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Liquid-Phase Synthesis of Polyamide Nucleic Acids (PNA)

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Abstract: Three liquid-phase processes for the elaboration of short orthogonally protected PNA have been devised. Two of these methods are similar to the convergent and divergent approaches in peptide synthesis. The third process consists in building a fully protected polyamide backbone, by using as many different and orthogonal protecting groups as there are different types of nucleic bases in the targeted polyPNA. Simultaneous and selective cleavage of one kind of protecting group allows the simultaneous attachment of several identical nucleobase units.

INTRODUCTION

PNAs (Peptide or Polyamide Nucleic Acids) are DNA or RNA mimics containing an achiral, uncharged pseudopeptidic backbone.[1] They recognize their complementary oligonucleotides with a remarkably high specificity and affinity,[2] and they are resistant to nucleases.[3] Therefore, PNAs are very interesting for medicinal chemistry as gene-targeted (antisens and antigen) drugs.[4] Even short oligomers (heptamers) can be sequence- and gene-selective antisens inhibitors of protein expression, as shown recently.[5] PNA syntheses follow well-established solid-phase procedures.[6-10] However, for short PNAs (up to decamers), liquid-phase strategies are more suitable, because they are more flexible owing to a larger choice of solvents and protecting groups, less expensive and more appropriate for large scale PNA syntheses. Liquid-phase syntheses are better adapted to functionalize PNA fragments prior to their covalent attachment to various molecules (including peptides, intercalators, steroids, oligonucleotides...), which are to increase the affinity and/or the specificity of the PNAs for their target.

We have explored three liquid-phase strategies (Scheme 1, path A, B and C) to synthetize PNA fragments. We report herein the synthesis of di-, tri-, tetra- and hexa-PNAs bearing the four natural A, C, G or U nucleobases, some of them (i.e. compounds **5dc** and **7dc** in Scheme 2) being fonctionalized on the terminal amino group of the polyamide backbone.

The first strategy (Scheme 1, path A) is analogous to the classic convergent stepwise approach in peptide synthesis. A similar submonomer solid-phase synthesis of PNA oligomers has previously been described.[10] The PNA moieties are successively elongated, *via* their carboxy end, using a three-step procedure. This procedure consists in condensing the PNA unit with the N-2-aminoethyl glycine ester backbone **2**, onto which is then connected the nucleobase acetic acid unit. Hydrolysis generates the carboxy function required for the next

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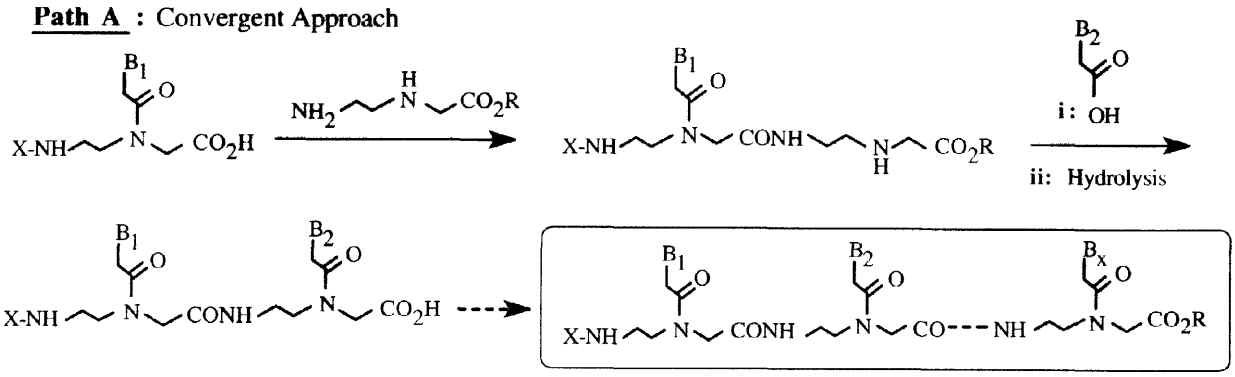
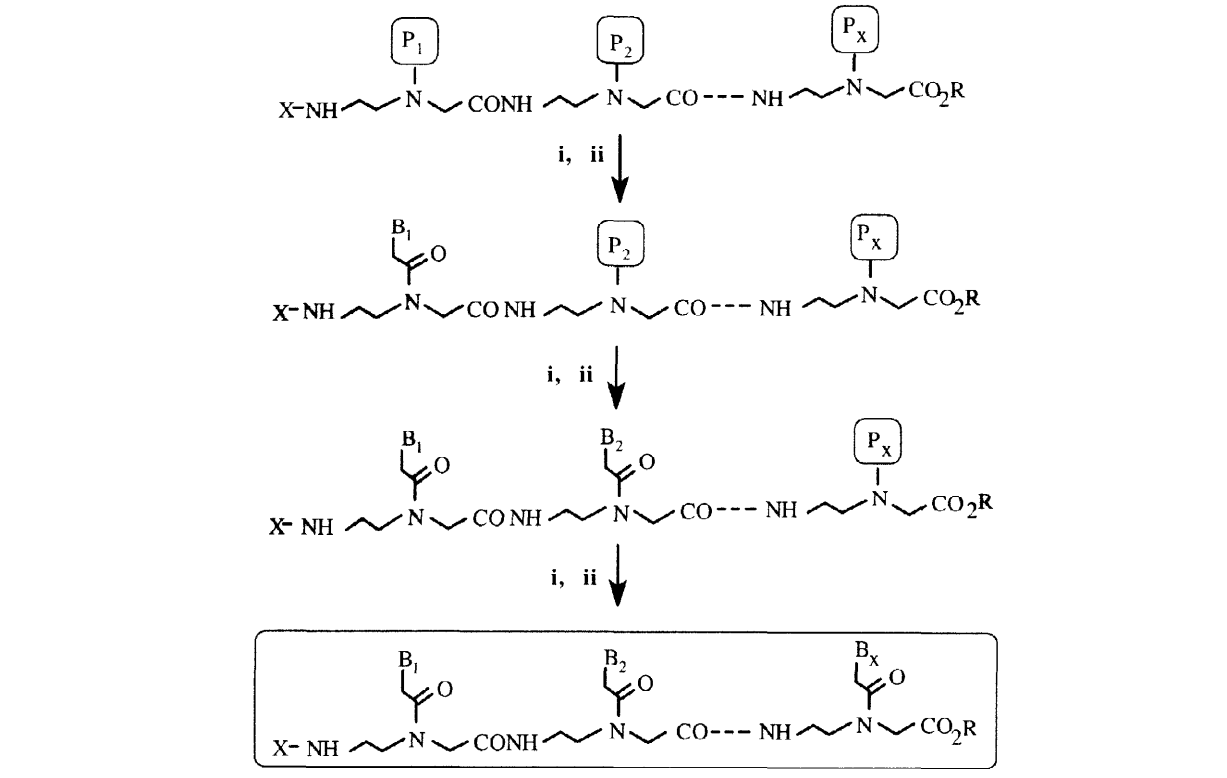
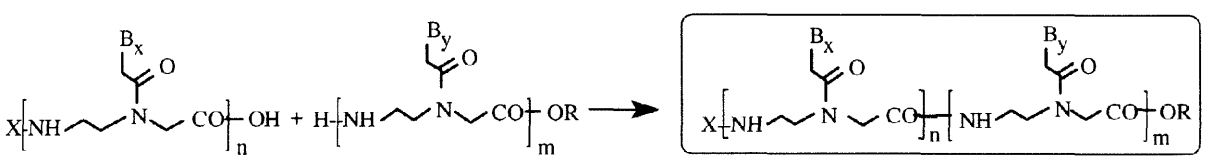
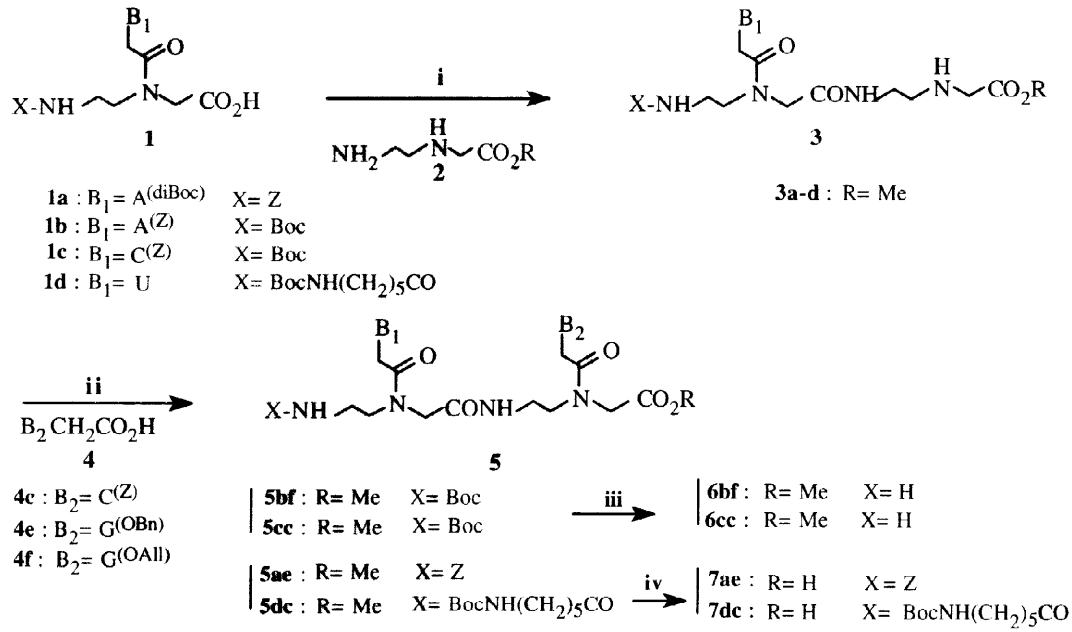


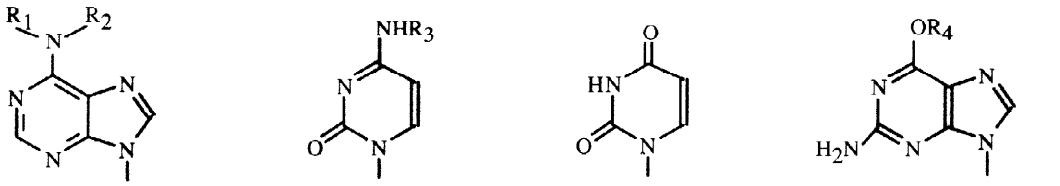




Scheme 1. Liquid-phase strategies for the synthesis of polyPNAs



Reagents and conditions: **(i) 1a-c**, DCC/HOSu, DMF, 12h then **2(2HCl)**, NMM, -15°C or **1d**, Bop, DMF, NMM, -15°C; **(ii) 4c, 4e** or **4f**, Brop, CH_2Cl_2, TEA; **(iii) 5bf** or **5cc**, TFA, CH_2Cl_2; **(iv) 5ae** or **5dc**, dioxane, LiOH 1N, 0°C.
With:



a: $A^{(diBoc)}$ R_1=Boc R_2= Boc
b: $A^{(Z)}$ R_1= H R_2= Z
c: $C^{(Z)}$ R_3= Z
d: U
e: $G^{(OBn)}$ R_4= Benzyl
f: $G^{(OAll)}$ R_4= Allyl

$A^{(diBoc)}$: N^6,N^6-di-tert-butyloxycarbonyl adenine ; $A^{(Z)}$: N^6-benzyloxycarbonyl adenine ;
$G^{(OBn)}$: 2-amino-6-(benzyloxy)-purine ; $G^{(OAll)}$: 2-amino-6-(allyloxy)-purine ;
$C^{(Z)}$: N^4-benzyloxycarbonyl cytosine ; U : uracil.

Scheme 2. General protocol for the synthesis of di-PNAs by the elongation strategy (path A)

elongation step. Part of our results concerning the synthesis of di-PNAs following this approach has already been described.[11]

The second strategy (Scheme 1, path B) consists first in building a fully protected polyamide backbone containing as many different and orthogonal protecting groups as they are different types of nucleic bases in the targeted polyPNA. After appropriate deprotection(s), successive and selective attachment(s) of the different nucleobase(s) unit(s) onto the backbone lead(s) to the desired PNA.

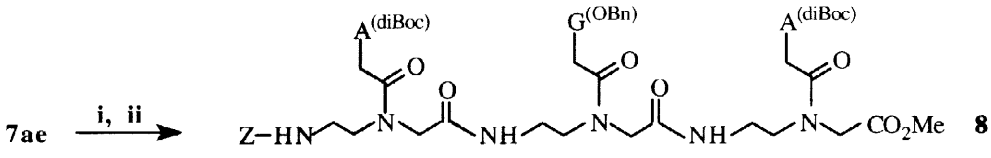
The third explored strategy (Scheme 1, path C) is analogous to the divergent approach in peptide synthesis. It consists in coupling two PNA fragments which have been obtained following one of the precedent strategies.

RESULTS

Path A: the convergent approach.

