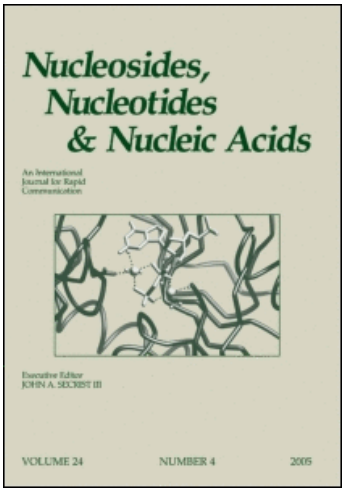

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Liquid Phase Synthesis of Peptide Nucleic Acid (or Polyamide Nucleic Acid) Dimers

Audrey Farese[a]; Sandrine Pairot[a]; Nadia Patino[a]; Vkronique Ravily[a]; Roger Condom[a]; Andreacute Aumelass[b]; Roger Guedj[a]
[a] Laboratoire de Chimie Bio-Organique, CNRS ESA 6001 Université de Nice Sophia-Antipolis, Nice Cedex 2, France [b] Centre de Biochimie Structurale, UMR C9955, FacultC de Pharmacie, 15 av. Ch. Flahaut, Montpellier Cedex, France

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LIQUID PHASE SYNTHESIS OF PEPTIDE NUCLEIC ACID (OR POLYAMIDE NUCLEIC ACID) DIMERS

Audrey Farese+, Sandrine Pairot+, Nadia Patino+, Véronique Ravily+, Roger Condom+*, André Aumelas$, Roger Guedj+

+ Laboratoire de Chimie Bio-Organique, CNRS ESA 6001
Université de Nice Sophia-Antipolis, F-06108 Nice Cedex 2, France
$ Centre de Biochimie Structurale, UMR C9955, Faculté de Pharmacie,
15 av. Ch. Flahaut, F-34060, Montpellier Cedex, France

Abstract: The liquid phase synthesis of "polyamide nucleic acid" (PNA) dimers containing the purine nucleic acid bases adenine and guanine has been achieved in good yields. This strategy was elaborated in order to circumvent difficult direct coupling of protected PNA monomers. This method can be applied to the liquid phase synthesis of short protected polyPNAs fragments, which can then selectively be deprotected.

Oligonucleotides that bind sequence specifically to double-stranded DNA or RNA are of major interest in molecular biology and medicinal chemistry, as gene-targeted drugs (antisense or antigene approaches)[1]. However, their use as therapeutic agents is limited because of their susceptibility to enzymatic degradation *in vivo* (overcome by synthesis of phosphorothioates), and their poor cellular penetration.

Nielsen *et al.*[2] have developed non-charged oligonucleotide analogues, poly (Polyamide Nucleic Acids) or poly(PNAs). In the PNA structure, the sugar backbone of DNA (or RNA) is replaced by a repeating N-(2-aminoethyl)glycine unit with a nucleobase attached through a methylenecarbonyl linker. PolyPNAs specifically recognize DNA or RNA fragments and form triplexes or duplexes via Watson-Crick and Hoogsteen interactions between complementary bases[3,4]. The affinity of PNA for DNA (or RNA) is higher than that of the oligonucleotide homologues, both because of the loss of negative charges and the rigidity of the polyamide backbone. Two major advantages of PNA as antisense agents, relative to oligonucleotides, are :

- their stability towards nucleases
- their lipophilicity, which permits cellular penetration[5].

Nielsen *et al.*[6] first described a synthesis of polyPNAs using solid-phase peptide techniques. However, the synthesis of short polyPNAs, for studying *in vitro* interactions of DNA fragments with other molecules (peptides, DNA, steroids,...), could also be performed in the liquid-phase. Since no epimerisation can occur, a liquid-phase synthesis could be carried out with a wide choice of solvents and reagents for each coupling steps. Thus, a wider range of protecting groups may be used in liquid-phase syntheses than in solid-phase syntheses. Moreover, liquid-phase synthesis offers the possibility of coupling larger fragments as well as monomers.

The direct condensation of two protected monomeric PNA units has been shown to be unsuccessful in the liquid-phase, even at low temperature and with a wide variety of coupling reagents[7]. In order to circumvent this difficult coupling, we have elaborated a general method for the synthesis of PNA dimers[7]. In this paper, we fully describe the synthesis of two PNA dimers containing the nucleic acid bases, adenine and guanine. This strategy may be used for the preparation of longer sequences.

RESULTS AND DISCUSSION

We have prepared dimers **5a** and **5b** in two steps from monomers **1a** and **1b** employing the strategy described in FIG. 1.

In this procedure, compound **2** constitutes the polyamide backbone of the second PNA unit. Its coupling with protected PNA monomers **1a** or **1b** affords compounds **3a** or **3b**, respectively, which are then condensed with protected base units **4b** or **4a** to yield the PNA dimers **5a** or **5b**. Alkaline hydrolysis of **5a** and **5b** produces **6a** or **6b**, respectively, which can then be subjected to the same stepwise processes to give further polyPNAs chain elongation.

The combination of protecting groups chosen for the nucleic acid bases (guanine: benzyl and adenine: ditertiobutyloxycarbonyl) and for the amino and carboxy groups of the backbone (benzyloxycarbonyl and methyl ester, respectively), allows the selective deprotection of adenine and guanine without deprotection of the backbone end groups: this could be of interest for biological studies since the cellular penetration of the short polyPNAs should be improved.

