAs is manifested in colour changes that can be induced thermally, by pH changes and through solvent variations.[4] Blue to red transitions also result from binding events in which lipophilic biospecific receptors are embedded in a host PDA matrix; such assemblies can be generated either by co-polymerisation of appropriately functionalised diacetylenes (e.g. carbohydrate-mediated recognition of influenza virus[3]) or by the use of lipid/PDA mixed vesicles (e.g. elucidation of peptide-membrane interactions[5]).

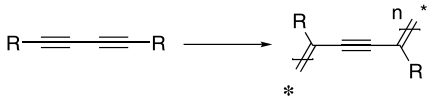
We envisage that nucleic acid biosensors should be accessible using PDA matrixes in which the receptor is a group with suitable intercalating or other DNA-binding properties. Previously, we have reported the preparation of lipophilic acridine-labelled diacetylenes[6] and PDA liposomes thereof.[7] Here, we report the synthesis of peptide nucleic acids (PNAs) and model PNA monomer analogues in the 1,3-diyne series. PNAs are DNA mimics in which the entire deoxyribose–phosphate backbone has been exchanged with a structurally homomorphous uncharged polyamide backbone composed of *N*-(2-aminoethyl)glycine units (Scheme 2).[8] An important feature of PNAs is that they bind with higher affinity and sequence specificity to both single-stranded DNA (ssDNA) and RNA than their natural oligonucleotide counterparts.[9]



Scheme 1. Preparation of PDAs from conjugated 1,3-diynes.

The objectives of the present work were as follows:

1. Synthesis of PNA monomer model compounds with diacetylenic or stearoyl moieties at the N- or C-terminus.
2. Solid-phase synthesis (sps) of PNA oligomers incorporating diacetylenic and (separately) stearoyl groups.
3. Production of new PDA liposomes.



Scheme 2. Structure of PNA. B = adenine; cytosine; guanine; thymine.

Keywords: nucleic acid biosensors; PNA; polydiacetylenes; liposomes.
* Corresponding authors. Tel.: +(44)-(0)131-451 8026; fax: +(44)-(0)131-451 3180 (N.M.H.); Tel.: +(44)-(0)131-451 8035; fax: +(44)-(0)131-451 3180 (P.N.P.); e-mail: n.m.howarth@hw.ac.uk; p.n.preston@hw.ac.uk

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1. Synthesis of PNA monomer model compounds[12]

1.1. C-Terminal diacetylenic derivatives

The two C-terminal diacetylenic derivatives, **3** and **4**, were successfully prepared from the protected thymine PNA monomer **1**[13,14] as outlined in Scheme 3. Ester hydroysis using the procedure reported by Dueholm et al.[15] afforded **2** in a 74% yield. Compound **2** was then converted in situ into its corresponding *N*-hydroxybenzotriazole (HOBt)- activated ester using HOBt and EDC. Subsequent addition of either *N*-(8-amino-3,6-dioxaoctyl)-10,12-pentacosadiynamide[7] or 1-amino-10,12-pentacosadiyne[10] and *N*,*N*-diisopropylethylamine gave the desired compounds **3** and **4**, respectively, in 66 and 38% yields after purification.

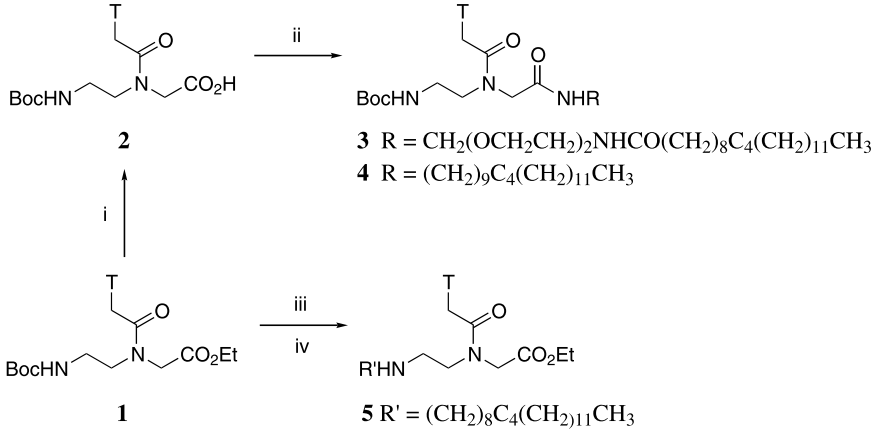
1.2. N-Terminal diacetylenic and stearoyl derivatives