Applying the elongation strategy (Scheme 1, path A), we obtained various N-protected or Boc-ε-aminocaproyl functionalized di-PNAs **5**, starting from the protected adenine (**1a-1b**), cytosine (**1c**) or uracil (**1d**) PNA monomer, in overall yields ranging from 42 to 65% (Scheme 2). Similarly, the tri-AGA-PNA **8** was synthetized by elongation of the di-AG-PNA **7ae** in 25% overall yield (Scheme 3).

The first step of the elongation process was performed through coupling of the PNA monomer or dimer with the N-2-aminoethyl glycine methyl ester backbone **2**, either (i) *via* a DCC/HOSu preactivation in the case of the A- or C-monomers **1a-c** (from 60 to 70% yields) and AG dimer **7ae** (50% yield), or (ii) *via* a Bop activation in the case of the N-functionalized U-monomer (**1d**; 60%). The second step consists in condensing, onto the free amine function remaining on the pseudo-peptidic backbone, either (i) the N^4-Z cytosine (**4c**), O-benzyl guanine (**4e**), or O-allyl guanine (**4f**) acetic acid unit for the di-PNAs **5** (Scheme 2), or (ii) the N^6,N^6-diBoc adenine acetic acid unit (**4a**) for the tri-PNA **8** (Scheme 3). In this condensation step, Brop was taken as coupling reagent in all cases (50-90% yields).

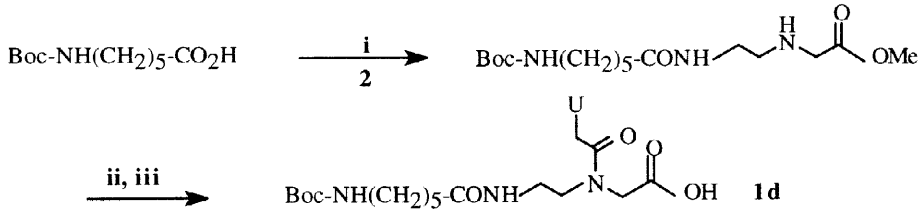


Reagents and conditions: **(i) 7ae**, DCC/HOSu, DMF, 12h then **2(2HCl)**, NMM, -15°C; **(ii) 4a**, Brop, CH_2Cl_2, TEA, 0°C.

Scheme 3. Synthesis of the tri-AGA-PNA **8** *via* the elongation strategy (path A)

Quasi-quantitative cleavage of the Boc group in dimers **5bf** and **5cc** with TFA / CH_2Cl_2 (1:1) led to the nucleobase-protected di-PNAs **6bf** and **6cc**, respectively (under such conditions, the Z and O-allyl protecting groups of the cytosine, adenine, and guanine nucleobases were not affected). Saponification of N-protected di-PNAs **5ae** and **5dc** afforded the nucleobase-protected acid derivatives **7ae** and **7dc** in 75% yields.

The syntheses of backbone **2**, monomers **1a-c** and base acetic units **4a-f** have been previously described.[6,11] Functionalized uracil monomer **1d** was prepared in 74% yield from the *N*-ε-Boc-ε-aminocaproic acid, as shown in Scheme 4.



Reagents and conditions: **(i)** *N*-ε-Boc-ε-aminocaproic acid, DCC/HOSu, DMF, 12h then **2(2HCl)**, NMM, -15°C; **(ii) 4d**, Brop, TEA, CH_2Cl_2, 0°C; **(iii)** Dioxane, LiOH (1N), 0°C.

Scheme 4. Preparation of the *N*-ε-Boc-ε-aminocaproyled uracil PNA momomer **1d**

This convergent strategy leads to fully orthogonally protected PNA fragments, which can be functionalized at the terminal amino function of the backbone, as in N-ε-Boc-ε-aminocaproylated dimer **5dc**. These PNA fragments can be selectively deprotected either on the amino- or carboxy-end of the polyamide backbone or on the nucleobases. This allows elongation from the N-amino or the C-carboxy termination of the PNA fragments in which the nucleobases are still protected, as in dimers **6** and **7**. Thus, the selective cleavage of the Boc group in **5dc**, by the action of TFA, is to liberate a reactive primary amine, for the covalent attachment of various molecules, whereas elongation from **7dc**, obtained by saponification of **5dc**, should afford a larger N-acylated PNA fragment. On the other hand, selective deprotection of the nucleobases, as in trimer **8**, will afford more lipophilic PNA fragments than the fully deprotected analogues; this should improve cell penetration of the active PNA drugs.

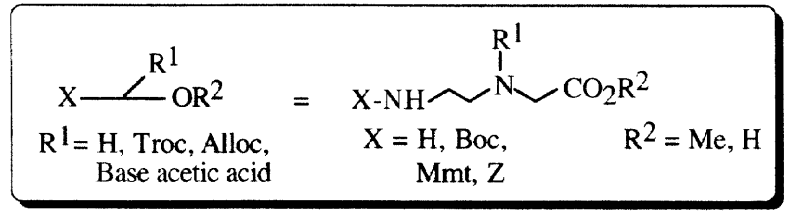
Path B: the protected polyamide backbone approach.

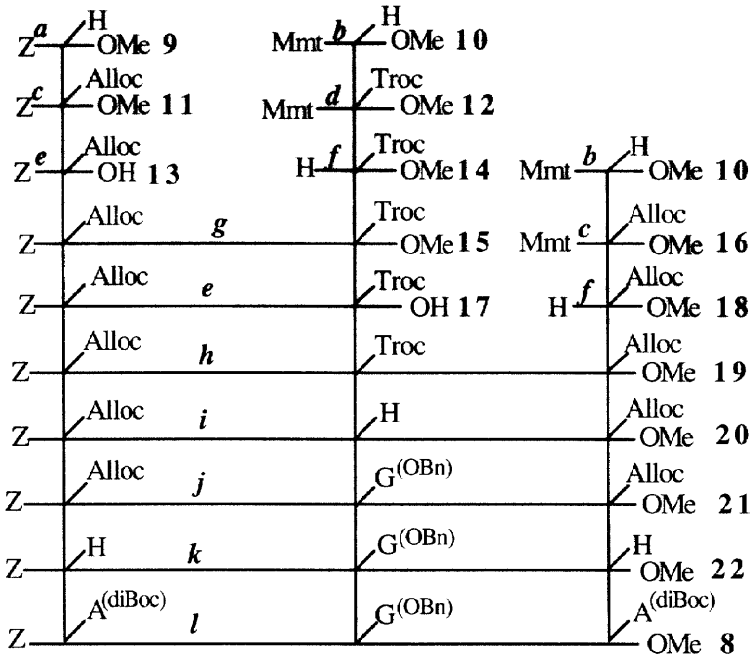
In order to evaluate the synthetic potential of the second strategy (Scheme 1, path B), the base-protected tri-AGA-PNA **8** (Scheme 5), tri-CCC-PNA **32** (Scheme 6) and tetra-UCCC-PNA **40** (Scheme 7) were synthetized. A convergent process was applied for the obtaining of the key trimeric Alloc- and/or Troc-protected polyamide backbones **19** or **29**, starting from the readily available compounds **9** and **10**, or **23**, respectively. Similarly, the tetrameric Fmoc- and Alloc-protected polyamide backbone **35** was obtained by elongation of **29**. The N-Z, N-Mmt and N-Boc aminoethylglycine monomeric synthons **9**, **10** and **23** were prepared in 70-75% yields from the readily available compound **2**.

The preparation of the tri-AGA-PNA **8** (Scheme 5) required the key trimeric backbone **19** which was obtained starting from synthons **13**, **14** and **18**. These latter derivatives were synthetized in high yields from **9** and **10**. The coupling reactions between **13** and **14** (leading to dimer **15**; step *g*) then between **17** and **18** (leading to trimer **19**; step *h*) were both performed with isobutyl chloroformate (70% yields). The selective Troc cleavage by means of cadmium in acetic acid on **19** (step *i*) then the condensation, with Brop reagent, of the guanine moiety **4e** onto the resulting **20** (step *j*) led to **21** in 85% overall yield. The cleavage of the two Alloc groups with $Pd[PPh_3]_4$ yielded **22** (step *k*, 75%). Simultaneous attachment of one adenine acetic acid unit **4a** onto each of the two amine functions of **22**, *via* a Brop activation (step *l*, 65% yield), afforded the fully protected tri-AGA-PNA **8**.

The tri-CCC-PNA **32** (Scheme 6), was synthetized from the key trimeric backbone **29**. This intermediate was obtained from synthons **25** and **26**, which were both prepared from **23**. The two coupling reactions (steps *e*, *f*) leading to **29** were performed by means of DCC/HOSu in nearly 80% yield. Quasi-quantitative deprotection of the three Alloc groups with $Pd[PPh_3]_4$ (step *g*) then yielded triamine **30**. Simultaneous attachment of the cytosine acetic acid unit **4c** onto each of the three amine functions of **30**, *via* a Brop activation (step *h*), afforded trimer **31** in 85% yield. Finally, the N-terminal amino group of compound **31** was deprotected with TFA / CH_2Cl_2, affording the tri-PNA fragment **32** as its TFA salt in quasi-quantitative yield.

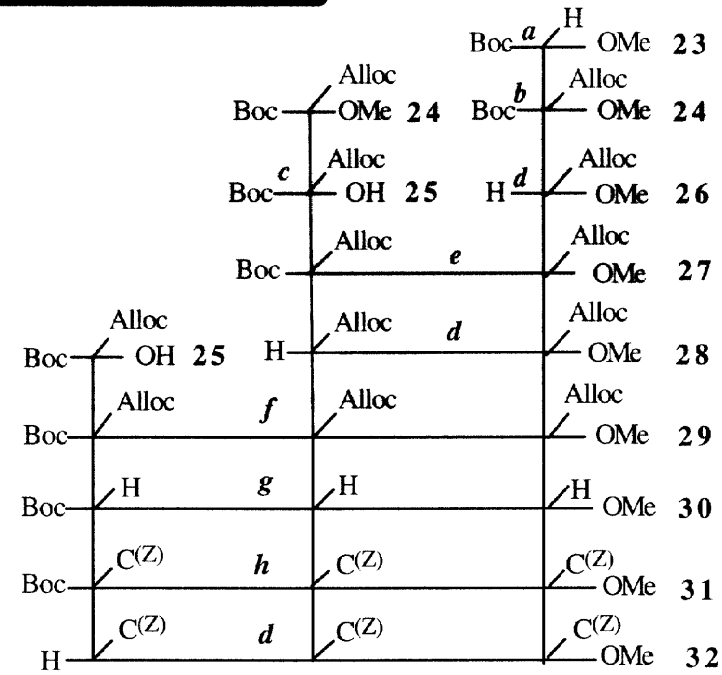
The preparation of the tetra-UCCC-PNA **40** (Scheme 7) required the synthesis of the key tetrameric Fmoc- and Alloc-protected backbone **35**. This compound was prepared by condensing Fmoc-protected monomer **33**[12] with Alloc-protected trimer **34**, obtained by Boc deprotection of **29**, *via* a DCC/HOSu preactivation (step *c*, 72% yield). Selective cleavage of Fmoc group on **35**, by means of diethylamine (step *d*), then condensation of uracil moiety **4d** onto **36** (step *e*) led to **37** in 70% overall yield. Quasi-quantitative deprotection of the three Alloc groups with $Pd[PPh_3]_4$ (step *f*) yielded **38**. The simultaneous attachment of the





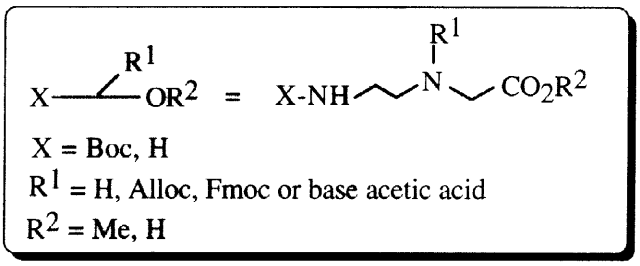
Reagents and conditions : **(a)** Z-OSu, **2(2HCl)**, CH_3CN, -15°C; **(b)** Mmt-Cl, CH_2Cl_2, **2(2HCl)**, TEA, -30°C; **(c)** Alloc-Cl, CH_2Cl_2, TEA, 0°C; **(d)** Troc-Cl, CH_2Cl_2, TEA, 0°C; **(e)** LiOH (1M), THF or MeOH; **(f)** TFA, CH_2Cl_2, 0°C; **(g)** *i.* **13**, Cl-COOiBu, CH_2Cl_2, TEA, -15°C *ii.* **14**, TEA, -15°C; **(h)** *i.* **17**, ClCOOiBu, CH_2Cl_2, TEA, -15°C *ii.* **18**, TEA, -15°C; **(i)** Cd, AcOH, DMF; **(j)** **4e**, Brop, CH_2Cl_2, TEA, 0°C; **(k)** $Pd[(PPh)_3]_4$, DEA, CH_2Cl_2; **(l)** **4a**, Brop, CH_2Cl_2, TEA, 0°C.