SYNTHESIS

Synthesis of PNA monomers 1a and 1b

The synthesis of adenine PNA monomer **1a** (Z-PNA(A-Boc$_2$)-OH) and of guanine PNA monomer **1b** (Z-PNA(G-Bn)-OH) is described in FIG. 2.

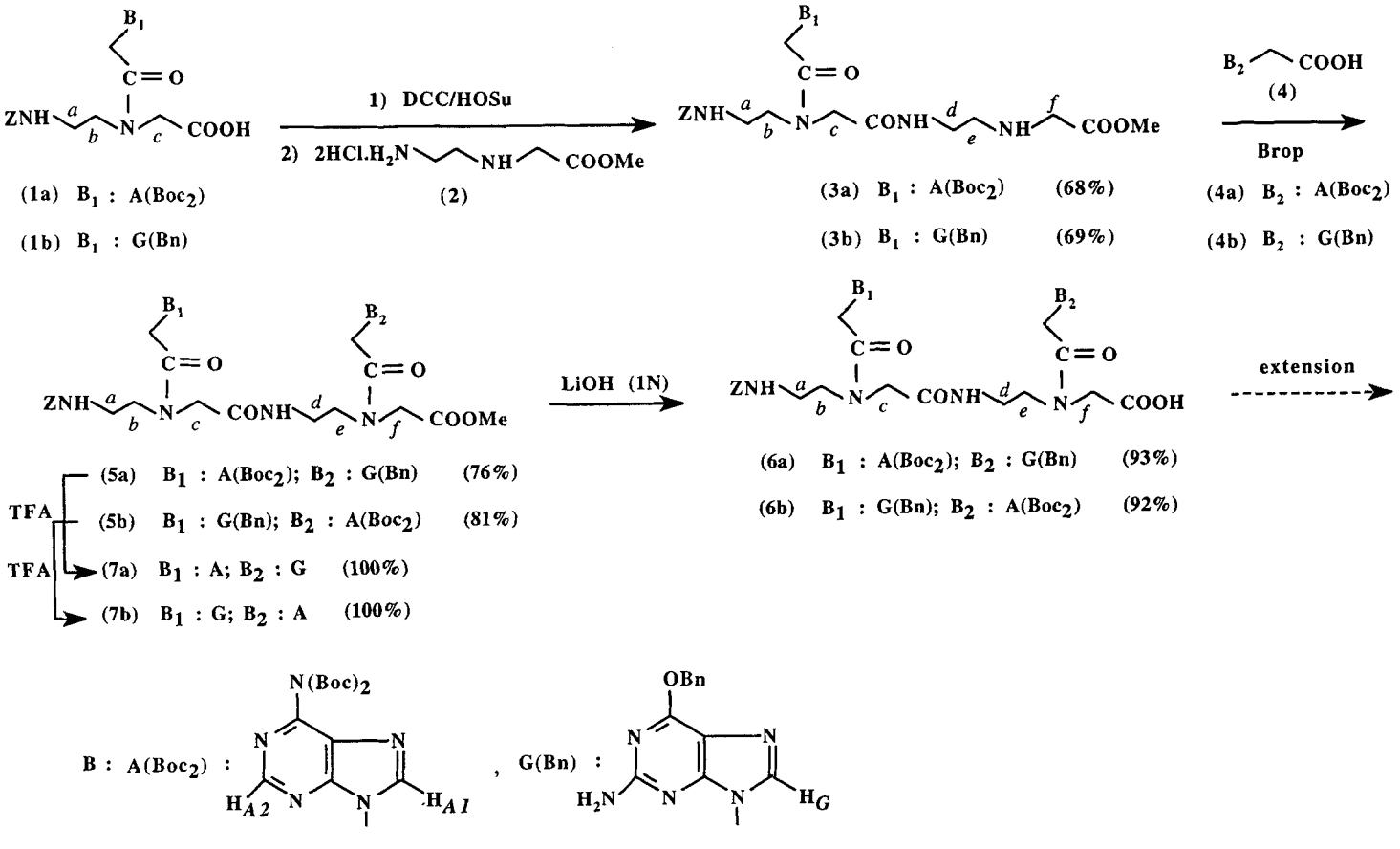

FIG. 1 : Synthesis of PNA dimers (NMR assigments are in italic)

