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Synthesis of (3*R*,6*R*)- and (3*S*,6*R*)-piperidinone PNA

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Two new conformationally restricted piperidinone PNA adenine monomers **12** and **13** have been synthesised using a stereoselective synthesis strategy analogous to a previously published strategy for pyrrolidinone analogues. In contrast to the pyrrolidinone case, epimerisation occurred during the final hydrolysis step. However, the diastereomeric mixture could be separated by RP-HPLC to give small amounts of pure **12** and **13**. These were built into a PNA dodecamer (once in a central position), and the thermal stability (T_m) of the modified oligomers hybridised to complementary DNA, RNA and PNA were measured. PNA modified with either **12** or **13** resulted in a decrease of the T_m compared to unmodified PNA and to pyrrolidinone modified PNA. Thus, any preorganisation for duplex formation of PNA with a six-membered piperidinone ring seems to be inferior to preorganisation with a five-membered ring in the pyrrolidinone PNA analogues studied earlier.

Introduction

Peptide nucleic acid (PNA) is an acyclic, pseudopeptide mimic of natural nucleic acids.[1] Only the nucleobases are retained, linked together by an achiral, uncharged amide backbone (Fig. 1). PNA is an excellent structural mimic of DNA and RNA; it binds tightly and with high sequence specificity to complementary oligonucleotides.[2a–g] PNA–DNA or PNA–RNA duplex formation is accompanied by a decrease in entropy.[2e] This entropy loss might be reduced by using a more rigid PNA analogue as has been attempted by using a variety of conformationally constrained chiral backbones.[3a–f] We have recently described pyrrolidinone PNA (pyr-PNA, Fig. 1) in which atoms of PNA are constrained by the introduction of a methylene bridge (see Fig. 1).[4] This bridge prevents rotation around the C–N bond of the amide unit connected to the base residue and