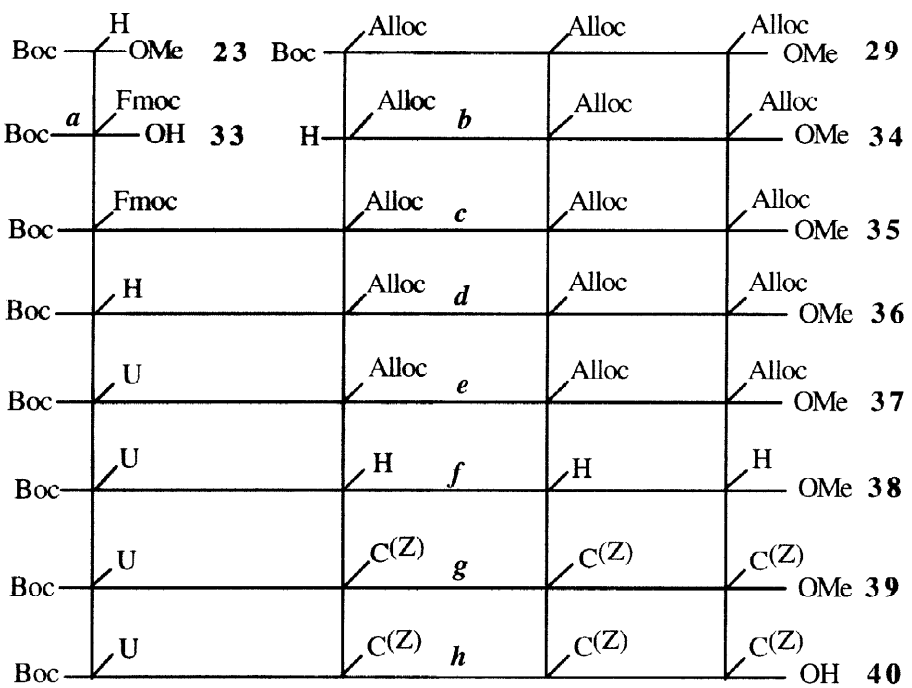
Scheme 5. Synthesis of the tri-AGA-PNA *via* path B



Reagents and conditions: **(a)** Boc_2O, DMAP, CH_2Cl_2, **2**, -10°C **(b)** Alloc-Cl, TEA, CH_2Cl_2, 0°C **(c)** LiOH (1N), dioxane **(d)** CH_2Cl_2, TFA **(e)** *i.* **25**, CH_2Cl_2, DCC/HOSu; *ii.* **26**, NMM, -15°C; **(f)** *i.* **25**, CH_2Cl_2, DCC/HOSu, 12h; *ii.* **28**, NMM; **(g)** $Pd[(PPh)_3]_4$, DEA, CH_2Cl_2; **(h)** **4c**, Brop, NMM.

Scheme 6. Synthesis of the tri-CCC-PNA *via* path B





Reagents and conditions: (**a**) *i.* NaOH (1N), dioxane; *ii.* Fmoc-Cl, then HCl (1N) (**b**) TFA/CH_2Cl_2 (**b**) TFA, CH_2Cl_2 (**c**) *i.* **33**, CH_2Cl_2, DCC/HOSu, 12h *ii.* **34**, NMM (**d**) CH_2Cl_2, DEA (**e**) **4d**, Brop, TEA, DMF, 0°C (**f**) $Pd[P(Ph)_3]_4$, DEA, CH_2Cl_2 (**g**) **4c**, TEA, Brop, CH_2Cl_2, 0°C (**h**) LiOH (1N), dioxane, 0°C.

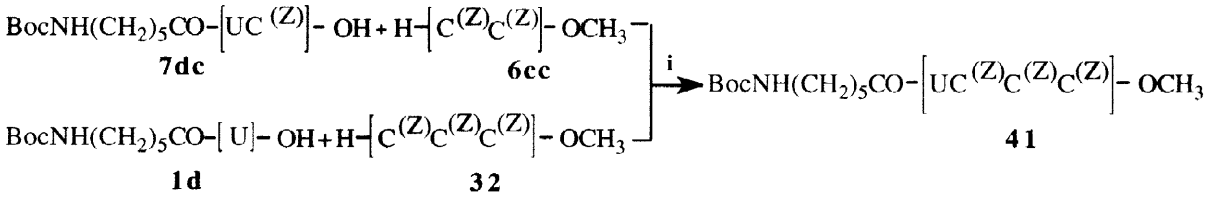
Scheme 7. Synthesis of the tetra-UCCC-PNA *via* path B

cytosine unit **4c** onto each of the three amine functions of **38**, *via* a Brop activation (step *g*), afforded **39** in 75% yield. Cautious saponification of the latter compound gave the carboxylic acid **40** (70% yield), which has been used for evaluating the synthetic potential of the divergent approach (see below).

Path C: the divergent approach.

Concerning the fragment condensation approach (Scheme 1, path C), we focused on the synthesis of tetra- and hexa-PNAs by condensing (i) a monomer with a trimer or two dimers together (Scheme 8), and (ii) a dimer with a tetramer (Scheme 9), respectively.

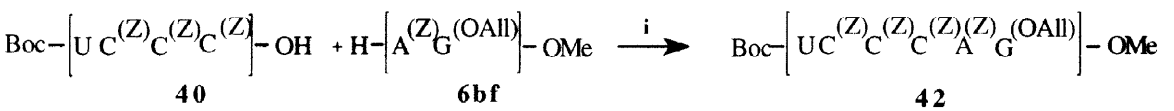
The N-acylated nucleobase-protected tetra-UCCC-PNA **41** was obtained in 70% yield through condensing either the di-UC-PNA **7dc** with the di-CC-PNA **6cc**, or the uracil monomer **1d** with the tri-CCC-PNA **32**, *via* a Bop activation in both cases (Scheme 8).



Reagents and conditions : (i) Bop, TEA, CH_2Cl_2, 0°C.

Scheme 8. Synthesis of the N-acylated tetra-UCCC-PNA **41** by fragment condensation (path C)

The base-protected hexa-UCCCAG-PNA **42** (Scheme 9) was synthesised by condensing the tetra-UCCC-PNA **40** and the di-AG-PNA **6bf** *via* a DCC/HOBt activation (60% yield).



Reagents and conditions : (i) **40**, DCC/HOSu, DMF, 12h then **6bf**, TEA, -15°C.

Scheme 9. Preparation of the hexa-UCCCAG-PNA **42** by fragment condensation (path C)

DISCUSSION

Three liquid-phase synthesis processes for the elaboration of PNA fragments have been devised and their potential evaluated.

The elongation strategy (Scheme 1, path A) is best adapted for the synthesis of orthogonally protected di- and tri-PNAs, as yields drastically decrease when the size of PNA fragments increases. Selective deprotection of either the N-amino or the C-carboxy end of the polyamide backbone furnishes synthons which can be used for a fragment condensation (Scheme 1, path C). PNA fragments **41** and **42** thus obtained should be selectively deprotected on the amine or on the carboxyl termini, for the synthesis of longer oligomers (**42**) or for the preparation of conjugates (**41**). Besides, the removal of all the protecting groups on PNAs can be performed, for instance, by action of HBr in glacial acetic acid. However, the scopes of both (path A and C) strategies are

mainly limited by the poor solubility of the protected PNAs (as **8**, **41** and **42**), making the reactions and the purification steps difficult to perform.

The fully protected polyamide backbone strategy (Scheme 1, path B) offers several advantages over the two others. The coupling reactions are carried out with high efficiencies and the reaction products are easily isolated and purified. This is due to the higher solubility of Fmoc-, Alloc- and Troc-protected polyamide derivatives in most common organic solvents, as compared to PNA fragments. These characteristics substantially facilitate the stepwise elongation of the polyamide backbone before the troublesome base acetic acid units are introduced (higher yields are observed for the synthesis of the tri-PNA **8** when applying this strategy rather than the elongation one). If one considers the number of different available orthogonal protecting groups, this strategy can be advantageously applied for the synthesis of PNAs bearing four different nucleobases. Moreover, the simultaneous and selective cleavage of one kind of the protecting groups in the fully protected polymeric backbone allows the simultaneous condensation of several identical base acetic acid units.

CONCLUSION

The tactical decision concerning the liquid-phase synthesis of a PNA depends on the fragment size as well as on the nature of the incorporated nucleobases. For di- and tri-PNAs, the elongation strategy can be applied successfully and a large choice of solvents, protecting groups and coupling reagents can be used at each step of their synthesis. Larger PNAs can be prepared by condensation of these di or tri-PNA; however, owing to the low solubility of even short and protected PNAs, the fully protected polyamide backbone strategy is best adapted as base acetic acid units are introduced at the last stages of the synthesis. This strategy is all the more convenient since there is several identical nucleobases in the targeted PNA. The choice of protecting groups can be broadened to non-carbamate protecting groups, the only constraint being their orthogonality. This method, as the former ones, allows to prepare N- or C-termini functionalized protected-nucleobases PNAs, with the aim of their covalent attachment to other molecules.

EXPERIMENTAL SECTION

General.

Unless otherwise stated, all reagents were obtained from commercial suppliers and used without further purification. All solvents were freshly distilled. The following abbreviations are employed : ε-amino caproic acid (ε-Ahx-OH), N-methylmorpholine (NMM), triethylamine (TEA), diethylamine (DEA), 4-dimethylamino-pyridine (DMAP), Bromo-tris-(dimethylamino)-phosphonium-hexafluorophosphate (Brop), benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium-hexafluorophosphate (Bop), N-hydroxy succinimide (HOSu), N,N'-dicyclohexylcarbodiimide (DCC), di-tert-butyldicarbonate (Boc_2O), tertio-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), allyloxycarbonyl (Alloc), trichloro-ethyloxycarbonyl (Troc), benzyloxycarbonyl (Z), trifluoroacetic acid (TFA), tetrakis(triphenylphosphine)-palladium(0) ($Pd[P(Ph)_3]_4$). Melting points were determined using an electrothermal digital melting point apparatus IA900 Series and are uncorrected. TLC were performed on 0.25-mm-thick silica gel plates (Merck, silica gel 60F254). Columns of chromatography were performed using Merck silica gel 60 (230-400 mesh ASTM). Analytical HPLC chromatograms were obtained using either a HP1100 (UV detector set at 254 nm) or a WATERS 996

Photodiode Array Detector (PDA, UV detector from 195 to 290nm) and a column (250*4 mm) packed with Lichrospher 100 RP-18 (5 μm). A gradient with water (0.1% TFA) as solvent A and acetonitrile (0.1% TFA) as solvent B was used with a flow=1 ml/min. For semi-preparative HPLC purification's, the HP1100 apparatus was used with a column (250*10mm) packed with Lichrospher 100 RP-18 (5 μm) and with a flow=2ml/min. ^1H (at 200 MHz) and ^{13}C (at 50.3 MHz) NMR spectra were recorded on a Bruker AC 200 Fourier Transform spectrometer and the chemical shifts δ are given in ppm. The NMR spectra of some compounds 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. Mass measurements were carried out on TSQ 7000 FINNIGAN MAT (ESI/MS) instrument. The elemental analyses were performed by the Service Central de Microanalyses of the CNRS.
The syntheses of backbone **2**, monomers **1a-c** and base acetic units **4a-f** have been previously described.[6,10] For the designation of protected polyamide compounds, see ref 13.