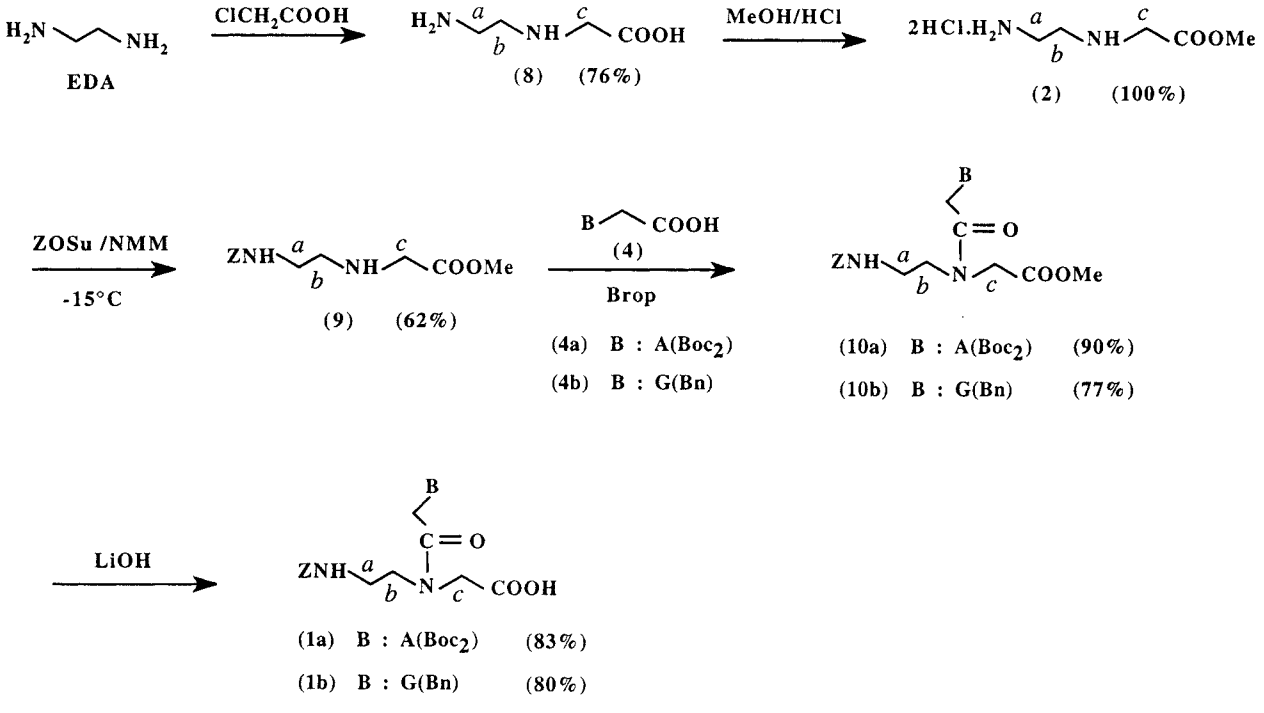

FIG. 2 : Synthesis of PNA monomers (NMR assigments are in italic)

Chloroacetic acid was condensed with ethylene diamine (EDA) to give compound **8** which was esterified using a literature procedure[8]. The ester **2** was then benzyloxycarbonylated with N-(benzyloxycarbonyloxy)-succinimide (ZOSu) at low temperature (-15°C) (to avoid its cyclisation which occurs in the presence of tertiary amines, even at 0°C). ZOSu reacted predominantly on the less hindered primary amine to give **9** as the major product (62%) when N-methylmorpholine (NMM) was used. When triethylamine (TEA) was employed, the formation of the dibenzyloxycarbonylated byproduct was enhanced.

The synthesis of adenine acetic acid unit **4a**, described in FIG. 3, was carried out in three steps. Condensation of methyl bromoacetate with adenine by first generating the anion with sodium hydride (NaH) led to **11** in 90% yield. The exocyclic amine function was then protected using $(Boc)_2O$/DMAP reagent (ditertiobutyl dicarbonate / dimethylaminopyridine) in acetonitrile. Knolfer *et al.*[9] have previously reported the synthesis of aromatic isocyanates from arylamines with $(Boc)_2O$ and a stoichiometric amount of DMAP. However, in our case, when a stoichiometric amount of DMAP was employed the diprotected compound **12** (56%) and a byproduct **13** containing three tertiobutyloxycarbonyl groups were obtained. Subsequent alkaline hydrolysis of **12** (LiOH 1N) afforded the N,N-diprotected adenine acetic acid unit **4a** (43% yield from adenine). The benzylated guanine acetic acid unit **4b** was prepared following the procedure described by Nielsen *et al.*[6].

Condensation of the backbone **9** and the base acetic acid units **4a** or **4b** (FIG. 2) was carried out using bromo tris(dimethylamino)phosphonium hexafluorophosphate (Brop reagent) to afford the PNA monomers **10a** or **10b** (90% and 77% yields, respectively). Alkaline hydrolysis (LiOH 1N) of the methyl ester of PNA monomers **10a** or **10b** respectively yielded **1a** or **1b** (83% and 80% yields respectively).

Synthesis of PNA dimers 6a, 6b and 7a, 7b

Condensation of the PNA monomers **1a** or **1b** with the backbone **2** was carried out by first preactivating the acid function with dicyclohexylcarbodiimide/N-hydroxysuccinimide (DCC/HOSu), and then coupling with **2**, at -15°C (FIG. 1).

The base acetic acid unit **4b** was coupled with **3a** using Brop reagent to give **5a** in 76% yield. Similarly, coupling of the base acetic acid unit **4a** with **3b** gave **5b** in 81% yield. The use of PyBrop (bromo-tris-pyrrolidino-phosphonium hexafluorophosphate) in place of Brop gave reduced yields of **5a** and **5b** (55% and 58%, respectively). Furthermore, the products obtained using Brop were easier to purify.