Two routes to the N-terminal PNA monomer model compounds have been employed. The N-terminal diacetylenic derivative **5** was prepared from the protected thymine PNA monomer **1** (Scheme 3). Removal of the Boc protecting group was accomplished using aqueous hydrochloric acid. Treatment of the isolated crude hydrochloride salt with *N*-succinimidyl-10,12-pentacosadiynoate[6] in the presence of *N*,*N*-diisopropylethylamine gave compound **5** in 50% yield after purification. We have found more recently that **5** can be obtained in a higher yield (90%) if 10,12-pentacosadiynoic acid fluoride[11] is used in the last step rather than *N*-succinimidyl-10,12-pentacosadiynoate.

The N-terminal diacetylenic derivatives **13**–**15** and N-terminal stearoyl analogue **16** have been synthesised using an alternative strategy starting from *N*-(2-aminoethyl)glycine methyl ester dihydrochloride **6**[16] (Scheme 4). Treatment of **6** with either *N*-succinimidyl-10,12-pentacosadiynoate[6] or *N*-succinimidyl stearoate in the presence of *N*,*N*-diisopropylethylamine afforded **7** and **8**, respectively, in 65 and 60% yields after purification. Compound **7** was then coupled to thyminyl acetic acid,[14] N^4-benzyloxycarbonyl cytosinyl acetic acid[15] and N^6-benzyloxycarbonyl adeninyl acetic acid[15] using either HBTU or HATU as the activating agent in the presence of *N*,*N*-diisopropylethylamine. After work-up and purification by flash chromatography, **9**, **10** and **11** were ioslated in 63, 71 and 80% yields. Similar conditions were applied in the coupling of **8** to thyminyl acetic acid to obtain **12** (86% yield). Finally, ester hydrolysis using lithium hydroxide afforded the desired compounds **13**, **14**, **15** and **16** in yields ranging from 84 to 97%.

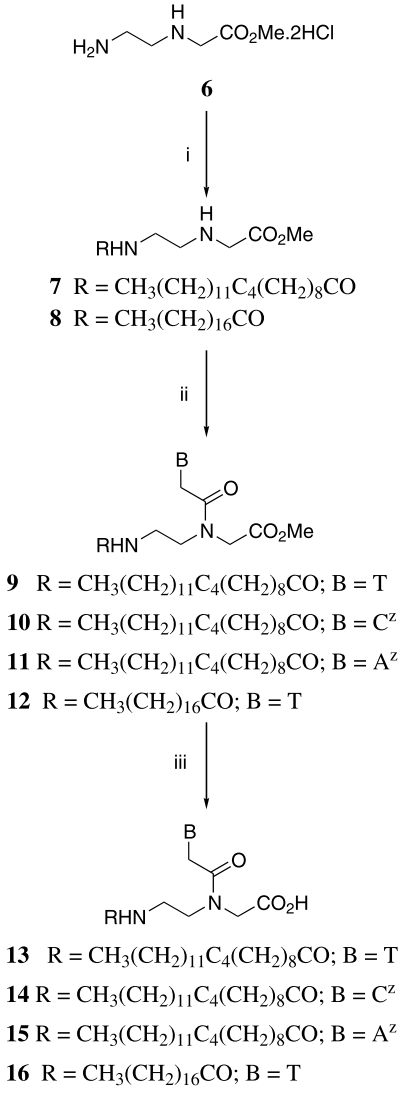
2. Synthesis of *N*-stearoyl and diacetylenic PNA oligomers

The PNA oligomer of sequence $CH_3(CH_2)_{16}CO$-$(T)_{10}$Lys-NH_2, **17**, has been prepared by sps[17,18] using the N^1-Boc-N^5-(2-chloro-Z)-L-lysine-derivatised 4-methylbenzhydrylamine (MBHA) resin. The Boc-protected thymine monomer **2** (Scheme 3) was coupled using HBTU as the activating agent in the presence of a slight excess of *N*,*N*-diisopropylethylamine. All the couplings were monitored using the Kaiser test[19] and in all cases only a single coupling was required. The stearoyl group was incorporated at the *N*-terminus of the oligomer in the final coupling step once the PNA T_{10}-mer had been assembled on the solid support. This was achieved using 3.33 equiv. of stearoyl fluoride[20] and 10 equiv. of *N*,*N*-diisopropylethylamine. At the end of the synthesis, the oligomer