N-[2-(N-ε-Boc-ε-aminocaproyl)aminoethyl]-N-[(uracil-1-yl)acetyl]glycine (1d)

i) A solution of *N*-ε-Boc-ε-aminocaproic acid (1.0g, 4.33mmol), HOSu (758mg, 6.59mmol) and DCC (982mg, 4.76mmol) in DMF (20ml) was stirred 12 hours at rt. After cooling to -15°C, **2(2HCl)** (976mg, 4.76mmol) and NMM (954μl, 8.66mmol) were added. The mixture was stirred at -15°C for 3 hours then allowed to warm to rt (ca. 2h). The dicyclohexylurea (DCU) was filtered off on celite, washed with EtOAc and the filtrate were concentrated under reduced pressure. The residue was taken up in an aqueous solution of 1M $KHSO_4$. The acidic solution was washed with EtOAc and then a solution of 10% $NaHCO_3$ was added until the pH 8-9. The product was extracted with EtOAc and the organic layers were washed with water, brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography (EtOAc/MeOH 8:2, v:v) to yield methyl N-[2-(N-ε-Boc-ε-aminocaproyl)aminoethyl]glycinate as an amorphous solid (1.3g, 87%). TLC (EtOAc/MeOH 8:2, v:v): Rf=0.25. ^1H NMR ($CDCl_3$) δ 7.20 (1H, t); 5.45 (1H, t); 3.70 (3H, s); 3.40 (2H, s); 3.30 (2H, q); 3.05 (2H, q); 2.75 (2H, t); 2.25 (1H, s); 2.20 (2H, t); 1.80-1.30 (6H, m); 1.40 (9H, s). ^{13}C NMR ($CDCl_3$) δ 173.7; 173.2; 156.5; 77.9; 52.0; 50.7; 48.6; 40.5; 39.1; 36.5; 29.8; 26.4; 25.4; 28.5. MS (ESI+) m/z 346.3 $(M+H)^+$, m/z 368.3 $(M+Na)^+$.

ii) To a cooled solution of the previous compound (1.0g, 2.90mmol), uracil acetic acid **4d** (543mg, 3.19mmol) and TEA (808.4μl, 5.80mmol) in CH_2Cl_2 (5ml) was added Brop (1.24g, 3.19mmol). The mixture was stirred for 2 hours at rt. CH_2Cl_2 was evaporated and the crude residue was taken up in EtOAc. The organic layer was washed successively with a 1M $KHSO_4$ solution , a 10% $NaHCO_3$ solution, brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo*. The residue was purified by column chromatography (EtOAc/MeOH 9:1, v:v) to afford the methyl ester of **1d** as an amorphous solid (1.15g, 80%). TLC (EtOAc/MeOH 8:2, v:v): Rf=0.41. ^1H NMR ($CDCl_3$) δ 7.40 (1H, d); 6.95-6.50 (1H, t); 5.65 (1H, d); 4.80 (1H, t); *4.55*, 4.40 (2H, s); 4.20, *4.05* (2H, s); 3.75, *3.70* (3H, s); 3.60-3.20 (4H, m); 3.10 (2H, q); 2.20 (2H, t); 1.70-1.20 (15H, m). ^{13}C NMR ($CDCl_3$) δ 173.9; 171.3; 170.4; 163.9; 157.5; 151.5; 146.1; 101.8; 79.0; 52.5; 49.2; 48.8; 48.3; 40.5; 37.3 ; 36.2 ; 29.7; 26.5; 25.2; 28.5. MS (ESI+) m/z 498.2 $(M+H)^+$.

iii) The methyl ester of **1d** (1.65g, 3.31mmol) was dissolved in dioxane (50ml) and 6,62ml of 1M aqueous LiOH was added at 0°C. The mixture was stirred for 1 hour, then slightly acidified with 1M aqueous HCl. The solvent was evaporated *in vacuo* and the residue was purified on Sephadex (LH-20, MeOH) to yield **1d** as an amorphous solid (1.60g, 100%). TLC (EtOAc/MeOH 1:1, v:v): Rf=0.49. HPLC (A/B 80:20 to 30:70 over 30min): Rt=9.12min, λ_{max}=211.4 nm. ^1H NMR (DMSO d_6) (two isomers) δ 11.30 (1H, bs); 8.65 (1H, t);

7.50 (1H, d); 6.85 (1H, t); 5.60 (1H, d); 4.65, *4.55* (2H, s); *3.75*, 3.70 (2H, s); 3.60-3.10 (4H, m); 3.00 (2H, q); 2.15 (2H, t); 1.70-1.20 (15H, m). ^{13}C (DMSO d_6) δ 172.2, *171.7* ; 171.8, *171.2* ; 167.1, *165.7* ; 163.6; 155.2; 150.8; 146.3; 101.7; 77.0; 48.4; 47.6; 39.4; 36.0; 35.1; 28.9; 25.6; 24.5; 27.9. MS (ESI-) m/z 482.3 $(M-H)^-$. Anal. calcd for $C_{21}H_{33}N_5O_8$: C 52.17, H 6.88, N 14.48. Found: C 52.31, H 6.81, N 14.35.

General procedure for the synthesis of methyl N-[2-(N-protected PNA monomer) aminoethyl] glycinate 3a-c:

A solution of monomer **1a**, **1b** or **1c** (0.50mmol), HOSu (86mg, 0.75mmol) and DCC (103mg, 0.50mmol) in DMF (10ml) was stirred for 12 hours at rt. The mixture was then cooled to -15°C and **2(2HCl)** (102,5mg, 0.50mmol) and NMM (110µl, 1.0mmol) were added. The mixture was stirred 3 hours at -15°C and 2 hours at rt. The solution was then cooled to 0°C, the precipitated DCU was filtered off and washed with EtOAc. The filtrate was concentrated under reduced pressure. The residue was partitioned between 1M aqueous $KHSO_4$ and EtOAc. The aqueous layer was then treated with $NaHCO_3$ until pH 8-9. The product **3** was extracted with EtOAc and the organic layer were washed with water, dried over $MgSO_4$ and evaporated under reduced pressure.

Compound **3a** was previously described[10].

Compound **3b** was obtained as an amorphous solid in 60% yield (192mg). TLC (EtOAc/MeOH 1:1, v:v): Rf=0.45. HPLC (A/B 80:20 to 30:70 over 30min): Rt=14.6min, λ_{max}=213.0 nm. ^{1}H NMR ($CDCl_3$) δ 8.75, *8.70* (1H, s); 8.15, *8.00* (1H, s); 7.70 (1H, s); 7.60-7.20 (5H, m); 5.85 (1H, t); 5.05 (2H, s); 4.95 (2H, s); 4.05, *3.85* (2H, s); 3.60, *3.55* (3H, s); 3.55-3.15 (8H, m); 2.75-2.55 (2H, m); 1.40 (9H, s). MS (ESI+) m/z 642.3 $(M+H)^+$.

Compound **3c** was obtained as an amorphous solid in 70 % yield (216mg). TLC (EtOAc/MeOH 1:1, v:v): Rf=0.69. HPLC (A/B 80:20 to 0:100 over 20min): Rt=9.95min. ^{1}H NMR ($CDCl_3$) (two isomers) δ 8.50 (1H, t); 7.85-6.60 (7H, m); 5.85 (1H, t); 5.05 (2H, s); 4.60, *4.45* (2H, s); *4.15*, 3.90 (2H, s); 3.70 (3H, s); 3.45-3.00 (8H, m); 2.80 (2H,m); 1,35 (9H, s). ^{13}C NMR ($CDCl_3$) δ 172.0; 166.7; *165.6*; 162.9; 155.0; 152.9; 150.7; 135.7; 129.0; 128.4; 127.9; 93.4; 79.1; 66.8; 52.6; *51.7*; 50.4; 50.2; *48.3*; 48.0; *39.6*; 38.4; 28.3. MS (ESI+) m/z 618.3 $(M+H)^+$.

Methyl N-[2-(N-uracil PNA)aminoethyl]glycinate (3d)

A mixture of **1d** (1.0g, 2.07mmol), **2(2HCl)** (471mg, 2.30mmol) and NMM (684µl, 6.21mmol) in 10ml of DMF was cooled to -15°C. Bop (916mg, 2.07mmol) was then added. The mixture was stirred for 3 hours at -15°C and 2 hours at rt. The mixture was concentrated under reduced pressure. The residue was taken up in EtOAc, the DCU was filtered off on celite and washed with EtOAc. The solvents were removed under reduced pressure and the residue was taken up in 1M aqueous $KHSO_4$. The acidic solution was washed with EtOAc and a solution of 10% $NaHCO_3$ was added until pH 8-9. This aqueous solution was then extracted with EtOAc. The organic layers were washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography (EtOAc/MeOH 1:1, v:v) to yield **3d** as an amorphous solid (742mg, 60%). TLC (AcOEt/MeOH 1:1, v:v): Rf=0.21. ^{1}H NMR ($CDCl_3$) (two isomers) δ 7.40 (1H, d); 6.95-6.50 (1H, t); 5.65 (1H, d); 4.80 (1H, t); *4.55*, 4.40 (2H, s); 4.20, *4.05* (2H, s); 3.75, *3.70* (3H, s); 3.65-3.10 (10H, m); 2.7 (2H, t); 2.20 (2H, t); 1.70-1.20 (15H, m). ^{13}C NMR ($CDCl_3$) δ 172.6; 169.8; 167.4; 163.5; 156.6; 152.4; 146.9; 102.1; 78.2; 52.5; 51.9; 49.5; 48.4; 48.3; 47.8; 40.3; 39.7; 38,6; 36.2; 29.8; 26.6; 25.1; 28.4. MS (ESI+) m/z 598.3 $(M+H)^+$.

General procedure for the synthesis of protected diPNAs X-[B_1B_2]-OMe (5)

To a cooled solution of **3a, 3b, 3c** or **3d** (0.62mmol), of the appropriate protected base-acetic acid unit **4** (0.62mmol) and of TEA (259µl, 1.86mmol) in CH_2Cl_2 (2ml) was added Brop (241mg, 0.62mmol). The mixture was stirred for 2 hours at rt, then CH_2Cl_2 was added. The solution was washed with 1M aqueous $KHSO_4$, with a 10% $NaHCO_3$ solution and finally with brine. The organic layer was dried over $MgSO_4$ and the solvent concentrated *in vacuo*. The residue was purified by column chromatography to give **5.**

Z-[$A^{(diBoc)}G^{(OBn)}$]-OMe (5ae) was previously described[10].

Boc-[$A^{(Z)}G^{(OAll)}$]-OMe (5bf)

5bf was obtained as a white solid (487mg, 90%). mp=137-139°C; TLC (AcOEt/MeOH 1:1, v:v): Rf=0.19. HPLC (A/B 80:20 to 30:70 over 30min): Rt=18.6min, λ_{max}=211.8 nm. ^{1}H NMR (DMSO d_6) δ 8.60 (1H, s); 8.30 (1H, s); 7.70 (1H, s); 7.60-7.20 (5H, m); 6.40 (2H, s); 6.10 (1H, m); 5.60-4.70 (10H, m); 4.50-3.50 (7H, m); 1.40 (9H, s). ^{13}C NMR ($CDCl_3$) (two isomers) δ *170.2*; 170.1; *169.7*; 169.5; 167.4; *166.5*; 158.8; 156.7; 153.1; 152.3; 151.5; 149.7; 146.6; 145.4; 139.8; 135.7; 131.6; 128.0; 127.9; 119.2; 117.7; 79.6; 67.6; 67.5; 51.7; 51.3; 49.8; 49.3; 48.1; 47.8; 42.2; 35.4; 35.3; 27.1. MS (ESI+) m/z 873.5 $(M+H)^+$. Anal. calcd for $C_{39}H_{48}N_{14}O_{10}$: C 53.66, H 5.54, N 22.46. Found: C 53.91, H 5.38, N 22.25.

Boc-[$C^{(Z)}C^{(Z)}$]-OMe (5cc)

5cc was obtained as an amorphous solid (476mg, 85%). TLC (AcOEt/MeOH 8:2, v:v): Rf=0.25. HPLC (A/B 80:20 to 0:100 over 20min): Rt=12.4min. ^{1}H NMR ($CDCl_3$) (two isomers) δ 7.85-7.45 (3H, m); 7.45-7.25 (10H, m); 7.25-7.00 (2H, m); 6.20, *6.05* (1H, t); 5.25-5.00 (4H, m); *4.75*, 4.60 (4H, s); 4.30, *4.05* (4H, s); 3.85-3.10 (11H, m); 1,40 (9H, s). ^{13}C NMR ($CDCl_3$) δ 171.0; 169.1; 168.0, *167.6*; *163.3*, 162.9; 156.3; 152.5; 150.1; 135.2; 128.6; 128.3; 128.1; 95.4; *79.7*, 79.0; 67.7; 52.6; 50.5; 50.2; 49.9; 48.9; 47.7; 38.8; 36.7; 28.4. MS (ESI+) m/z 903.3 $(M+H)^+$. Anal. calcd for $C_{42}H_{50}N_{10}O_{13}$: C 55.87, H 5.58, N 15.51. Found: C 54.08, H 5.45, N 15.28.

***N*-ε-Boc-ε-Ahx-[$UC^{(Z)}$]-OMe (5dc)**

5dc was obtained as a white amorphous powder (384mg, 70%). TLC (AcOEt/MeOH 1:1, v:v): Rf=0.59. HPLC (A/B 80:20 to 60:40 over 30min): Rt=26.6min. ^{1}H NMR ($CDCl_3$) (two isomers) δ 8.20 (1H, t); 8.00 (2H, m); 7.70 (1H, d); 7.60-7.30 (5H, m); 7.05 (1H, d); 6.80 (1H, t); 5.60 (1H, d); 5.25 (2H, s); 5.00-4.65 (4H, m); 4.65-3.20 (15H, m); 3.00 (2H, q); 2.20 (2H, t); 1.60-1.05 (15H, m). ^{13}C NMR (DMSO d_6) δ *173.2*, 173.0; *170.7*, 170.6; *169.1*, 168.7; 168.1, *167.9*; 163.9; 163.1; *155.5*, 155.0; 153.1; 150.9; 150.3; 146.4; 135.5; 128.6; 128.3; 128.0; 100.6; 94.1; *79.0*, 77.7; 66.9; 52.3, *51.8*; 50.6; 50.1; 49.6; 48.3; 47.7; 47.2; 40.3; 37.6; 36.9; 36.0; 29.9; 26.8; 25.3; 28.4. MS (ESI+) m/z 905.7 $(M+Na)^+$. Anal. calcd for $C_{40}H_{54}N_{10}O_{13}$: C 54.41, H 6.16, N 15.86. Found: C 54.16, H 6.07, N 16.02.