Compounds **7a** and **7b** were obtained from **5a** and **5b** by a selective deprotection of the nucleic bases (Bn and Boc) with trifluoroacetic acid (TFA). Methyl esters of the PNA dimers **5a** and **5b** were saponified with lithium hydroxide (LiOH 1N)

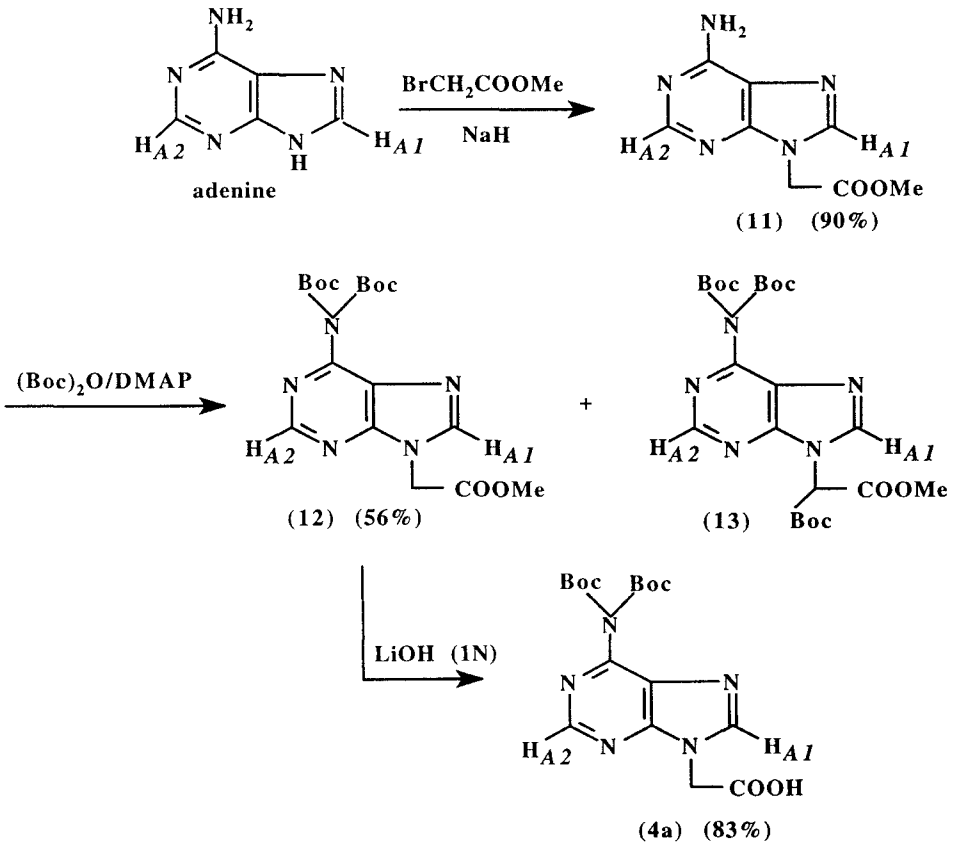

FIG. 3 : Synthesis of the adenine acetic acid unit (NMR assigments are in italic)

to give acids **6a** and **6b** in 93% and 92% yields, respectively. Further elongation of **6a** and **6b** are possible using identical procedures.

This new synthesis strategy is general and can be applied to the liquid-phase synthesis of a wide variety of short protected polyPNAs fragments. The particular combination of protecting groups utilized also offers a large degree of selectivity in the deprotection steps.

EXPERIMENTAL SECTION

Unless otherwise stated, all reagents were obtained from commercial suppliers and used without further purification. All solvents were freshly distilled. Melting points

were determined using Büchi capillary melting point apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded on a Brucker WP200 (200 MHz) Fourier transform spectrometer. The NMR spectra of PNA monomers and PNA dimers displayed a doubling of signals caused by the presence of an equilibrium mixture of the E and Z isomers generated by the substituted amide bond.

TLC were performed on 0.25-mm-thick silical gel plates (Merck, silica gel 60F254). Merck grade 60 silica gel, 230-400 mesh was used for column chromatography.

Mass measurements were carried out on INCOS 500 E FINNIGAN MAT (EI, 70eV, direct introduction) and TSQ 7000 FINNIGAN MAT (ESI / MS) instruments.

HPLC chromatograms were obtained using a HP 1100 and a water (1/1000 TFA) / acetonitrile (1/1000 TFA) 90:10 to 70:30 gradient over 40mn (UV detector at 254nm) with a column (250x4mm) packed with Lichrospher 100 RP-18 (5μm).

The elemental analyses of carbon, hydrogen, nitrogen and halogen were done at Vernaison (France) by CNRS.

N-((N,N'-(Ditertiobutyloxycarbonyl)adenin-9-yl)acetyl)-N-(2-(benzyloxy carbonyl)aminoethyl)glycine (1a)

In 20ml of tetrahydrofuran (THF), 1.20g (1.87mmol) of **10a** were dissolved and 7.5ml of LiOH (1N) were added at 0°C. After 45min of stirring at 0°C, a solution of citric acid (10%) was added until pH=7. The solution was evaporated to dryness, the residue was taken up in a solution of $NaHCO_3$ (10%), washed with ethyl acetate (EtOAc) and then acidified to pH=3 at 0°C with a citric acid solution (10%). The product was extracted with EtOAc, the combined organic layers were washed with water and then dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and precipitated with diethylether to yield the title compound **1a** (970mg, 83%) as white crystals. mp 120-122°C; ^1H NMR (CD_3OD) (two isomers) δ 8.80; *8.85* (1H, H_{A2}, s), 8.40; *8.50* (1H, H_{A1}, s), 7.40-7.15 (5H, Ph(Z), m), 5.20; *4.95* (2H, CH_2-Ad, s), 5.05; *4.75* (2H, CH_2(Z), s), 4.25; *4.05* (2H, CH_2c, s), 3.80-3.30 (4H, CH_2a, CH_2b, m), 1.25; *1.20* (18H, 2Boc, s); MS (ESI+) (m/z) 628 $(M+H)^+$, 650 $(M+Na)^+$; Anal. calcd for $C_{29}H_{37}N_7O_9$: C 55.50, H 5.90, N 15.63. Found: C 54.89, H 5.75, N 14.90.