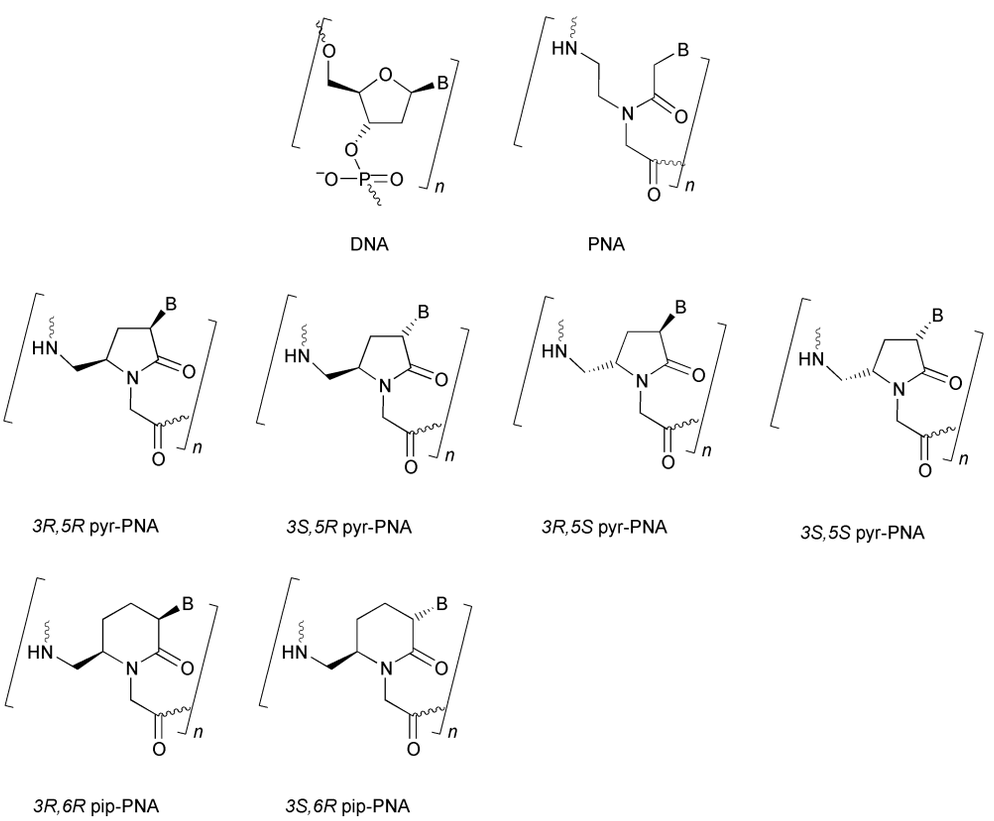

Fig. 1

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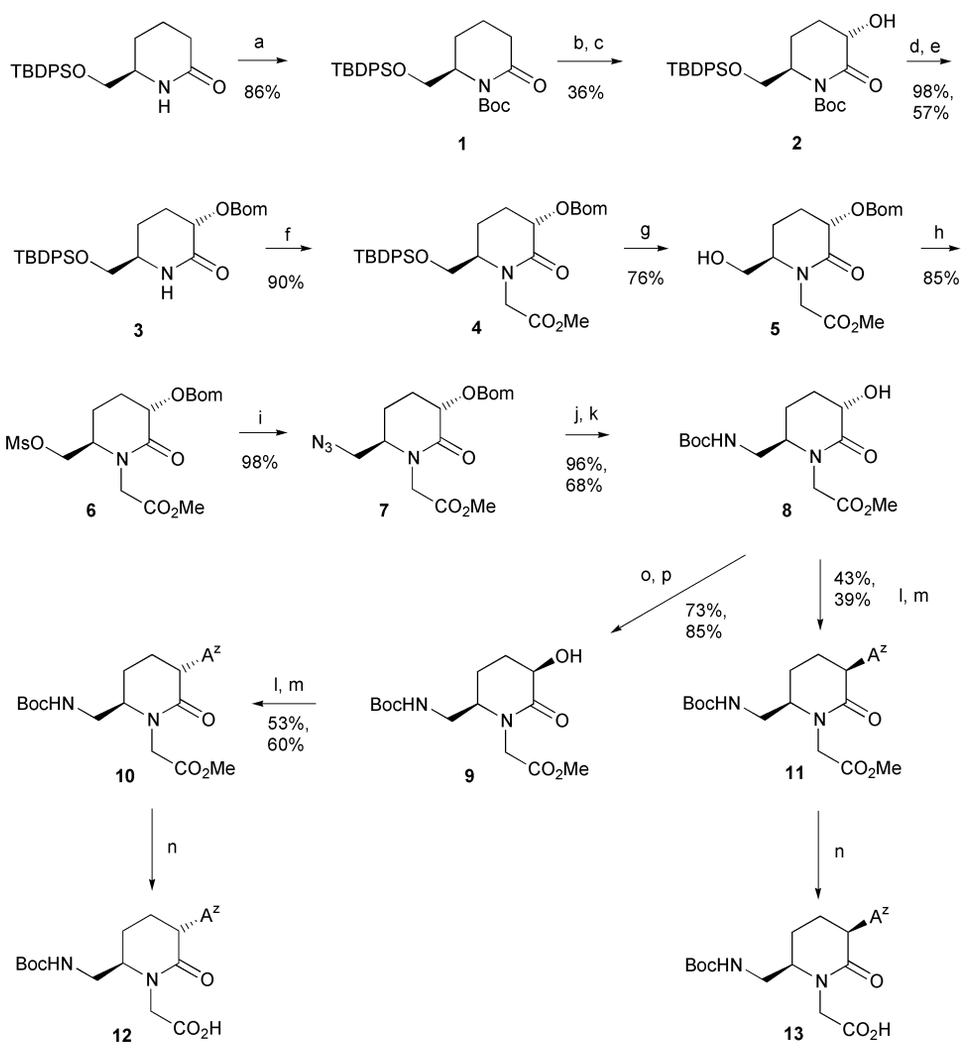
pre-organises PNA in the rotameric conformation prevailing in PNA–DNA, PNA–RNA and PNA–PNA duplexes as well as PNA_2–DNA triplexes.[5a–d] The results from the pyr-PNA study, which involved only adenine monomers, showed that PNA oligomers containing the (3*S*,5*R*) isomer had the highest affinity towards RNA. However, the affinity was slightly decreased (ΔT_m per modification −1 to −3 °C) compared to unmodified PNA.[4] This decrease in affinity could well be the result of unfavourable constraints of the PNA in terms of duplex formation caused by the introduction of the five-membered pyrrolidinone ring. Indeed, model building seemed to suggest that a six-membered piperidinone PNA monomer (pip-PNA, Fig. 1) could better adjust to a duplex structure. We now present our results on the syntheses and properties of two such monomers: [(3*R*,6*R*)-pip-PNA and (3*S*,6*R*)-pip-PNA (Fig. 1, B = adenin-9-yl)].