TFA. H-[$A^{(Z)}G^{(OAll)}$]-OMe (6bf) and TFA. H-[$C^{(Z)}C^{(Z)}$]-OMe (6cc)

0.114mmol of **5bf** (100mg) or **5cc** (111mg) were stirred at 0°C in a (1:1, v:v) solution of CH_2Cl_2/TFA (2ml) for 1 hour. The solvent was then evaporated to dryness *in vacuo*. The residue was worked up with EtOAc, filtered off to give **6bf** (97mg, 95%) or **6cc** (100mg, 95%) as amorphous solids.

Compound **6bf**: TLC ($MeOH/H_2O$/AcOH 50:49:1, v:v:v): Rf=0.64. HPLC (A/B 80:20 to 30:70 over 30min): Rt=8.31min, λ_{max}=207.4 nm. MS (ESI+) m/z 773.3 $(M)^+$.

Compound **6cc**: TLC (MeOH/AcOH 99:1, v:v): Rf=0.43. HPLC (A/B 80:20 to 0:100 over 20min): Rt=9.8min. MS (ESI+) m/z 803.3 $(M+H)^+$.

Z-[$A^{(diBoc)}G^{(OBn)}$]-OH (5g) and N-ε-Boc-ε-Ahx-[$UC^{(Z)}$]-OH (5h)

5ae (588mg, 0.576mmol) or **5dc** (501mg, 0.576mmol) was dissolved in 2ml of dioxane and 864µl of 1M aqueous LiOH was added at 0°C. After 1 hour, the pH was adjusted to 7 with a 1M aqueous HCl solution. The dioxane was evaporated under reduced pressure and the residue was taken up in EtOAc. The organic layer was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure, to give **7ae** and **7dc**.

Compound **7ae** was previously described[10].

Compound **7dc** was obtained as a white amorphous powder by triturating with Et_2O (375mg, 75%). TLC (AcOEt/MeOH 1:1, v:v): Rf=0.38. ^1H NMR (DMSO d_6) δ 10.80 (1H, bs); 8.20 (1H, t); 8.00 (2H, m); 7.70 (1H, d); 7.40 (5H, m); 7.10 (1H, d); 6.75 (1H, t); 5.60 (1H, d); 5.20 (2H, s); 5.00-4.40 (4H, m); 4.35-3.75 (6H, m); 3.70-3.00 (6H, m); 2.90 (2H, q); 2.15 (2H, t); 1.60-1.05 (15H, m). ^{13}C NMR (DMSO d_6) δ *172.6*, 173.0; 170.7; 170.5; *168.6*, 168.1; 167.6, *167.0*; 163.1; 155.5; 155.0; 153.0; 150.9; 146.3; 135.8; 128.5; 128.2; 128.0; 100.5; 93.8; 77.2; 66.4; 49.8; 49.7; 49.5; 48.6; 48.4; 47.9; 47.1; 46.0; 36.6; 36.0; 35.2; 29.3; 26.0; 24.7; 28.2. MS (ESI-) m/z 867.4 $(M-H)^-$.

Z-[$A^{(diBoc)}G^{(OBn)}A^{(diBoc)}$]-OMe (8)

i) **7ae** (110mg, 0.110mmol) and HOSu (19mg, 0.165mmol) were placed in DMF (4ml) at 0°C. DCC (23mg, 0.110mmol) was then added. The mixture was stirred at rt 12 hours, cooled to -15°C for 15 min then **2(2HCl)** (27mg, 0.132mmol) and NMM (30µl, 0.275mmol) were introduced. The solution was stirred at -15°C for 3 hours and 2 hours at rt. The precipitated DCU was filtered off on celite and washed with EtOAc. The filtrate was evaporated *in vacuo*. The residue was taken up in 10% aqueous solution of citric acid. The acidic layer was washed with EtOAc then treated with $NaHCO_3$ until pH 8-9. The aqueous solution was extracted with EtOAc. The organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The amino compound was obtained as an amorphous solid (50mg, 41%). TLC (AcOEt/MeOH 1:1, v:v): Rf=0.60. HPLC (A/B 80:20 to 0:100 over 30min): Rt=16.6min. ^1H NMR (CD_3OD) (two isomers) δ 8.75, *8.70* (1H, s); 8.35, *8.30* (1H, s); *7.80*, 7.70 (1H, s); 7.60-7.15 (10H, m); 5.60-5.05 (4H, m); 5.00-4.80 (4H, m); 4.25-4.00 (4H, m); *3.75*, 3.70 (3H, s); 3.65-3.10 (10H, m); 2.85-2.80 (2H, m); 1.40 (18H, s). MS (ESI+) m/z 1123.5 $(M+H)^+$, m/z 1145.5 $(M+Na)^+$.

ii) The previous compound (50mg, 0.045mmol), **4a** (17.5mg, 0.045mmol) and TEA (18.6µl, 0.134mmol) were placed under stirring in CH_2Cl_2 (0.5ml) at 0°C. Brop (17.3mg, 0.045mmol) was then added. The mixture was stirred 2 hours at rt and the solvent was concentrated *in vacuo*. The residue was taken up in EtOAc and the organic layer was washed with a 10% aqueous solution of citric acid, with 10% aqueous $NaHCO_3$ solution, with water then brine. The solvent was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/MeOH 8:2, v:v) to give **8** (25mg, 37%) as an amorphous solid. TLC (EtOAc/MeOH 6:4, v:v): Rf=0.35. HPLC (A/B 80:20 to 0:100 over 30min): Rt=21.3min. ^1H NMR ($CDCl_3$) δ 8.85 (1H,s); 8.25 (1H,s); 7.60-7.10 (13H, s); 5.95 (1H, bs); 5.45 (2H, s); 5.05-4.75 (6H, m); 4.10-3.75 (9H, m); 3.55-3.15 (12H, m); 1.45 (18H, s). ^{13}C NMR ($CDCl_3$) δ 172.8; 171.3; 171.1; 170.5; 170.3; 168.1; 161.0; 159.4; 156.9; 154.2; 153.4; 152.1; 150.5; 150.2; 150.0; 146.2; 140.4; 136.6; 136.4; 128.5; 128.0; 114.9; 84.2; 84.1; 68.0; 66.1; 52.3; 51.5; 50.9; 50.2; 48.2; 47.8; 47.4; 43.5; 43.3; 39.5; 38.9; 38.1; 28.0. MS (ESI+) m/z 1498.6 $(M+H)^+$, m/z 1520.6 $(M+Na)^+$. Anal. calcd for $C_{69}H_{87}N_{21}O_{18}$: C 55.30, H 5.85, N 19.63. Found: C 55.02, H 5.95, N 19.84.

Methyl N-[2-(N-Benzyloxycarbonyl)aminoethyl]glycinate (9)

To a cold solution (-15°C) of N-(benzyloxycarbonyloxy)-succinimide (1.22g, 4.88mmol) in 20ml of acetonitrile were added **2**(2HCl) (1.0g, 4.88mmol) and NMN (1.35ml, 12.2mmol). The mixture was stirred 3 hours at -15°C then 2 hours at rt. The solvent was concentrated *in vacuo* and the crude residue was taken up in a 1M aqueous $KHSO_4$ solution. The aqueous layer was washed with EtOAc then a 10% $NaHCO_3$ solution was added until pH 9. After extraction with EtOAc, the organic layer was washed with water, with brine then dried over $MgSO_4$. The solvent was evaporated *in vacuo*, to give **9** as an amorphous solid (947mg, 73%). TLC (EtOAc): Rf=0.38. HPLC (A/B 90:10 to 70:30 over 40min): Rt=18.1min. ^{1}H NMR ($CDCl_3$) δ 7.15 (5H,m); 6.20 (1H,bs); 4.90 (2H, s); 3.50 (3H, s); 3.20 (2H, s); 3.10 (2H, m); 2.55 (2H, m); 1.80 (1H, bs). ^{13}C NMR ($CDCl_3$) δ 172.0; 156.0; 136.0; 127.5; 127.0; 65.5; 51.0; 49.3; 47.8; 40.0. MS (ESI+) m/z 267.1 $(M+H)^+$.

Methyl N-[2-(N-Monomethoxytrityl)aminoethyl]glycinate (10)

To a cold solution (-30°C) of **2**(2HCl) (3.0g, 14.3mmol) in CH_2Cl_2 (100ml) were added monomethoxytrityl chloride (4.5g, 14.3mmol) and TEA (6.2ml, 43.9mmol). The mixture was stirred 2 hours at -30°C. The solvent was concentrated *in vacuo* and the crude residue was taken up in EtOAc. The organic layer was washed with water, with brine then dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (Hexane/EtOAc 1:1, v:v) to give **10** (4.4g, 74%) as an amorphous solid. TLC (Hexane/EtOAc 1:1): Rf=0.26. ^{1}H NMR ($CDCl_3$) δ 7.60–6.80 (14H,m); 3.85 (3H, s); 3.75 (3H, s); 3.40 (2H, s); 2.80 (2H, m); 2.35 (2H, m); 1.90 (1H, bs). ^{13}C NMR ($CDCl_3$) δ 173.2; 158.0; 145.6; 138.5; 130.0-126.3 (13C); 113.3; 70.3; 55.4; 51.9; 50.7; 49.9; 43.2. MS (ESI+) m/z 427.2 $(M+Na)^+$.

Methyl N-[2-(N-Z)aminoethyl]-N-[Alloc] glycinate (Z-[Alloc]-OMe) (11)

9 (800mg, 3.0mmol) and TEA (419µl, 3.0mmol) were placed in CH_2Cl_2 (10 ml) at 0°C. A solution of allyl chloroformate (414µl, 3.9mmol) in CH_2Cl_2 (2ml) was added dropwise. The mixture was stirred at rt for 1 hour. The solvent was then evaporated *in vacuo*. The residue was taken up in EtOAc and the organic layer was washed successively with a 1M aqueous $KHSO_4$ solution, a 10% aqueous $NaHCO_3$ solution, brine and finally dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by column chromatography (Hexane/EtOAc 7:3 to 2:3, vv) to afford **11** as an amorphous solid (750mg,71%). TLC (Hexane/EtOAc 1:1, v:v): Rf=0.36. HPLC (A/B 80:20 to 0:100 over 30min): Rt=15.0min. ^{1}H NMR ($CDCl_3$) δ 7.20 (5H, s); 5.90-5.65 (2H, m); 5.35-4.95 (4H, m); 4.45 (2H, d); 3.90 (2H, s); 3.60 (3H, s); 3.45-3.15 (4H, m). ^{13}C NMR ($CDCl_3$) δ 172.1; 156.5; 156; 136.2; 132.1; 127.8-127.2; 118.1; 66.9; 65.3; 52.5; 50.1; 47.5; 38.8. MS (ESI+) m/z 373.2 $(M+Na)^+$.

Methyl N-[2-(N-Mmt)aminoethyl]-N-[Troc] glycinate (Mmt-[Troc]-OMe) (12)

10 (3.67g, 9.1mmol) and TEA (1.25ml, 9.1mmol) were placed in CH_2Cl_2 (45 ml) at 0°C. A solution of trichloroethyloxycarbonyl chloride (1.50ml, 10.9mmol) in CH_2Cl_2 (5ml) was added dropwise. The mixture was stirred at 0°C for 2 hours. The solvent was then evaporated *in vacuo*. The residue was taken up in EtOAc and the organic layer was washed with water and brine and finally dried ($MgSO_4$). The solvent was evaporated *in vacuo* and the residue was purified by column chromatography (Hexane/EtOAc 6:4, v:v) to afford **12** as a colourless oil (513mg, 97%). TLC (Hexane/EtOAc 6:4, v:v): Rf=0.54. ^{1}H NMR ($CDCl_3$) (two isomers) δ 7.50–6.80 (14H,m); 4.75,*4.70* (2H,s); 4.15,*4.05* (2H,s); 3.80 (3H, s); 3.75, *3.70* (3H, s); 3.55 (2H, m); 2.35 (2H, m); 1.90 (1H, bs). ^{13}C NMR ($CDCl_3$) δ 173.2; 158.1; 155.0; 146.3; 146.2; 138.1; 129.8-126.4; 113.3;

95.5; 75.5; 70.5; 55.3; 52.4; 50.7; 50.1; 49.3; 42.4. MS (ESI+) m/z 601.1 (M+Na; $^{35}Cl)^+$, m/z 603.2 (M+Na ; $^{37}Cl)^+$, m/z 617.1 (M+K ; $^{35}Cl)^+$, m/z 619.2 (M+K; $^{37}Cl)^+$.