Scheme 3. Boc = $(CH_3)_3COCO$; T = thyminyl. *Reagents and conditions*: i. 1 M (aq.) LiOH, THF, rt; ii. (a) 1.1 equiv. EDC, 1.1 equiv. HOBt, DMF, 0°C–rt (b) 0.94 equiv. $CH_3(CH_2)_{11}C_4(CH_2)_8CONH(CH_2CH_2O)_2CH_2NH_2$[7] or 0.67 equiv. $CH_3(CH_2)_{11}C_4(CH_2)_9NH_2$,[10] 1 equiv. DIPEA, DMF, 0°C–rt; iii. 6 M (aq.) HCl, DCM, rt; iv. 1 equiv. $CH_3(CH_2)_{11}C_4(CH_2)_8CO_2N(COCH_2)_2$,[6] DMF, rt, or 1 equiv. $CH_3(CH_2)_{11}C_4(CH_2)_8COF$,[11] 2:1 DMF:DCM, rt, 3 equiv. DIPEA.

was cleaved from the solid support using trifluoromethanesulfonic acid (TFMSA) under identical conditions to those employed by Christensen et al.[17] The crude oligomer was purified using RP-HPLC (R.T. = 28 min; buffer A = 0.1% TFA in water; buffer B = 0.1% TFA in acetonitrile; flow rate = 1 mL/min; λ_{max} = 260 nm). The identity of the oligomer was verified by MALDI-TOF mass spectrometry which showed a principal peak at 3096.2 Da, corresponding to the Na^+ adduct of the desired oligomer.



Scheme 4. $A^Z = N^6$-Benzyloxycarbonyladeninyl; $C^Z = N^4$-benzyloxycarbonylcytosinyl; T = thyminyl. *Reagents and conditions*: i. 1 equiv. $CH_3(CH_2)_{11}C_4(CH_2)_8CO_2N(COCH_2)_2$[7] or 1 equiv. $CH_3(CH_2)_{16}CO_2N(COCH_2)_2$, 3 equiv. DIPEA, DMF, rt; ii. 1.2 equiv. $R'CH_2CO_2H$,[14,15] 1.2 equiv. HBTU or HATU, 3 equiv. DIPEA, DMF, rt; iii. 1 M (aq.) LiOH, THF, rt.

17 $CH_3(CH_2)_{16}CO$-$(T)_{10}$-$LysNH_2$[†]
18 $CH_3(CH_2)_{16}CO$-$(T)_{10}$-$AspNH_2$
19 $CH_3(CH_2)_{11}C_4(CH_2)_8CO$-$(T)_{10}$-$LysNH_2$
20 AcAsp-$(T)_{10}$-$LysNH_2$
21 AcAsp-$(T)_{10}$-Lys$(CO(CH_2)_{16}CH_3)NH_2$
22 AcAsp-$(T)_{10}$-Lys$(CO(CH_2)_8C_4(CH_2)_{11}CH_3)NH_2$

Similarly prepared[21] was **18**, $[M+Na]^+ = 3082.1$ Da, but the final coupling step on the solid support to prepare the related diacetylene derivative **19** failed. However, a post-synthetic modification method involving acylation of the lysine side chain was used successfully to attach a diacetylenic group; thus, capped PNA **20** in 1:1 DMF/pyridine was allowed to react with the appropriate acyl fluoride (10 equiv.) with DIPEA (30 equiv.) in 1:1 DCM:DMF to afford **21**, $[M+Na]^+ = 3252.5$ Da, and **22**, $[M+Na]^+ = 3342.7$ Da, both in 67% yield.

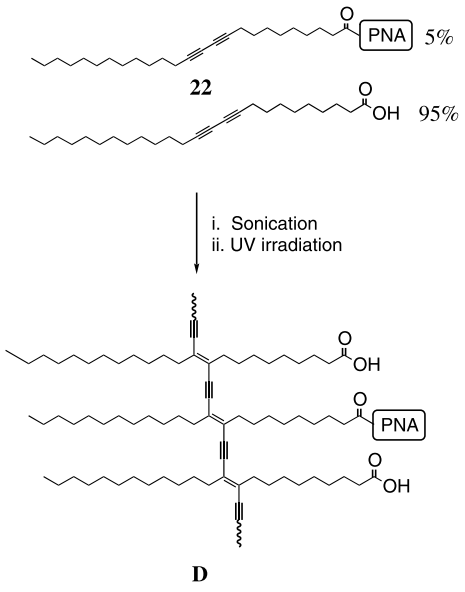
3. Formation of liposomes

Liposomes of selected lipophilic products, including derivatives with diacetylene groups as a component, were prepared in deionised water[22] using a 25 KHz probe sonicator at 80°C, followed by filtration (0.8 μm nylon filter) and cooling to 4°C. Photo-polymerisation of the N_2-purged solutions with a UV lamp (254 nm) afforded deep blue solutions of PDA-liposomes. The following examples, **A**–**E**, were stable to precipitation at room temperature.