Results and discussion

Synthesis of the (3*S*,6*R*)- and (3*R*,6*R*)-monomer esters 10 and 11

The adenine monomers were prepared as outlined in Scheme 1. The starting material, (6*R*)-6-(*tert*-butyldiphenylsilyloxymethyl)piperidin-2-one,[6] was Boc protected at nitrogen to give **1**. Diastereoselective hydroxylation of **1** was carried out as described for the 5-membered analogue,[4] giving a 95 : 5 mixture of diastereoisomers. The major isomer was assigned as the (3*S*)-**2** (*trans*) isomer by analogy with the outcome for the corresponding 5-membered analogue[4] and the known predominance of formation of the *trans*-isomer (*trans* : *cis* ratio 14 : 1) when the enolate of **1** is alkylated with Me_3SnCH_2I.[7] Protection of the hydroxy group as its benzyloxymethyl (Bom) ether was followed by removal of the Boc group to give **3**. The lactam nitrogen was alkylated and the silyl protecting group was removed with $Et_3N{\cdot}3HF$ in THF to produce reasonably pure **5**. This hydroxy ester was immediately mesylated to **6** as it is unstable due to lactone formation. Conversion into the azide **7** was followed by hydrogenation to the amine which was Boc protected in a one-pot procedure using 10% Pd/C as the catalyst. The Bom protecting group was stable to this treatment but was removed by hydrogenation using Pearlman's catalyst to give **8**. Under Mitsunobu conditions, the configuration at C-3 was inverted to yield the pure diastereomer **9**. The content of **8** in **9** was less than 1% according to ^{13}C NMR where the region of the strong Boc CH_3 signal of **9** (δ_C 27.9) was devoid of the corresponding signal of **8** (δ_C 27.5). Apparently, the minor diastereomer carried over from **2** had been removed by one or several of the column chromatography steps employed in the transformation of **5** to **9**. The hydroxy groups in **8** and **9** were substituted with adenine under Mitsunobu conditions. ^{13}C NMR of these intermediates strongly indicated that the correct N^9 isomers had formed. The exocyclic amine in the nucleobases were Z-protected using Rapoport's reagent[8] to give **10** and **11**, respectively. According to RP-HPLC, **10** was pure, but some



Scheme 1 Synthesis of (3*S*,6*R*)-**12** and the (3*R*,6*R*)-**13** pip-PNA A monomers. a) Boc_2O, DMAP, CH_3CN; b) HMDS, BuLi, THF; c) MoOPH; d) Bom chloride, DIEA, CH_2Cl_2; e) TFA–CH_2Cl_2 (1 : 1); f) NaH, $BrCH_2CO_2CH_3$, THF; g) $(C_2H_5)_3N{\cdot}3HF$, THF; h) MsCl, pyridine; i) NaN_3, DMF; j) H_2, 10% Pd/C, Boc_2O, AcOEt; k) H_2, 10% $Pd(OH)_2$/C, MeOH; l) adenine, PPh_3, DEAD, dioxane; m) *N*-benzyloxycarbonyl-*N*′-methylimidazolium triflate, CH_2Cl_2; n) LiOH, THF, then HCl; o) $PhCO_2H$, PPh_3, DEAD, dioxane; p) NaOMe, MeOH.

impurities from the Mitsunobu reagents were present in **11**. Both compounds were diastereomerically pure according to ^{1}H NMR.

Synthesis of the (3*S*,6*R*)- and (3*R*,6*R*) monomer acids 12 and 13

The methyl esters **10** and **11** were hydrolysed using standard conditions for the hydrolysis of PNA monomer methyl esters: LiOH in aq. THF at 0 °C. After 2 min, TLC revealed one new spot in each case corresponding to the hydrolysis product, but unexpectedly two spots after 60 min when the hydrolyses were complete. The two spots had identical R_f values whether **10** or **11** was hydrolysed, and each corresponded to one of the single spots seen after 2 min. Apparently the esters or the acid salts epimerise at C-3 under the hydrolysis conditions, although epimerisation did not occur during hydrolysis of the five-membered ring pyr-PNA analogues.[4] Attemps to purify the diastereomers by repeated column chromatography on silica failed to give pure products, probably because they epimerised slowly on the column, but small amounts of pure **12** (6 mg) and **13** (2 mg) were finally obtained after preparative RP-HPLC. The proof of **12** being the (3*S*,6*R*)- and **13** the (3*R*,6*R*)-isomer rests on TLC evidence. Thus, **12** had the same R_f value as the new spot which appeared after 2 min hydrolysis of **10**, and **13** corresponded to the new spot appearing after 2 min hydrolysis of **11**.