N-((2-Amino-6(benzyloxy)purin-9-yl)acetyl)-N-(2-(benzyloxycarbonyl) aminoethyl)glycine (1b)

Following the above procedure, **1b** (324mg, 80%) was obtained from **10b** (417mg, 0.76mmol) as white crystals. mp 168-170°C; ^1H NMR (CD_3OD) (two

isomers) δ 7.75; *7.80* (1H, H_G, s), 7.65-7.20 (10H, Ph(Z), Ph(OBn), m), 5.60 (2H, CH_2(OBn), s), 5.20; *5.10* (2H, CH_2(Z), s), 5.15; *5.00* (2H, CH_2- Gu, s), 4.10; *4.25* (2H, CH_2c, s), 3.70-3.40 (4H, CH_2a, CH_2b, m); MS (ESI+) (m/z) 534 $(M+H)^+$; Anal.calcd for $C_{26}H_{27}N_7O_6$: C 58.54, H 5.07, N 18.39. Found: C 59.12, H 5.09, N 17.98.

N-(2-Aminoethyl)glycine methyl ester.2HCl (2)

Compound **2** was prepared from **8** following the procedure described by Heimer *et al.*[8]; ^1H NMR (DMSO-d_6) δ 9.90 (2H, NH_2^+, bs), 8.60 (3H, NH_3^+, bs), 4.15 (2H, CH_2c, s), 3.80 (3H, CH_3, s), 3.30 (4H, CH_2a, CH_2b, m); MS (EI) (m/z) 133 $(M)^{+\cdot}$.

Z-PNA(A-Boc2)NHCH2CH2NHCH2COOMe (3a)

A solution of 300mg (0.48mmol) of **1a**, 83mg (0.72mmol) of HOSu and 99mg (0.48mmol) of DCC in 10ml of dimethylformamide (DMF) was stirred at room temperature overnight. The mixture was then cooled to -15°C and 98mg (0.48mmol) of **2** and 0.14ml of NMM were added. The solution was stirred for 3 hours at -15°C and 2 hours at room temperature. Subsequently, the suspension was cooled to 0°C, the dicyclohexylurea (DCU) was filtered and washed with EtOAc, and the filtrate was concentrated under reduced pressure. The residue was taken up in an aqueous solution of 1N $KHSO_4$. The acidic solution was washed with EtOAc and then a solution of $NaHCO_3$ (10%) was added until the pH=8-9. The product was extracted with EtOAc and the organic layers were washed with water, dried over $MgSO_4$ and then evaporated under reduced pressure. **3a** was isolated as white crystals in 68% yield (236mg). mp 105-107°C; TLC (EtOAc/MeOH, 8:2, v:v): Rf=0.25; ^1H NMR ($CDCl_3$) (two isomers) δ 8.70; *8.75* (1H, H_{A2}, s), 8.00; *8.15* (1H, H_{A1}, s), 7.30-7.00 (6H, NH, Ph(Z), m), 6.00 (1H, NH(Z), s), 5.05 (2H, CH_2(Z), s), 4.95 (2H, CH_2-Ad, s), 3.85; *4.05* (2H, CH_2c, s), 3.60; *3.55* (3H, CH_3, s), 3.55-3.15 (8H, CH_2a, CH_2b, CH_2d, CH_2f, m), 2.75-2.55 (2H, CH_2e, m), 1.40 (18H, 2Boc, s); MS (ESI+) (m/z) 742 $(M+H)^+$, 764 $(M+Na)^+$; Anal. calcd for $C_{34}H_{47}N_9O_{10}$: C 55.06, H 6.34, N 17.00. Found: C 55.81, H 6.31, N 17.12.

Z-PNA(G-Bn)NHCH2CH2NHCH2COOMe (3b)

Following the above procedure, **3b** (210mg, 69%) was obtained as white crystals from **1b** (250mg, 0.47mmol). mp 101-104°C; TLC (EtOAc/MeOH, 1:1, v:v): Rf=0.56; ^1H NMR (CD_3OD) (two isomers) δ 7.70; *7.80* (1H, H_G, s), 7.60-7.15 (10H, Ph(Z), Ph(OBn), m), 5.55 (2H, CH_2(OBn), s), 5.10 (2H, CH_2(Z), bs), 5.05

(2H, CH_2-Gu, bs), 4.05; *4.30* (2H, CH_2c, s), 3.75; *3.70* (3H, CH_3, s), 3.60-3.35 (8H, CH_2a, CH_2b, CH_2d, CH_2f, m), 2.75; *2.80* (2H, CH_2e, t, J=6.0Hz), 1.40 (18H, 2Boc, s); MS (ESI+) (m/z) 648 $(M+H)^+$, 670 $(M+Na)^+$; Anal. calcd for $C_{31}H_{37}N_9O_7$: C 57.50, H 5.72, N 19.47. Found: C 57.92, H 5.69, N 19.28.