A *poly*-$CH_3(CH_2)_{11}C_4(CH_2)_8CO_2H$ **23** (100%) λ_{max} 626, 582, 229 nm
B *poly*-**13** (100%) λ_{max} 623, 574, 522, 269 nm
C *poly*-{**23** (95%)+**13** (5%)} λ_{max} 627, 583, 254 nm
D *poly*-{**23** (95%)+**22** (5%)} λ_{max} 624, 580, 259 nm
E *poly*-**23** (95%)+**16** (5%) λ_{max} 631, 587, 254 nm

Polymerised carboxylic acid **23** forms a liposome **A** with an electronic spectrum typical of PDAs (e.g. see Ref. 3). The thyminyl-containing PDA-liposome **B** includes an absorption band, 269 nm, assignable to the pyrimidine ring (the higher energy band <229 nm, observed in **A**, being obscured). Liposomes formed from mixtures of **23** (95%) and a thymine-containing lipid, **13**, **16** or **22** (5%), show spectral evidence for incorporation of the latter component both in **C** and **D**, with polymerisable 1,3-diyne functions in the minor constituent, and also in **E**, with only an unreactive stearoyl tail in **16**. Thus, the formation of PDA-liposomes containing PNA head-groups with potential for biosensor applications has been achieved (Scheme 5).

[†] We employ the following nomenclature: $CH_3(CH_2)_{16}CO$, $CH_3(CH_2)_{11}C_4(CH_2)_8CO$ and Ac denote the acyl group attached to the N-terminal; T the thyminyl PNA monomer unit; Lys and Asp refer to lysine and aspartic acid, respectively, and NH_2 indicates a C-terminal amide.



Scheme 5. Formation of the mixed assembly derived from polymerisable diacetylenes 10,12-pentacosadiynoic acid (95%) and **22** (5%).

Acknowledgements

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10. Synthesis and spectroscopic data for $CH_3(CH_2)_{11}C_4(CH_2)_9NH_2$: 10,12-Pentacosadiynoyl amide[7] and excess Red-Al® were stirred in toluene, 0–20°C, 17 h; quenching with NaOH aq. and extraction into ethyl acetate afforded the product as a colourless solid, 72%. ^{1}H NMR ($CDCl_3$): δ ppm 0.80 (t, $J=6.2$ Hz, 3H, CH_3), 1.2–1.7 (m, 34H, CH_2), 2.18 (t, $J=6.6$ Hz, 4H, C_4CH_2), 2.8 (br s, 2H, NH_2), 3.55 (t, $J=6.4$ Hz, 2H, NCH_2); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ ppm 14.1, 19.1, 22.6, 25.6, 28.2, 28.8, 29.0, 29.3, 29.4, 29.6, 31.8, 32.7, 63.0.
11. Synthesis and spectroscopic data for $CH_3(CH_2)_{11}C_4(CH_2)_8COF$: 10,12-Pentacosadiynoic acid, 2 equiv. cyanuric fluoride and 1 equiv. pyridine were heated at reflux in DCM, 3 h; aq./DCM work-up gave the product as a colourless, oily solid, 99%. ^{1}H NMR ($CDCl_3$): δ ppm 0.85 (t, $J=6.4$ Hz, 3H, CH_3), 1.2–1.7 (m, 30H, CH_2), 1.7 (m, 2H, CH_2), 2.25 (t, $J=6.7$ Hz, 4H, C_4CH_2), 2.50 (dt, $J=0.9$, 4.9 Hz, 2H, CH_2); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ ppm 14.1, 19.1 (×2), 22.7, 28.2, 28.3, 28.6, 28.7, 28.8, 28.9, 29.1, 29.3, 29.5, 29.6 (×3), 31.9 65.2, 65.4, 77.2, 77.6, 163.5 {d, $^1J(^{13}C–^{19}F)=361$ Hz}; ^{19}F NMR ($CDCl_3$): δ ppm 44.9. IR (Nujol) cm^{-1}: 1836 (νCO). HRMS (EI): m/z 394.3480 $[M+NH_4]^+$, $C_{25}H_{45}NOF$ calcd 394.3485.
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