Oligomer synthesis

The monomers (3*S*,6*R*)-pip-PNA **12** and (3*R*,6*R*)-pip-PNA **13** were incorporated into the middle position (italic) of the 12-mer PNA sequence H-TAC-TC*A*-TAC-TCT-LysNH_2 using standard PNA coupling procedures,[9] and the oligomers were purified to high homogeneity by RP-HPLC. Some epimerisation may have occurred during solid phase synthesis, but the CD spectra of the PNA–PNA duplexes were markedly different, so extensive epimerisation can be ruled out. The same sequence with the five-membered analogues (3*R*,5*R*)-pyr-PNA and (3*S*,5*R*)-pyr-PNA incorporated were prepared for comparison.

Thermal stability

The T_m's of the PNA–RNA, PNA–DNA and PNA–PNA duplexes are given in Table 1. Both pip-PNA modified 12-mers (entry 2 and 3) hybridised to RNA, but with a large decrease in T_m compared to the unmodified PNA (entry 1). Contrary to the (3*S*,5*R*) pyr-PNA modified sequence (entry 5), which was bound better to RNA than its diastereomers both in the present (compare with entry 4) and in the previously published cases,[4] the (3*S*,6*R*) pip-PNA **12** modified sequence (entry 2) did not bind significantly better to RNA than its diastereomer (entry 3). The data for binding to DNA are difficult to interpret because two transitions occurred in most cases, but the modification clearly decreased the binding to DNA. Binding to PNA is compromised as well, albeit somewhat less pronounced for both pip-PNA modified sequences.

Conclusion

Two new conformationally restricted piperidinone PNA adenine monomers **12** and **13** have been synthesised and incorporated into a PNA dodecamer (once in a central position). Modifying PNA with either **12** or **13** resulted in a large decrease in duplex stability with RNA (ΔT_m 10–11.5 °C) as well as with DNA, and a smaller decrease with PNA. In particular, **12** with the similar (3*S*,6*R*) configuration to that of the best of the pyr-PNA [(3*S*,5*R*) configuration] bound much less efficiently to RNA. Therefore, any expected preorganisation of the PNA single strand induced by these cyclic pip-PNA analogues thus seems to be inferior to the preorganisation by the cyclic pyr-PNA analogues studied earlier in terms of producing a hybridisation-competent conformation. However, it cannot be excluded that PNA oligomers composed exclusively or predominantly of pip-PNA hybridise better than the mixed backbone oligomers **12** and **13** studied here. Therefore, more data in terms of sequence context and backbone context (number and position of pip-PNA modifications in an otherwise unmodified PNA "background") are required to fully evaluate the properties of pip-PNA. Nonetheless, the present results stress that only a narrow ensemble of hybridisation competent PNA backbone conformations are available for potent RNA and/or DNA binding.

Table 1 Melting temperatures (T_m/°C) of PNA–RNA, PNA–DNA and PNA–PNA duplexes[a,b]

		T_m		
Entry	Monomer incorporated	RNA	DNA	PNA
1	Unmodified PNA	59.0	(23)[c] + 49.5	75.5
2	(3*S*,6*R*)-pip-PNA **12**	47.5	24.5 + (36.5)	69.0
3	(3*S*,6*R*)-pip-PNA **13**	49.0	30.5	68.5
4	(3*S*,5*R*)-pyr-PNA	44.0	24 + (38)	65.5
5	(3*S*,5*R*)-pyr-PNA	54.0	(25) + 41	69.5

[a] Measured in aqueous buffer containing 100 mM NaCl, 10 mM phosphate, 0.1 mM EDTA, pH 7.0; heating rate: 1 K min^{-1}. UV absorbance measured at 260 nm. [b] The PNA sequence was H-TACTC*A*TACTCT-LysNH_2, with the monomer at the italic position. The complementary sequences were 5′-d(AGAGTATGAGTA), 5′-AGAGUAUGAGUA, or H-AGAGTATGAGTA-NH_2 for DNA, RNA and PNA, respectively. [c] Transition with low hyperchromicity in brackets.