(N,N'-(Ditertiobutyloxycarbonyl)adenin-9-yl)acetic acid (4a)

In 15ml of dioxane, 2.0g (6.5mmol) of **12** were dissolved and 7ml of LiOH (1N) were added. The mixture was stirred at 0°C for 45min, then 10ml of water were added. Dioxane was evaporated under reduced pressure. The aqueous layer was washed with EtOAc and then acidified at 0°C with a solution of citric acid (10%) to pH=3. After extraction with EtOAc, the organic layer was washed with water, then with brine and dried over $MgSO_4$. After evaporation of the solvent and trituration in diethylether, 1.6g of **4a** were obtained as white crystals (83%). mp 176-178°C; TLC (EtOAc/MeOH, 1:1, v:v): Rf=0.40; ^1H NMR (CD_3OD) δ 8.90 (1H, H_{A2}, s), 8.50 (1H, H_{A1}, s), 5.20 (2H, CH_2, s), 1.40 (18H, 2Boc, s); MS (CI) (m/z) 394 $(M+H)^+$; Anal. calcd for $C_{17}H_{23}N_5O_6$: C 51.90, H 5.85, N 17.81. Found: C 51.85, H 6.12, N 17.42.

Z-PNA(A-Boc$_2$)PNA(G-Bn)OMe (5a)

To a solution of 460mg (0.62mmol) of **3a**, 186mg (0.62mmol) of **4b**, and 0.35ml of TEA in 2.0ml of CH_2Cl_2, were added 241mg (0.62mmol) of Brop at 0°C. The mixture was stirred at room temperature for 1 hour. CH_2Cl_2 was evaporated and the crude residue was taken up in EtOAc. The organic layer was washed with a solution of citric acid (10%), then with an aqueous solution of $NaHCO_3$ (10%), and finally with water. The organic layer was dried over $MgSO_4$ and then the solvent was removed by evaporation. The residue was purified using column chromatography (EtOAc) and then recrystallized from CH_2Cl_2/Et_2O to afford **5a** as white crystals in 76% yield (480mg). mp 100-103°C; TLC (EtOAc-MeOH 8:2): Rf=0.38; ^1H NMR (CD_3COCD_3) (two isomers) δ 8.80; *8.75* (1H, H_{A2}, s), 8.35; *8.30* (1H, H_{A1}, s), 7.75; *7.80* (1H, H_G, s), 7.60-7.10 (11H, NH, Ph(Z), Ph(OBn), m), 6.00 (1H, NH(Z), bs), 5.50; *5.55* (2H, CH_2(OBn), s), 5.10; *5.30* (2H, CH_2-Gu, s), 5.05-4.80 (4H, CH_2(Z), CH_2-Ad, m), 4.20-3.90 (4H, CH_2f, CH_2c, m), 3.70 (3H, CH_3, s), 3.70-3.10 (8H, CH_2e, CH_2d, CH_2a, CH_2b, m), 1.40 (18H, 2Boc, s); MS (ESI+) (m/z) 1023 $(M+H)^+$, 1045 $(M+Na)^+$; Anal. calcd for $C_{48}H_{58}N_{14}O_{12}$: C 56.36, H 5.68, N 19.18. Found: C 57.02, H 5.72, N 18.96.

Z-PNA(G-Bn)PNA(A-Boc$_2$)OMe (5b)

Following the above procedure, using DMF instead of CH_2Cl_2, **5b** (283mg, 81%) was obtained as white crystals from **3b** (214mg, 0.33mmol) and **4a** (130mg,

0.33mmol). mp 86-89°C; TLC (EtOAc/MeOH, 8:2, v:v): Rf=0.42; ^{1}H NMR (CD_3COCD_3) (two isomers) δ 8.80; *8.75* (1H, H_{A2}, s), 8.40; *8.45* (1H, H_{A1}, s), 7.70; *7.80* (1H, H_G, s), 7.60-7.10 (11H, NH, Ph(Z), Ph(OBn), m), 6.00 (1H, NH(Z), bs), 5.50; *5.55* (2H, CH_2(OBn), s), 5.10; *5.30* (2H, CH_2-Gu, s), 5.05-4.80 (4H, CH_2(Z), CH_2-Ad, m), 4.20-3.90 (4H, CH_2*f*, CH_2*c*, m), 3.70 (3H, CH_3, s), 3.70-3.10 (8H, CH_2*e*, CH_2*d*, CH_2*a*, CH_2*b*, m), 1.40 (18H, 2Boc, s); MS (ESI+) (m/z) 1023 $(M+H)^+$, 1045 $(M+Na)^+$; Anal. calcd for $C_{48}H_{58}N_{14}O_{12}$: C 56.36, H 5.68, N 19.18. Found: C 56.04, H 5.72, N 19.00.

Z-PNA(A-Boc2)PNA(G-Bn)OH (6a)

In 2ml of THF, 127mg (0.125mmol) of **5a** were dissolved and 0.4ml of LiOH (1N) were added at 0°C. The mixture was stirred for 1 hour, then 20ml of a citric acid solution (10%) were introduced and the product was taken up in EtOAc. The organic layer was washed with water until neutral pH, dried over $MgSO_4$ and the EtOAc was evaporated under reduced pressure. Trituration in diethylether led to **6a** (118mg, 93%) as a white crystalline product. mp 220-223°C (dec); TLC (EtOAc/MeOH, 1:1, v:v): Rf=0.30; ^{1}H NMR (CD_3OD) (two isomers) δ 8.70; *8.75* (1H, H_{A2}, s), 8.40; *8.35* (1H, H_{A1}, s), 7.70; *7.75* (1H, H_G, s), 7.55-7.10 (10H, Ph(Z), Ph(OBn), m), 5.55-4.95 (4H, CH_2(OBn), CH_2-Gu, m), 4.90-4.75 (4H, CH_2(Z), CH_2-Ad, m), 4.25-3.85 (4H, CH_2*f*, CH_2*c*, m), 3.65-3.15 (8H, CH_2*a*, CH_2*b*, CH_2*c*, CH_2*d*, m), 1.35 (18H, 2Boc, s); MS (ESI-) (m/z) 1007 $(M-H)^-$; Anal. calcd for $C_{47}H_{56}N_{14}O_{12}$: C 55.95, H 5.55, N 19.44. Found: C 56.79, H 5.41, N 20.18.