N-[2-(N-Z)aminoethyl]-N-[Alloc] glycine (Z-[Alloc]-OH) (13)

Compound **11** (650mg, 1.86mmol) was dissolved in THF (4ml) and 1M aqueous LiOH (3.7ml) was added at 0°C. This mixture was stirred for 1 hour, then acidified with 1M aqueous $KHSO_4$ until pH 4. The product was extracted with EtOAc, the organic layers were washed with water until neutral pH, with brine then dried over $MgSO_4$. The solvent was evaporated *in vacuo*, to yield **13** as an amorphous powder (580mg, 93%). TLC (EtOAc/MeOH 1:1, v:v): Rf=0.29. HPLC (A/B 80:20 to 0:100 over 30min): Rt=12.0min. ^1H NMR ($CDCl_3$) δ 7.30 (5H, s); 5.90-5.55 (2H, m); 5.25-4.95 (4H, m); 4.50 (2H, d); 3.90 (2H, s); 3.45-3.15 (4H, m). ^{13}C NMR ($CDCl_3$) δ 173.1; 156.8; 156.2; 136.5; 132.1; 128.0-127.2; 118.3; 67.1; 65.3; 52.0; 48.4; 39.8. MS (ESI-) m/z 335.1 $(M-H)^-$.

TFA. methyl N-[2-aminoethyl]-N-[Troc]glycinate (TFA.H-[Troc]-OMe) (14)

Compound **12** (1.0g, 1.73mmol) was dissolved in 17ml of TFA / CH_2Cl_2 (1:1, v:v). The mixture was stirred at rt for 4 hours. The solvent was evaporated under reduced pressure and the crude residue purified by column chromatography (EtOAc 100% to MeOH 100%). **14** was obtained as a colourless resin (610mg, 84%). TLC (EtOAc/MeOH 1:1, v:v): Rf=0.50. HPLC (A/B 80:20 to 0:100 over 30min): Rt=26.6min. ^1H NMR ($CDCl_3$) δ 7.40 (3H, bs); 4.75 (2H, s); 4.15 (2H, s); 3.75 (3H, s); 3.50 (2H, m); 2.40 (2H, m). ^{13}C NMR ($CDCl_3$) δ 171.7; 156.5; 95.2; 75.5; 52.4; 50.0; 47.3; 46.8. MS (ESI+) m/z 307.1 (M; $^{35}Cl)^+$, m/z 313.2 (M; $^{37}Cl)^+$.

Z-[Alloc-Troc]-OMe (15)

To a cold solution (-15°C) of **13** (486mg, 1.45mmol) and TEA (302µl, 2.17mmol) in CH_2Cl_2 (5ml) was added dropwise a solution of isobutyl chloroformate (207µl, 1.6mmol) in of CH_2Cl_2 (2ml). After 15 min stirring, **14** (610mg, 1.45mmol) and TEA (302µl, 2.17mmol) were added at -15°C. The mixture was stirred for 3 hours at this temperature then allowed to warm to rt (ca. 1h). The solvent was evaporated under reduced pressure. The residue was taken up in EtOAc and washed successively with a 1M aqueous $KHSO_4$ solution, a 10% aqueous $NaHCO_3$ solution, brine and finally dried ($MgSO_4$). Evaporation of the solvent under reduced pressure led to an oil that was purified by column chromatography (Hexane/EtOAc 3:7 to EtOAc/MeOH 8:2, v:v) giving **15** (620mg, 70%) as a colourless resin. TLC (EtOAc 100%): Rf=0.40. HPLC (A/B 80:20 to 0:100 over 30min): Rt=17.7min. ^1H NMR ($CDCl_3$) δ 7.35 (5H, s); 7.05 (1H, bs); 6.15 (1H, bs); 5.80 (1H, bs); 5.15 (4H, m); 4.65 (2H, s); 4.45 (2H, d); 4.00 (2H, s); 3.75 (5H, m); 3.35 (8H, m). ^{13}C NMR ($CDCl_3$) δ 171.5; 169.5; 156.8; 156.4; 156.2; 136.5; 132.1; 128.0-127.2; 118.3; 95.2; 75.5; 67.1; 65.3; 52.4; 50.9; 49.9; 48.2; 47.6; 40.9; 38.6. MS (APCI+) m/z 625.3 (M+H; $^{35}Cl)^+$, m/z 631.2 (M+H; $^{37}Cl)^+$.

Methyl N-[2-(N-Mmt) aminoethyl]-N-[Alloc] glycinate (Mmt-[Alloc]-OMe) (16)

Compound **10** (4.29g, 10.6mmol) and TEA (1.47ml, 10.6mmol) were placed in CH_2Cl_2 (60ml) at 0°C. A solution of allyl chloroformate (1.46ml, 13.8mmol) in CH_2Cl_2 (7ml) was added dropwise. The mixture was stirred at 0°C for 2 hours. The solvent was then evaporated *in vacuo*. The residue was taken up in EtOAc and the organic layer was washed with water and brine and finally dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue was purified by column chromatography (Hexane/EtOAc 6:4, v:v) to afford **16** as a colourless resin (4.7g, 90%). TLC (Hexane/EtOAc 6:4, v:v): Rf=0.44. ^1H NMR ($CDCl_3$) (two isomers) δ 7.55–6.80 (14H, m); 5.95 (1H, m); 5.30 (2H, m); 4.65 (2H, m); 4.05 (2H, m); 3.80 (3H, s); 3.70 (3H, s); 3.50 (2H, m); 2.40 (2H, m); 1.95 (1H, bs). ^{13}C NMR ($CDCl_3$) δ 170.4; 158.0; 156.6; 146.3; 146.2; 138.2; 132.9; 129.9-126.4; 113.4; 117.7, *117.3* ; 70.5; 66.6, *66.4* ; 55.3; 52.2; 49.5; 42.4. MS (ESI+) m/z 511.2 $(M+Na)^+$, m/z

527.2 $(M+K)^+$.

Z-[Alloc-Troc]-OH (17)

Compound **15** (125mg, 0.2mmol) was dissolved in MeOH (2ml) and 1M aqueous LiOH (0.5ml) was added at 0°C. The mixture was stirred for 2 hours, then acidified with a 1M aqueous $KHSO_4$ solution until pH 4. The product was extracted with EtOAc, the organic layers were washed with water until neutral pH and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to yield **17** as a colourless resin (110mg, 90%). TLC (EtOAc/MeOH 8:2, v:v): Rf=0.35. HPLC (A/B 80:20 to 0:100 over 30min): Rt=15.4min. ^1H NMR ($CDCl_3$) δ 7.40 (5H, s); 7.10 (1H, bs); 6.20 (1H, bs); 5.80 (1H, m); 5.30-5.05 (4H, m); 4.65 (2H, s); 4.45 (2H, d); 4.05 (2H, s) 3.90 (2H, s); 3.50-3.20 (8H, m). ^{13}C NMR ($CDCl_3$) δ 172.3; 169.4; 156.7; 156.4; 156.3; 136.4; 132.0; 128.0-127.2; 118.4; 95.3; 75.8; 67.1; 65.2; 51.2; 50.1; 48.3; 47.6; 40.7; 38.5. MS (ESI+) m/z 611.3 (M+H, $^{35}Cl)^+$, m/z 616.3 (M+H, $^{37}Cl)^+$.

TFA. methyl N-[2-aminoethyl]-N-[Alloc]glycinate (TFA.H-[Alloc]-OMe) (18)

18 was obtained (1.39g, 90%) from 16 (2.29g, 4.71mmol) following the above procedure for the preparation of 14. TLC (EtOAc/MeOH 1:1, v:v): Rf=0.55. 1H NMR (CDCl3) δ 7.60 (3H, bs); 5.80 (1H, m); 5.20 (2H, m); 4.55 (2H, m); 4.05 (2H, m); 3.80-3.50 (5H, s); 3.15 (2H, m). ^{13}C NMR ($CDCl_3$) δ 171.7; 156.6; 132.1; 118.6, 118.1; 67.1, 66.7; 52.7; 50.5; 47.2; 40.8, 38.6. MS (ESI+) m/z 217.1 $(M+H)^+$.

Z-[Alloc-Troc-Alloc]-OMe (19)

19 was obtained from **17** (110mg, 0.18mmol) and **18** (60mg, 0.18mmol), following the above procedure for the preparation of **15**. Column chromatography (Hexane/EtOAc 3:7 to EtOAc/MeOH 8:2, v:v) led to **19** as an amorphous solid (100mg, 70%). TLC (EtOAc): Rf=0.26. HPLC (A/B 80:20 to 0:100 over 20min): Rt=17.3min. ^1H NMR ($CDCl_3$) δ 7.40-7.00 (7H, m); 6.30 (1H, bs); 5.80 (2H, m); 5.30-4.95 (6H, m); 4.75-4.40 (6H, m); 3.85-3.60 (9H, m); 3.50-3.15 (12H, m). ^{13}C NMR ($CDCl_3$) δ 172.2; 170.1; 169.5; 157.1; 156.7; 156.2, 156.0; 136.4; 132.3, 132.0; 128.1-127.3; 118.2, 117.9; 95.1; 75.4; 67.1; 66.7; 65.4; 52.8; 51.9; 50.5; 49.9; 49.3; 48.1; 47.6; 40.9; 38.9; 38.5. MS (ESI+) m/z 831.3 (M+Na, $^{35}Cl)^+$, m/z 837.3 (M+Na, $^{37}Cl)^+$ m/z 847.3 (M+K, $^{35}Cl)^+$, m/z 853.3 (M+K, $^{37}Cl)^+$.

Z-[Alloc-H-Alloc]-OMe (20)

To a 1:1 (v:v) solution of DMF / glacial acetic acid (1ml) were added **19** (65mg, 80.3μmol) and cadmium dust (300mg, 2.67mmol). The mixture was stirred 2 hours at rt, then cadmium was filtered off on celite and washed with EtOAc. The filtrate was concentrated *in vacuo* and the residue was taken up in a 10% aqueous $NaHCO_3$ solution. This aqueous solution was extracted with EtOAc then the organic layer was washed with water, dried over $MgSO_4$ and evaporated. Compound **20** (colourless resin, 50mg, 95%) was used in the next step without further purification. TLC (EtOAc/MeOH, 8:2, v:v): Rf=0.35.HPLC (A/B 90:10 to 70:30 over 40min): Rt=6.6min. ^1H NMR (CD_3OD) δ 7.25 (5H, m); 5.85 (2H, m); 5.35-4.95 (6H, m); 4.60-4.40 (4H, m); 4.10-3.80 (4H, m); 3.70 (3H, s); 3.55-3.05 (12H, m); 2.60 (2H, m). ^{13}C NMR (CD_3OD) δ 171.3; 170.3; 170.1; 157.3; 157.0, 156.3; 136.6; 132.3; 132.1; 128.1-127.4; 116.9, 116.4; 66.2; 66.0; 51.7; 51.1; 50.9; 49.0; 48.5; 48.1; 47.8; 47.3; 38.7; 37.0. MS (ESI+) m/z 635.5 $(M+H)^+$.

Z-[Alloc-G$^{(OBn)}$-Alloc]-OMe (21)

A solution of **20** (50mg, 0.079mmol), 2-amino-6-(benzyloxy)-purine acetic acid **4e** (26mg, 0.087mmol), TEA (33μl, 0.236mmol) in CH_2Cl_2 (0.3ml) was stirred at 0°C. Brop (33.7mg, 0.087mmol) was then added. After 18 hours stirring, the mixture was diluted with chloroform (10ml). The organic layer was washed successively with a 1M aqueous $KHSO_4$ solution, a 10% aqueous $NaHCO_3$ solution, brine and dried over $MgSO_4$. The

solvent was evaporated *in vacuo* and **21** was obtained as an amorphous powder (65mg, 90%). TLC (AcOEt/MeOH 8:2, v:v): Rf=0.30. HPLC (A/B 80:20 to 0:100 over 30min): Rt=16.1min. ^{1}H NMR ($CDCl_3$) δ 7.45 (3H, m); 7.25 (10H, m); 6.45 (1H, bs); 5.75 (2H, m); 5.45 (2H, s); 5.30-4.85 (8H, m); 4.60-4.30 (4H, m); 4.00-3.50 (9H, m); 3.50-3.15 (12H, m). ^{13}C NMR ($CDCl_3$) δ 173.1; 171.9; 170.2; 170.0; 161.0; 159.4; 157.3; 157.0; 156.9; 154.2; 140.4; 136.6; 136.4; 132.3; 132.1; 128.5-128.0; 116.9; 116.4; 114.9; 68.2; 66.5; 66.2; 66.1; 52.7; 51.5; 50.9; 50.2; 49.2; 48.5; 47.7; 43.4; 39.7; 38.5; 38.0. MS (ESI+) m/z 916.4 $(M+H)^+$, m/z 938.5 $(M+Na)^+$, m/z 954.4 $(M+K)^+$.