Experimental

General

Benzyl chloromethyl ether,[10] (6*R*)-6-[(*tert*-butyldiphenylsilyl)-oxymethyl]piperidin-2-one,[6] oxodiperoxymolybdenum(pyridine)hexamethylphosphoric triamide (MoOPH),[11] and *N*-benzyloxycarbonyl-*N*′-methylimidazolium triflate[12] were prepared according to literature procedures. All other reagents were purchased from Sigma-Aldrich and used without purification. Solvents were HPLC-grade from LAB-SCAN. Acetonitrile, *N*,*N*-dimethylformamide, benzene, toluene, dioxane, methylene chloride and pyridine were dried over 4 Å molecular sieves. Tetrahydrofuran was dried by distillation from sodium–benzophenone. TLC was run on Merck 5554 silica 60 aluminium sheets. Column chromatography was performed as flash chromatography on Merck 9385 silica gel 60 (0.040–0.063 mm). Reactions were carried out under nitrogen except in the case of hydrogenations. FABMS were recorded in the positive ion mode. Elemental analyses were performed at the Microanalytical Laboratory, Department of Chemistry, University of Copenhagen. NMR spectra were obtained on a 300 or 400 MHz spectrometer. δ-Values are in ppm relative to DMSO-d_6 (2.50 for proton and 39.5 for carbon) or $CDCl_3$ (7.29 for proton and 76.9 for carbon) or CD_3OD (3.35 for proton and 49.1 for carbon). RP-HPLC was run on a Waters 486 HPLC system with diode array detector, abs. 260 nm, Waters 19 × 300 mm C-18 column, eluents 0.1% aq. TFA (buffer A) and CH_3CN–H_2O 9 : 1 v/v (buffer B).

(6*R*)-*N*-*tert*-Butoxycarbonyl-6-[(*tert*-butyldiphenylsilyl)-oxymethyl]piperidin-2-one (1)

Boc_2O (3.3 g, 15.0 mmol) and then DMAP (61 mg, 0.5 mmol) were added to a stirred solution of (6*R*)-6-[(*tert*-butyldiphenylsilyl)oxymethyl]piperidin-2-one[6] (3.68 g, 10.0 mmol) in CH_3CN (14 ml) at rt. The solution was stirred at rt overnight.

More Boc_2O (3.3 g, 15.0 mmol) was added and the solution again stirred overnight. The reaction mixture was quenched by the addition of 10% aq. citric acid (25 ml) and CH_2Cl_2 (50 ml). The aqueous phase was extracted with CH_2Cl_2 (2 × 50 ml). The organic phases were combined, washed with brine (25 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by chromatography (a stepwise gradient of AcOEt in hexane from 1 : 4 to 1 : 0 v/v). Yield: 4.02 g of **1** as a white solid (86%), mp 74–75 °C, R_f 0.80 (AcOEt). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.66–7.39 (10H, m, Ph), 4.18 (1H, m, H-6), 3.78 (1H, m, $SiOCH_A$), 3.62 (1H, m, $SiOCH_B$), 2.48–2.29 (2H, m, H-3), 2.02–1.89 (3H, m, H-4 + H-5), 1.65 (1H, m, H-4 or H-5), 1.38 (9H, s, Boc), 0.97 (9H, s, Bu^{t}Si). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 171.2, 152.5, 135.1, 135.0, 132.5, 132.3, 129.9, 127.9, 127.8, 81.7, 64.5, 55.7, 34.4, 27.5, 26.5, 24,5. 18.7, 17.6 (Found: C, 69.4; H, 8.1; N, 2.9. Calc. for $C_{27}H_{37}NO_4Si$: C, 69.3; H, 8.0; N, 3.0%).