Z-PNA(G-Bn)PNA(A-Boc2)OH (6b)

Following the above procedure, **6b** (200mg, 92%) was obtained as white crystals from **5b** (200mg, 0.215mmol). mp 224-227°C (dec); TLC (EtOAc/MeOH, 1:1, v:v): Rf=0.30; ^{1}H NMR (CD_3OD) (two isomers) δ 8.70; *8.75* (1H, H_{A2}, s), 8.40; *8.35* (1H, H_{A1}, s), 7.70; *7.75* (1H, H_G, s), 7.55-7.10 (10H, Ph(Z), Ph(OBn), m), 5.55-4.95 (4H, CH_2(OBn), CH_2-Gu, m), 4.90-4.75 (4H, CH_2(Z), CH_2-Ad, m), 4.25-3.85 (4H, CH_2*f*, CH_2*c*, m), 3.65-3.15 (8H, CH_2*a*, CH_2*b*, CH_2*c*, CH_2*d*, m), 1.35 (18H, 2Boc, s); MS (ESI-) (m/z) 1007 $(M-H)^-$; Anal. calcd for $C_{47}H_{56}N_{14}O_{12}$: C 55.95, H 5.55, N 19.44. Found: C 55.12, H 5.71, N 18.97.

Z-PNA(A)PNA(G)OMe (7a)

In a solution of 0.5ml of TFA and 1.5ml of CH_2Cl_2, 93mg (0.091mmol) of **5a** were placed. The mixture was stirred at room temperature for 3 hours and the TFA was evaporated under reduced pressure. After trituration in EtOAc/Et_2O (1:1, v:v),

compound **7a** (65mg) was isolated as beige crystals in a nearly quasi-quantitative yield. mp 188-190°C (dec); TLC (iPrOH/AcOH/H_2O, 4:1.3:2.5, v:v:v): Rf=0.69; HPLC Rt=22.8min; MS (ESI+) (m/z) 734 $(M+H)^+$, 756 $(M+Na)^+$; Anal. calcd for $C_{33}H_{37}N_{14}O_{10}F_3$: C 46.81, H 4.37, N 23.17. Found: C 46.41, H 4.54, N 23.31.

Z-PNA(G)PNA(A)OMe (7b)

7b (57mg) was obtained from **5b** (80mg, 0.078mmol) in a nearly quantitative yield as white crystals, following the above procedure. mp 200-202°C (dec); TLC (iPrOH/AcOH/H_2O, 4:1.3:2.5, v:v:v): Rf=0.71; HPLC Rt=22.7min; MS (ESI+) (m/z) 734 $(M+H)^+$, 756 $(M+Na)^+$; Anal. calcd for $C_{33}H_{37}N_{14}O_{10}F_3$: C 46.81, H 4.37, N 23.17. Found: C 45.05, H 4.56, N 22.98.

N-(2-Aminoethyl)glycine (8)

This compound has been prepared following the procedure described by Heimer *et al.*[10]; ^1H NMR (D_2O) δ 3.20 (2H, CH_2c, s), 2.95 (2H, CH_2b, t, J=6.0Hz), 2.80 (2H, CH_2a, t, J=6.0Hz).

Methyl-N-(2-(benzyloxycarbonyl)aminoethyl)glycinate (9)

A solution of 1.22g (4.88mmol) of ZOSu in 20ml of CH_3CN was prepared. The mixture was cooled to -15°C. After 15min at -15°C, 1.0g (4.88mmol) of **2** and 1.35ml of NMM were added. The solution was stirred for 3 hours at -15°C and for 2 hours at room temperature. The solvent was evaporated under reduced pressure. The crude product was purified using column chromatography (EtOAc) to afford **9** (800mg, 62%) as a colorless resin. TLC (EtOAc): Rf=0.38; ^1H NMR ($CDCl_3$) δ 7.35 (5H, Ph(Z), s), 5.40 (1H, NH(Z), bs), 5.10 (2H, CH_2(Z), s), 3.75 (3H, CH_3, s), 3.40 (2H, CH_2c, s), 3.25 (2H, CH_2a, t, J=6.0Hz), 2.75 (2H, CH_2b, t, J=6.0Hz), 1.75 (1H, NH, bs); MS (ESI+) (m/z) 267 $(M+H)^+$, 289 $(M+Na)^+$; Anal. calcd for $C_{13}H_{18}N_2O_4$: C 58.64, H 6.76, N 10.52. Found: C 59.01, H 6.70, N 10.01.