Z-[H-G$^{(OBn)}$-H]-OMe (22)

Compound **21** (65mg, 0.071mmol) and DEA (238µl, 2.13mmol) were dissolved in CH_2Cl_2 (0.5ml) at 0°C, and tetrakis(triphenylphosphine)-palladium(0) (9mg, 0.071mmol) was added. The mixture was stirred for 1h at rt. The solvent was concentrated under reduced pressure and the crude residue was purified by column chromatography (EtOAc/MeOH 8:2 to 0:100, v:v) to afford **22** as a colourless resin (40mg, 75%). TLC (EtOAc/MeOH 1:1, v:v): Rf=0.33. HPLC (A/B 80:20 to 0:100 over 30min): Rt=10.9min. ^{1}H NMR ($CDCl_3$) δ 7.60-7.10 (13H, m); 6.00 (1H, bs); 5.50 (2H, s); 5.05 (2H, s); 4.80 (2H, s); 3.90 (3H, s); 3.60 (3H, s); 3.50-2.90 (12H, m); 2.75-2.25 (6H, m). ^{13}C ($CDCl_3$) δ 172.9; 171.7; 170.3; 170.0; 160.9; 159.3; 156.7; 154.3; 140.2; 136.8; 136.5; 128.5-128.0; 115.0; 68.3; 66.1; 52.5; 51.0; 49.3; 43.5; 41.5; 40.7; 40.2; 39.5; 38.0; 37.4; 36.5. MS (ESI+) m/z 748.6 $(M+H)^+$, m/z 770.6 $(M+Na)^+$.

Z-[A$^{(diBoc)}$G$^{(OBn)}$A$^{(diBoc)}$]-OMe (8)

A solution of **22** (40mg, 0.054mmol), N^6,N^6-di-tert-butyloxycarbonyl adenine acetic acid **4a** (41mg, 0.118mmol), TEA (37µl, 0.268mmol) in CH_2Cl_2 (0.3ml) was stirred at 0°C. Brop (46mg, 0.118mmol) was then added. After 18 hours stirring, the mixture was diluted with chloroform (10ml). The organic layer was washed successively with 1M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$ solution, brine and dried on $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography (EtOAc/MeOH 8:2 to 0:100, v:v) to afford **8** as a colourless resin (50mg, 62%). TLC (EtOAc/MeOH 6:4, v:v): Rf=0.35. HPLC (A/B 80:20 to 0:100 over 30min): Rt=21.3min. ^{1}H NMR ($CDCl_3$) δ 8.85 (1H,s); 8.25 (1H,s); 7.60-7.10 (13H, s); 5.95 (1H, bs); 5.45 (2H, s); 5.05-4.75 (6H, m); 4.10-3.75 (9H, m); 3.55-3.15 (12H, m); 1.45 (18H, s). ^{13}C NMR ($CDCl_3$) δ 172.8; 171.3; 171.1; 170.5; 170.3; 168.1; 161.0; 159.4; 156.9; 154.2; 153.4; 152.1; 150.5; 150.2; 150.0; 146.2; 140.4; 136.6; 136.4; 128.5; 128.0; 114.9; 84.2; 84.1; 68.0; 66.1; 52.3; 51.5; 50.9; 50.2; 48.2; 47.8; 47.4; 43.5; 43.3; 39.5; 38.9; 38.1; 28.0. MS (ESI+) m/z 1498.6 $(M+H)^+$, m/z 1520.6 $(M+Na)^+$. Anal. calcd for $C_{69}H_{87}N_{21}O_{18}$: C 55.30, H 5.85, N 19.63. Found: C 55.65, H 5.69, N 19.40.

Methyl N-[2-(N-Boc)aminoethyl]glycinate (23)

To a cooled solution (-15°C) of DMAP (2.98g, 24.39mmol) in CH_2Cl_2 (10ml) was slowly added a solution of Boc_2O (5.32g, 24.39mmol) in CH_2Cl_2 (5ml). **2**(2HCl) (5.0g, 24.39mmol) and NMM (5.4ml, 48.80mmol) were then added and the mixture was stirred for 3 hours at -15°C then 2 hours at rt. The solvent was evaporated under reduced pressure and the crude residue taken up in a 1M aqueous $KHSO_4$ solution. The aqueous layer was washed with EtOAc then a 10% $NaHCO_3$ solution was added until pH 9. After extraction with EtOAc, the organic layer was washed with brine, dried over $MgSO_4$ then evaporated. The residue was purified by column chromatography (EtOAc/MeOH 9:1, v:v) to yield **23** as an amorphous solid (4.0g, 72%). TLC (AcOEt/MeOH 8:2, v:v): Rf=0.51. ^{1}H RMN ($CDCl_3$) δ 4.95 (1H, t); 3.65 (3H, s); 3.35 (2H, s); 3.15 (2H, q); 2.70 (2H, t); 2.20

(1H, bs); 1.35 (9H, s). ^{13}C NMR ($CDCl_3$) δ 172.9; 156.2; 78.9; 51.7; 50.3; 48.8; 40.2; 28.4. MS (ESI+) m/z 233.1 $(M+H)^+$.

Methyl N-[2-(N-Boc)aminoethyl]-N-[Alloc] glycinate (Boc-[Alloc]-OMe) (24)

Compound **24** was obtained as an amorphous solid (2.4g, 88%) from **23** (2.0g, 8.61mmol) following the procedure described for the preparation of **11**. TLC (Hexane/EtOAc 1:1, v:v): Rf=0.48. ^{1}H NMR ($CDCl_3$) δ 6.00-5.70 (1H, m); 5.35-5.05 (2H, m); 4.55 (2H, t); 3.95 (2H, s); 3.65 (3H, s); 3.40 (2H, t); 3.20 (2H, q); 1.35 (9H, s). ^{13}C NMR ($CDCl_3$) δ 171.1, 170.5; 156.1; 132.7; 117.8, 117.3; 79.2; 66.4; 52.2; 49.9, 49.7; 49.0, 48.6; 39.2; 28.4. MS (ESI+) m/z 317.2 $(M+H)^+$, m/z 339.3 $(M+Na)^+$.

N-[2-(N-Boc)aminoethyl]-N-[Alloc]glycine (Boc-[Alloc]-OH) (25)

25 was obtained as an amorphous solid (1.9g, 100%) from **24** (2.0g, 6.33mmol), following the procedure described for the preparation of **13**. TLC (EtOAc/MeOH 1:1, v:v): Rf=0.23. HPLC (A/B 80:20 to 0:100 over 20min): Rt=8.0min. ^{1}H NMR ($CDCl_3$) δ 6.00-5.70 (1H, m); 5.35-5.05 (2H, m); 4.55 (2H, t); 3.95 (2H, s); 3.65 (3H, s); 3.40 (2H, t); 3.20 (2H, q); 1.35 (9H, s); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.5; 156.1; 132.7; 117.8; 117.3; 79.2; 66.4; 52.2; 49.9, 49.7; 49.0, 48.6; 39.2; 28.4. MS (ESI-) m/z 301.1 $(M-H)^-$.

TFA. methyl N-[2-aminoethyl]-N-[Alloc]glycinate (TFA.H-[Alloc]-OMe) (26)

Compound **26** was obtained from **24** (2,0g, 6.33mmol) following the procedure for the preparation of **14**. **26** was crystallised from Et_2O (2.0g, 96%). mp=84-85°C. TLC (AcOEt/MeOH 1:1, v:v): Rf=0.22. MS (ESI+) m/z 217.1 $(M)^+$.

Boc-[Alloc-Alloc]-OMe (27)

Compound **25** (2.0g, 6.62mmol) and HOSu (1.14g, 9.93mmol) were placed in CH_2Cl_2 (20ml) at 0°C. DCC (1.5g, 7.28mmol) was then added. The reaction mixture was stirred for 12 hours at rt then cooled at -15°C. Compound **26** (2.20g, 6.62mmol) and TEA (1.84ml, 13.24mmol) were added. The solution was stirred for 3 hours at -15°C then allowed to warm to rt (ca. 1h). The solvent was evaporated under reduced pressure. The residue was taken up in EtOAc, DCU was filtered off on celite and washed with EtOAc. The filtrate was washed successively with a 1M aqueous $KHSO_4$ solution, a 10% aqueous $NaHCO_3$ solution, brine and finally dried ($MgSO_4$). The solvent was evaporated *in vacuo*. The residue was purified by column chromatography (EtOAc 100%), giving **27** as an oil (2.7g, 82%). TLC (EtOAc 100%): Rf=0.43. ^{1}H NMR ($CDCl_3$) δ 7.25-7.00 (1H, bs); 6.00-5.70 (2H, m); 5.70-3.35 (1H, bs); 5.30-5.05 (4H, m); 4.60-4.40 (4H, m); 3.95 (2H, s); 3.80 (2H, s); 3.70 (3H, s); 3.50-3.10 (8H, m); 1.40 (9H, s). ^{13}C NMR ($CDCl_3$) δ 171.5; 156.0; 155.9; 133.0; 117.5; 79.0; 66.4; 52.4; 52.0; 49.0; 39.2; 38.8; 28.4. MS (ESI+) m/z 501.2 $(M+H)^+$, m/z 523.2 $(M+Na)^+$.

TFA. H-[Alloc-Alloc]-OMe (28)

The TFA salt **28** was obtained from compound **27** (2.65g, 5.3mmol) following the procedure for the preparation of **26**. Triturating of the residue in Et_2O yielded **28** as an amorphous solid (2.45g, 90%). TLC (AcOEt/MeOH 1:1, v:v): Rf=0.30. HPLC (A/B 80:20 to 0:100 over 20min): Rt=8.1min. MS (ESI+) m/z 401.2 $(M)^+$.

Boc-[Alloc]$_3$-OMe (29)

Compound **29** was obtained from **25** (2.0g, 6.62mmol) and **28** (3.4g, 6.62mmol) following the above procedure for **27**. Column chromatography (EtOAc/MeOH 9:1, v:v) gave **29** as an oil (3.3g, 73%). TLC (AcOEt 100%): Rf=0.16. ^{1}H NMR ($CDCl_3$) δ 8.00, 7.60 (1H, bs); 7.35-7.00 (1H, bs); 5.80-5.40 (4H, m); 5.15-4.75 (6H, m); 3.50 (3H, s); 3.35-2.85 (12H, m); 1.40 (9H, s). ^{13}C NMR ($CDCl_3$) δ 171.0; 170.6; 169.6; 156.1; 132.8; 132.7; 132.5; 117.8; 117.3; 78.9; 66.4; 52.3; 51.7; 49.5; 39.2; 38.2; 28.4. MS (ESI+) m/z 685.3 $(M+H)^+$, m/z 707.4 $(M+Na)^+$.

Boc-[H]$_3$-OMe (30)

Compound **30** was obtained from **29** (1.0g, 1.46mmol), following the above procedure for **22**. After 30 min, the solvent was evaporated under reduced pressure. The residue was taken up in water, the aqueous layer was washed with EtOAc and evaporated *in vacuo*. The triamine **30** was obtained as an oil in quantitative yield (631mg). ^{1}H NMR ($CDCl_3$) δ 7.85 (1H, t); 7.70 (1H, t); 5.80 (1H, t); 3.70 (3H, s); 3.50-3.00 (10H, m); 2.90-2.60 (8H, m); 1.40 (9H, s). MS (ESI+) m/z 433.2 $(M+H)^+$, m/z 455.3 $(M+Na)^+$.

Boc-[C$^{(Z)}$C$^{(Z)}$C$^{(Z)}$]-OMe (31)

A solution of **30** (500mg, 1.157mmol), N^4-Z cytosine acetic acid **4c** (1.16g, 3.82mmol), TEA (0,65ml, 4.63mmol) in CH_2Cl_2 (5ml) was stirred at 0°C. Brop (1.5g, 3.82mmol) was then added. After 2 hours stirring, the mixture was diluted with chloroform (20ml). The organic layer was washed successively with a 1M aqueous $KHSO_4$ solution, a 10% aqueous $NaHCO_3$ solution, brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography (AcOEt/MeOH 1:1, v:v). **31** was obtained as an amorphous powder (1.1g, 70%). TLC (AcOEt/MeOH 1:1, v:v): Rf=0.51. ^{1}H NMR ($CDCl_3$) (two isomers) δ 7.90-7.45 (5H, m); 7.45-7.05 (18H, m); 6.20, *6.05* (1H, t); *5.30*, 5.00 (6H, s); *4.65*, 4.55 (6H, s); 4.30, *4.05* (4H, s); 3.85-3.05 (15H, m); 1.40 (9H, s). ^{13}C NMR ($CDCl_3$) δ 172.2; 169.1; 169.1; 167.4; 166.9; 163.0; 156.1; 155.3; 155.0; 152.4; 152.4; 150.2; 135.4; 128.6; 128.2; 127.7; 95.7; 79.3; 66.5; 52.3; 52.0; 51.3; 49.8; 48.7; 47.9; 47.6; 39.2; 38.8; 36.6; 28.4. MS (ESI+) m/z 1288.2 $(M+H)^+$, m/z 1310.2 $(M+Na)^+$. Anal. calcd for $C_{60}H_{69}N_{15}O_{18}$: C 55.94, H 5.40, N 16.30. Found: C 55.71, H 5.38, N 16.28.

TFA.H-[C$^{(Z)}$C$^{(Z)}$C$^{(Z)}$]-OMe (32)

32 was obtained as an amorphous solid (960mg, 96%) from **31** (1.0g, 0.78mmol) following the procedure described for the preparation of **26**. MS (ESI+) m/z 1301.3 $(M+H)^+$.