(3*S*,6*R*)-*N*-*tert*-Butoxycarbonyl-6-[(*tert*-butyldiphenylsilyl)-oxymethyl]-3-hydroxypiperidin-2-one (2)

BuLi (10.2 ml, 25.5 mmol, 2.5 M in hexane) was added dropwise to a solution of hexamethyldisilazane (5.39 ml, 25.5 mmol) in THF (20 ml) at −78 °C. The solution was stirred at −78 °C for 30 min. A solution of **1** (3.99 g, 8.51 mmol) in THF (20 ml) was added over a period of 5 min with stirring. The temperature of the reaction mixture was slowly raised from −78 to −40 °C during the next 60 min, before MoOPH (7.39 g, 17.0 mmol) was added in two portions. The green solution was stirred between −40 and −30 °C for 30 min and the reaction was then quenched by the addition of half-saturated aq. NH_4Cl (40 ml). The THF was evaporated off and the aqueous phase was extracted with AcOEt (3 × 80 ml). The organic phases were combined, washed with brine (80 ml), dried (Na_2SO_4) and evaporated *in vacuo* to give 6.7 g of a crude product which was purified by chromatography (a stepwise gradient of AcOEt–hexane from 2 : 3 to 1 : 0 v/v). Yield 1.46 g (36%) of **2** as a slightly yellow solid, mp 80 °C, R_f 0.44 (AcOEt–heptane 2 : 3 v/v). The product was contaminated with 5% of the (3*R*,6*R*)-isomer as judged by ^{1}H-NMR. ^{1}H-NMR (300 MHz, DMSO-d_6) [data for the minor isomer are given in brackets]: δ 7.61–7.39 (10 H, m, Ph), 5.57 (1H, d, *J* 4.4, OH), [5.43 (1H, d, *J* 3.3, OH)], 4.19 (1H, m, H-3 or H-6), 4.00 (1H, m, H-3 or H-6), 3.73 (1H, m, $SiOCH_A$), 3.57 (1H, m, $SiOCH_B$), 2.12 (2H, m, H-4 or H-5), 1.98 (1H, m, H-4 or H-5), 1.63 (1H, m, H-4 or H-5), 1.37 (9H, s, Boc), 0.98 (9H, s, Bu^{t}Si). ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ 173.4, 152.6, 135.0, 132.4, 132.2, 129.9, 127.9, 82.0, 68.4, 64.6, 56.0, 27.5, 27.0, 26.5, 21.2, 18.7 (Found: C, 66.6; H, 7.8; N, 2.8. Calc. for $C_{27}H_{37}NO_5Si$: C, 67.05; H, 7.7; N, 2.9%).

(3*S*,6*R*)-3-Benzyloxymethoxy-6-[(*tert*-butyldiphenylsilyl)-oxymethyl]piperidin-2-one (3)