Methyl-N-((N,N'-(ditertiobutyloxycarbonyl)adenin-9-yl)acetyl)-N-(2-(benzyloxycarbonyl)aminoethyl)glycinate (10a)

A mixture of 1.1g (2.82mmol) of **4a**, 750mg (2.82mmol) of **9** and 1.5ml of TEA were placed in 3ml of CH_2Cl_2 and cooled to 0°C. Brop, 1.1g (2.82mmol) was added and the mixture was stirred at room temperature for 4 hours. The solvent was then evaporated under reduced pressure and the residue was taken up in EtOAc. The organic layer was washed with a solution of $KHSO_4$ (1N), then with a solution of $NaHCO_3$ (10%) and finally with water. The organic layer was dried over $MgSO_4$ and EtOAc was

evaporated *in vacuo*. The residue was purified using column chromatography (EtOAc) and 1.62g (90%) of **10a** were isolated as colorless resin. TLC (EtOAc): Rf=0.40; ^{1}H NMR ($CDCl_3$) (two isomers) δ 8.75; *8.80* (1H, H_{A2}, s), 8.10; *8.25* (1H, H_{A1}, s), 7.45-7.05 (5H, Ph(Z), m), 6.30; *5.70* (1H, NH, bs), 5.15 (2H, CH_2(Z), s), 5.05 (2H, CH_2-Ad, s), 4.25; *4.10* (2H, CH_2c, s), 3.80; *3.65* (3H, CH_3, s), 3.65-3.30 (4H, CH_2a, CH_2b, m), 1.40 (18H, 2Boc, s); MS (ESI+) (m/z) 642 $(M+H)^+$, 664 $(M+Na)^+$; Anal. calcd for $C_{30}H_{39}N_7O_9$: C 56.16, H 6.08, N 15.29. Found: C 56.02, H 5.77, N 16.01.

Methyl-N-((2-amino-6-(benzyloxy)purin-9-yl)acetyl)-N-(2-(benzyloxy carbonyl)aminoethyl)glycinate (10b)

Following the above procedure, **10b** (417mg, 77%) was obtained as white crystals from **4b** (293mg, 0.98mmol) and **9** (260mg, 0.98mmol). mp 132-135°C; TLC (EtOAc): Rf=0.23; ^{1}H NMR ($CDCl_3$) (two isomers) δ 7.45; *7.40* (1H, H_G, s), 7.35-7.10 (10H, Ph(Z), Ph(OBn), m), 6.15 (1H, NH, bs), 5.50 (2H, CH_2(OBn), s), 5.10; *5.05* (2H, CH_2(Z), s), 4.75; *4.70* (2H, CH_2-Gu, s), 4.20; *4.00* (2H, CH_2c, s), 3.70; *3.65* (3H, CH_3, s), 3.65-3.25 (4H, CH_2a, CH_2b, m), 1.40 (18H, 2Boc, s); MS (ESI+) (m/z) 548 $(M+H)^+$, 570 $(M+Na)^+$; Anal. calcd for $C_{27}H_{29}N_7O_6$: C 59.23, H 5.30, N 17.91. Found: C 59.31, H 5.25, N 18.01.

Methyl(6-adenin 9-yl)acetate (11)

In 300ml of DMF under N_2, 5.0g (37mmol) of adenine and 0.90g (37mmol) of sodium hydride (NaH) were placed. The mixture was stirred at room temperature for 2 hours, then 3.5ml (37mmol) of methyl bromoacetate were introduced dropwise. The solution was stirred at room temperature for 10 hours and DMF was evaporated under reduced pressure. After trituration in water and filtration, the product was dried *in vacuo*. 6.9g of **11** were obtained as a white powder (90%). mp 238-240°C; TLC (EtOAc/MeOH, 1:1, v:v): Rf=0.54; ^{1}H NMR (DMSO-d_6) δ 8.20 (2H, H_{A1}, H_{A2}, s), 7.35 (2H, NH_2, bs), 5.15 (2H, CH_2, s), 3.70 (3H, CH_3, s); MS (EI) (m/z) 207 $(M)^{+\cdot}$, 208 $(M+H)^{+\cdot}$.

Methyl(N-(ditertiobutyloxycarbonyl)adenin-9-yl)acetate (12)

In 3ml of CH_3CN, 1.6g (7.25mmol) of $(Boc)_2O$ and 0.9g (7.25mmol) of DMAP were placed. After 15min, a solution of 500mg (2.42mmol) of **11** in 10ml of CH_3CN was added. The mixture was stirred overnight at room temperature, and the solvent was evaporated under reduced pressure. The crude residue was taken up in CH_2Cl_2, the organic layer was washed with a solution of $KHSO_4$ (0.5N), followed by

water until neutral and then dried over $MgSO_4$ and the solvent was evaporated to dryness. The residue was purified using column chromatography (Hexane/EtOAc 1:1) to obtain **12** which was crytallized from Et_2O/Hexane to give 550mg (56%) as beige crystals and **13** as a resin.

Compound 12 : mp 100-102°C; TLC (EtOAc): Rf=0.60; ^1H NMR ($CDCl_3$) δ 8.90 (1H, H_{A2}, s), 8.20 (1H, H_{A1}, s), 5.10 (2H, CH_2, s), 3.80 (3H, CH_3, s), 1.40 (18H, 2Boc, s); MS (CI) (m/z) 408 $(M+H)^+$; Anal. calcd for $C_{18}H_{25}N_5O_6$: C 53.07, H 6.14, N 17.20. Found: C 53.37, H 5.98, N 16.89.

Compound 13 : TLC (EtOAc): Rf=0.80; ^1H NMR ($CDCl_3$) δ 8.85 (1H, H_{A2}, s), 8.50 (1H, H_{A1}, s), 6.25 (1H, CH, s), 3.85 (3H, CH_3, s), 1.50 (9H, Boc(-CH), s), 1.40 (18H, 2Boc(-N), s); MS (EI) (m/z) 508 $(M+H)^{+\cdot}$.

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