N-[2-N-Boc aminoethyl]-N-[Fmoc]glycine (Boc-[Fmoc]-OH) (33)

To an ice-cooled solution of **23** (574.0mg, 2.47mmol) in THF (3ml) was added 1M aqueous NaOH (5.0ml). After 1 hour stirring, a solution of 9-fluorenylmethyl chloroformate (830.4mg, 3.21mmol) in THF (4ml) was slowly added at 0°C. The mixture was stirred for 30 min, and acidified with a 1M aqueous $KHSO_4$ solution until pH 6. The product was then extracted with EtOAc. The organic layers were washed with water, dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude residue was purified by column chromatography (Hexane/EtOAc 7:3, v:v to EtOAc 100%) to yield **33** (971.0mg, 90%) as an amorphous solid. TLC (AcOEt/MeOH 8:2, v:v): Rf=0.31. ^{1}H NMR (DMSO d_6) δ 8.05-7.30 (8H, m); 7.10 (1H, t); 4.40-4.10 (3H, m); *3.85*, 3.60 (2H, s); 3.35 (2H, q); 3.10 (2H, t); 1.40 (9H, s); ^{13}C NMR (DMSO d_6) (two isomers) δ 171.0, *170.4*; 156.1, *156.0*; 155.9, *155.5*; 144.1, 144.0; 141.5; 141.4; 127.9; 127.3; 125.3; 120.0; 79.0; 66.4; 50.0; 49.9; 49.1; 49.0; 47.4; 40.0; 28.5. MS (ESI-) m/z 439.2 $(M-H)^-$.

TFA. H-[Alloc]$_3$-OMe (34)

34 was obtained (484mg, 95%) from **29** (500mg, 0.73mmol) following the above procedure for the preparation of **26**. HPLC (A/B 80:20 to 0:100 over 30min): Rt=11.9min, λ_{max}=204.8 nm. MS (ESI+) m/z 585.3 $(M)^+$.

Boc-[Fmoc-(Alloc)$_3$]-OMe (35)

Compound **35** was obtained as an amorphous solid (326mg, 72%) from **33** (200mg, 0.45mmol) and **34** (314mg, 0.45mmol), following the procedure described for the preparation of **29**. ^{1}H NMR ($CDCl_3$) δ 8.50-8.10 (1H, bs); 7.95-7.75 (1H, bs); 7.75-7.00 (8H, m); 6.00-5.65 (3H, m); 5.65-5.50 (1H, bs); 5.30-5.00 (6H, m); 4.65-4.40 (6H, m); 4.40-4.25 (2H, m); 4.25-4.10 (1H, bs); 4.05-3.05 (8H, m); 3.65 (3H, s); 3.55-2.95 (16H, m). ^{13}C

($CDCl_3$) δ 173.7; 170.5; 169.6; 156.6; 156.2; 144.1; 141.4; 132.8; 132.5; 127.8; 127.3; 125.2; 120.1; 118.0; 79.1; 67.9; 66.6; 52.5; 52.0; 49.8; 49.0; 47.4; 39.3; 38.5; 28.6. MS (ESI+) m/z 1007.5 $(M+H)^+$, m/z 1029.6 $(M+Na)^+$. Anal. calcd for $C_{49}H_{66}N_8O_{15}$: C 58.44, H 6.61, N 11.13. Found: C 58.76, H 6.47, N 10.92.

Boc[H-(Alloc)$_3$]-OMe (36)

To a solution of **35** (300mg, 0.298mmol) in CH_2Cl_2 (2ml) was added DEA (596µl, 4.47mmol). After stirring for 30 min, the solvent was evaporated *in vacuo*. The residue was taken up in a 1M aqueous $KHSO_4$ solution and washed with EtOAc. The aqueous layer was neutralised with a 10% aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$. Concentration under reduced pressure gave the corresponding amine **36** (192mg, 82%), which was employed in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 8.50-7.80 (2H, m); 7.60-7.20 (1H, bs); 6.00-5.60 (4H, m); 5.35-5.00 (6H, m); 4.55 (6H, s); 4.00 (2H, s); 3.80 (4H, s); 3.70 (3H, s); 3.40 (12H, s); 3.25-3.00 (4H, m); 2.75-2.50 (2H, s); 1.40 (9H, s). ^{13}C NMR ($CDCl_3$) δ 172.3; 170.0; 156.4; 156.134; 132.714; 132.5; 177.9; 117.7; 117.4; 79.0; 66.6; 66.4; 52.5; 51.9; 49.6; 49.0; 52.4; 40.8; 38.3; 37.6; 28.5. MS (ESI+) m/z 785.4 $(M+H)^+$, m/z 807.4 $(M+Na)^+$.

Boc-[U-(Alloc)$_3$]-OCH$_3$ (37)

Compound **36** (200mg, 0.255mmol), uracil acetic acid **4d** (47.6mg, 0.280mmol) and TEA (88.8µl, 0.637mmol) were placed at 0°C in CH_2Cl_2 (3ml). Brop (108.7mg, 0.280mmol) was then added. The mixture was stirred for 2 hours at rt, and CH_2Cl_2 (10ml) was added. The organic layer was then washed successively with a 1M aqueous $KHSO_4$ solution, a 10% aqueous $NaHCO_3$ solution, brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue purified by column chromatography (EtOAc/MeOH 8:2 to 1:1, v:v) to give **37** as an amorphous solid (203mg, 85%). TLC (AcOEt/MeOH 8:2, v:v): Rf=0.34. ^{1}H NMR ($CDCl_3$) δ 8.50-7.30 (3H, m); 7.20 (1H, d); 6.00 (1H, bs); 5.95-5.70 (3H, m); 5.60 (1H, d); 5.30-5.00 (6H, m); 4.70-4.30 (8H, m); 4.10-3.75 (8H, m); 3.70 (3H, s); 3.55-3.20 (16H, s); 1.40 (9H, s). ^{13}C NMR ($CDCl_3$) δ 173.7; 170.9; 170.3; 168.5; 167.6; 164.3; 156.3; 156.0; 151.4; 146.0; 132.7; 132.5; 117.5; 117.1; 101.7; 79.3; 66.4; 66.2; 51.4; 50.2; 48.42; 48.3; 38.1; 28.4. MS (ESI+) m/z 937.4 $(M+H)^+$, m/z 959.5 $(M+Na)^+$.

Boc-[U-(H)$_3$]-OMe (38)

Compound **38** was obtained as an amorphous solid (109mg, 100%) from **37** (150mg, 0.16mmol), following the procedure described for the preparation of **30**. ^{1}H NMR ($CDCl_3$) δ 7.75-7.35 (3H, m); 7.15 (1H, d); 6.10 (1H, bs); 5.60 (1H, d); 4.55, 4.45 (2H, s); 4.10, 3.90 (2H, s); 3.70 (3H, s); 3.60-3.00 (16H, m); 2.80-2.60 (6H, m); 1.50 (9H,s). ^{13}C ($CDCl_3$) δ 173.0; 172.6; 169.1; 168.0; 164.8; 156.5; 151.9; 145.9; 102.2; 79.6; 52.3; 51.9; 50.2; 49.1; 48.6; 48.2; 38.9; 36.9; 28.6. MS (ESI+) m/z 685.3 $(M+H)^+$, m/z 686.3 $(M+2H)^+$, m/z 687.3 $(M+3H)^+$, m/z 707.3 $(M+Na)^+$, m/z 708.3 $(M+1+Na)^+$, m/z 709.3 $(M+2+Na)^+$.

Boc-[UC$^{(Z)}$C$^{(Z)}$C$^{(Z)}$]-OMe (39)

A solution of **38** (100mg, 0.146mmol), N^4-Z cytosine acetic acid **4c** (132.8mg, 0.438mmol), TEA (81.4µl, 0.584mmol) in CH_2Cl_2 (5ml) was stirred at 0°C. Brop (170mg, 0.438mmol) was then added. After 2 hours stirring, the mixture was diluted with chloroform (10ml). The organic layer was washed successively with a 1M aqueous $KHSO_4$ solution, a 10% aqueous $NaHCO_3$ solution, brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue triturated in EtOAc to give **38** as an amorphous powder (170mg, 75%). TLC (AcOEt/MeOH 1:1, v:v): Rf=0.28. HPLC (A/B 80:20 to 30:70 over 30min): Rt=23.7min, λ_{max}=210.0 nm. ^{1}H NMR (DMSO d_6) δ 10.80 (3H, bs); 8.10-7.70 (4H, m); 7.35 (15H, s); 7.15-6.80 (3H, m); 5.55 (1H, d); 5.15

(6H, s); 5.00-3.70 (16H, m); 3.65 (3H, s); 3.65-3.00 (16H, m); 1.40 (9H, s). ^{13}C NMR (DMSO d_6) (two isomers) δ 169.9, *169.7*; 168.9; 168.7; 167.8; *167.3*, 166.3; 163.9; 163.3; *155.8*, 155.7; *155.3*, 154.0; 153.3; 150.9; 150.4; 146.3; 136.0; 128.5; 128.2; 128.0; 100.8; 94.0; *78.1*, 77.8; 66.5; 52.3, *51.9*, 50.0; 48.8; 48.4; 47.8, *47.2*; 37.5; 36.8; 35.8; 28.2. MS (ESI+) m/z 1562.9 $(M+Na)^+$. Anal. calcd for $C_{70}H_{81}N_{19}O_{22}$: C 54.58, H 5.30, N 17.28. Found: C 54.20, H 5.07, N 17.50.

Boc-[UC$^{(Z)}$C$^{(Z)}$C$^{(Z)}$]-OH (40)

The methyl ester **39** (200mg, 0.13mmol) was dissolved in dioxane (4ml) and of 1M aqueous LiOH (0.78ml) was added at 0°C. The solution was stirred until disappearance of the starting material on HPLC. The pH was subsequently adjusted to 5 using a 1M aqueous HCl solution. The mixture was then evaporated to dryness. The residue was triturated in water and filtration afforded **40** (140mg, 70%) as an white amorphous solid. TLC (MeOH/AcOH 99:1, v:v): R=0.22. HPLC (A/B 80:20 to 30 :70 over 30min): Rt=22.7min, λ_{max} =214.8 nm. MS (ESI-) m/z 1524.6 $(M-H)^-$.

N-ε-Boc-ε-Ahx-[UC$^{(Z)}$C$^{(Z)}$C$^{(Z)}$]-OMe (41)

i) To a cooled solution (0°C) of dimer **7dc** (87mg, 0.10mmol), dimer **6cc** (110mg, 0.12mmol) and TEA (56µl, 0.40mmol) in CH_2Cl_2 (0.3ml) was added Bop reagent (44.2mg, 0.10mmol). The reaction was stirred for 2 hours at rt. The mixture was diluted with CH_2Cl_2, washed successively with a 1M aqueous $KHSO_4$ solution, a 10% aqueous $NaHCO_3$ solution, brine, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography (MeOH 100%). **41** was obtained as an amorphous solid (116mg, 70%). TLC (MeOH/H_2O/AcOH 50:49:1, v:v:v): Rf=0.20. HPLC (A/B 80:20 to 30:70 over 30min): Rt=24.5min, λ_{max}=207.0 nm. MS (ESI+) m/z 1676.2 $(M+Na)^+$. Anal. calcd for $C_{76}H_{92}N_{20}O_{23}$: C 55.20, H 5.61, N 16.94. Found: C 55.66, H 5.41, N 16.62.

ii) **41** was prepared (230mg, 70%) starting from monomer **1d** (97mg, 0.2mmol) and trimer **32** (260mg, 0.2mmol), following the same procedure than above.

Boc-[UC$^{(Z)}$C$^{(Z)}$C$^{(Z)}$A$^{(Z)}$G$^{(OAll)}$]-OMe (42)

Hexamer **42** was obtained (126mg, 60%) from **40** (140.5g, 0.092mmol) and **6bf** (90mg, 0.102mmol), following the procedure described for the preparation of **29**. HPLC (A/B 80:20 to 30.70 over 30min): Rt=23.6min, λ_{max} = 206.4 nm. MS (ESI+) m/z 2281.5 $(M+H)^+$. Anal. calcd for $C_{103}H_{117}N_{33}O_{29}$: C 54.23, H 5.17, N 20.26. Found: C 54.64, H 4.99, N 20.01.

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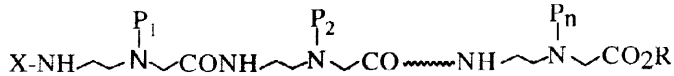
12. Compound **33** was best obtained by alkaline hydrolysis of **23** then *in situ* protection with Fmoc-Cl rather than by the alkaline hydrolysis of the methyl ester of **33** (under these conditions, we observed the unexpected Fmoc cleavage).

13. The designation X-[P_1 P_2..P_n]-OR is used for polyamide compounds of type :



$P_{1\text{-}n}$ can be : the Fmoc, Alloc or Troc protecting group, an hydrogen atom or a nucleic base acetic acid unit.