Compound **2** (1.45 g, 3.01 mmol) was dried by co-evaporation from CH_3CN–CH_2Cl_2 1 : 1 v/v and then redissolved in CH_2Cl_2 (7 ml). Bom-Cl (1.25 ml, 9.0 mmol) and then diisopropylethylamine (DIEA) (1.6 ml, 9.0 mmol) were added at 0 °C. The solution was stirred at rt overnight. More CH_2Cl_2 (50 ml) was added and the solution was extracted with half-saturated aq. NH_4Cl (2 × 25 ml). The organic phase was washed with brine (25 ml) and evaporated *in vacuo*. Purification by chromatography (AcOEt–hexane 2 : 3 v/v) afforded the Bom-protected intermediate as a clear oil. Yield 1.78 g (98%), pure on TLC, R_f 0.71 (AcOEt–hexane 2 : 1 v/v). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.63–7.56 (4H, m, Ph), 7.49–7.38 (6H, m, Ph), 7.34–7.27 (5H, m, Ph), 4.84 (2H, m, OCH_2O), 4.58 (2H, s, CH_2-Ph), 4.18 (1H, m, H-3 or H-6), 4.14 (1H, m, H-3 or H-6), 3.76 (1H, m, $SiOCH_A$), 3.59 (1H, m, $SiOCH_B$), 2.21–2.07 (2H, m, H-4 or H-5), 1.91 (1H, m, H-4 or H-5), 1.75 (1H, m, H-4 or H-5), 1.37 (9H, s, Boc), 0.97 (9H, s, Bu^{t}Si). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 170.7, 152.4, 138.0, 135.1, 132.4, 132.3, 130.0, 128.3, 128.2, 128.0, 127.9, 127.7, 127.5, 93.5, 82.2, 72.7, 69.2, 64.9, 55.8, 27.5, 26.6, 25.2, 21.2, 18.7. FABMS *m*/*z* 603.9 (M + H). This purified intermediate (1.76 g, 2.92 mmol) was dissolved in CH_2Cl_2 (2.9 ml). TFA (2.9 ml) was added dropwise at 0 °C during 1 min and the solution was stirred for 8 min. The reaction was quenched by the slow addition of saturated aq. $NaHCO_3$ (40 ml). The aqueous phase was extracted with CH_2Cl_2 (50 ml) and AcOEt (2 × 50 ml). The combined organic phases were dried ($MgSO_4$) and evaporated *in vacuo*. Purification by chromatography (AcOEt–hexane 2 : 1 v/v) afforded 0.87 g (57%) of **3** a clear oil which was pure on TLC, R_f 0.41 (AcOEt–hexane 2 : 1 v/v). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.63–7.60 (4H, m, Ph), 7.49–7.41 (6H, m, Ph), 7.36–7.27 (6H, m, Ph and NH), 4.91 (1H, d, *J* 6.8, OCH_AO), 4.83 (1H, d, *J* 6.8, OCH_BO), 4.59 (2H, s, CH_2-Ph), 3.95 (1H, m, H-3), 3.62 (1H, m, $SiOCH_A$), 3.53–3.46 (2H, m, $SiOCH_B$ and H-6), 2.02–1.98 (2H, m, H-4 or H-5), 1.68–1.65 (2H, m, H-4 or H-5), 1.00 (9H, s, Bu^{t}Si). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 170.5, 138.2, 135.1, 132.9, 132.8, 129.9, 128.3, 128.0, 127.7, 127.5, 94.0, 71.2, 68.9, 66.1, 53.1, 26.7, 26.3, 22.4, 18.9. FABMS *m*/*z* 503.8 (M + H).

(3*S*,6*R*)-3-Benzyloxymethoxy-6-[(*tert*-butyldiphenylsilyl)-oxymethyl]-*N*-[methoxycarbonylmethyl]piperidin-2-one (4)

Compound **3** (2.40 g, 4.81 mmol) was dissolved in THF (25 ml). NaH (60% suspension in mineral oil, 0.39 g, 9.62 mmol) and then methyl bromoacetate (0.93 ml, 9.62 mmol) were added at 0 °C and the reaction mixture stirred overnight at rt. The reaction mixture was quenched by the slow addition of half-saturated aq. NH_4Cl (50 ml) at 0 °C and extracted twice with AcOEt (2 × 50 ml). The combined organic phases were washed with brine (50 ml), dried (Na_2SO_4) and evaporated *in vacuo* to give the crude product as an oil which was purified by chromatography (AcOEt–hexane 2 : 1 v/v). Yield: 2.50 g (90%) of **4** as a clear oil, pure on TLC, R_f 0.67 (AcOEt). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.61–7.57 (4 H, m, Ph), 7.48–7.41 (6H, m, Ph), 7.34–7.28 (5H, m, Ph), 4.93 (1H, d, *J* 6.6, OCH_AO), 4.80 (1H, d, *J* 6.6, OCH_BO), 4.58 (2H, m, CH_2-Ph), 4.11 (1H, d, *J* 17.0, NCH_ACO), 4.05 (1H, m, H-3), 3.84 (1H, d, *J* 17.0, NCH_BCO), 3.65 (3H, m, H-6 and $SiOCH_2$), 3.58 (3H, s, CH_3), 2.00 (2H, m, H-4 or H-5), 1.82 (1H, m, H-4 or H-5), 1.71 (1H, m, H-4 or H-5), 0.98 (9H, s, Bu^{t}Si). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 169.9, 169.4, 138.2, 135.1, 132.6, 132.4, 130.1, 130.0, 128.2, 128.0, 128.0, 127.7, 127.4, 93.7, 70.6, 68.9, 64.5, 58.4, 51.7, 46.7, 26.6, 25.2, 21.2, 18.7. FABMS *m*/*z* 576.0 (M + H).

(3*S*,6*R*)-3-Benzyloxymethoxy-6-hydroxymethyl-*N*-methoxycarbonylmethylpiperidin-2-one (5)

Compound **4** (2.50 g, 4.34 mmol) was dried by co-evaporation from CH_3CN–CH_2Cl_2 1 : 1 v/v and then redissolved in THF (22 ml). $Et_3N{\cdot}3HF$ (2.75 ml, 17 mmol) was slowly added and the reaction was stirred at 50 °C for 3 h. The solvent was evaporated off and the crude product purified by chromatography (a stepwise gradient of 5–10% MeOH in CH_2Cl_2). Yield 1.11 g (76%) of **5** as a clear oil, TLC, R_f 0.60 (minor), R_f 0.54 (major) (CH_2Cl_2–MeOH 9 : 1 v/v). The product was contaminated with approx. 10% of the lactone of **5** as judged by ^{1}H-NMR. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.37–7.28 (5H, m, Ph), 4.95 (1H, d, *J* 6.6, OCH_AO), 4.86–4.80 (2H, m, OCH_BO and OH), 4.60 (2H, m, CH_2-Ph), 4.21 (1H, d, *J* 14.1, NCH_ACO), 4.04–3.98 (2H, m, NCH_BCO and H-3), 3.63 (3H, s, CH_3), 3.50–3.42 (3H, m, OCH_2 and H-6), 2.06–1.96 (2H, m, H-4 or H-5), 1.77–1.68 (2H, m, H-4 or H-5). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 169.9, 169.8, 138.2, 128.7, 127.7, 127.5, 93.7, 70.8, 68.9, 62.2, 58.8, 51.7, 46.8, 25.3, 21.3. FABMS *m*/*z* 338.1 (M + H).

(3*S*,6*R*)-3-Benzyloxymethoxy-*N*-methoxycarbonylmethyl-6-(methylsulfonyloxymethyl)piperidin-2-one (6)

Compound **5** (1.68 g, 4.98 mmol) was dried by evaporation from CH_3CN–CH_2Cl_2 1 : 1 v/v and then redissolved in CH_2Cl_2 (23 ml). Et_3N (0.96 ml, 6.9 mmol) and methanesulfonyl chloride (0.97 g, 8.5 mmol) were added at 0 °C. The reaction mixture was stirred at 0 °C for 45 min and then quenched by addition of half-saturated aq. $NaHCO_3$ (50 ml) and CH_2Cl_2 (75 ml). The aqueous phase was extracted with CH_2Cl_2 (75 ml) and the combined organic phases evaporated. The crude product was purified by chromatography (2% MeOH in CH_2Cl_2) to give 1.74 g (85%) of **6** as a clear oil, pure on TLC, R_f 0.26 (MeOH-CH_2Cl_2 2 : 98 v/v). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.38–7.28 (5H, m, Ph), 5.11 (1H, d, *J* 7.0, OCH_AO), 4.90 (1H, d, *J* 7.0, OCH_BO), 4.69 (2H, s, CH_2-Ph), 4.32–4.09 (5H, m, H-3, N-CH_2 and MsO-CH_2), 3.82 (1H, m, H-6), 3.77 (3H, s, O-CH_3), 3.06 (3H, s, S-CH_3), 2.27 (1H, m, H-4 or H-5), 2.30 (1H, m, H-4 or H-5), 1.95 (1H, m, H-4 or H-5), 1.85 (1H, m, H-4 or H-5). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 170.2, 169.4, 137.6, 128.3, 127.8, 127.6, 94.2, 70.4, 69.8, 68.9, 56.9, 52.2, 47.8, 37.5, 25.1, 21.6. FABMS *m*/*z* 416.0 (M + H).

(3*S*,6*R*)-6-Azidomethyl-3-benzyloxymethoxy-*N*-methoxycarbonylmethylpiperidin-2-one (7)

Compound **6** (1.70 g, 4.10 mmol) was dissolved in DMF (21 ml) and NaN_3 (1.33 g, 20.5 mmol) was added. The solution was stirred at 80 °C overnight. The solvent was evaporated off and the resulting oil partitioned between half-saturated aq. $NaHCO_3$ (50 ml) and AcOEt (75 ml). The aqueous phase was extracted with AcOEt (2 × 75 ml). The combined organic phases were washed with brine, dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by chromatography (a stepwise gradient of 2–10% MeOH in CH_2Cl_2) to give 1.45 g (98%) of **7** as an oil, pure on TLC, R_f 0.47 (AcOEt–hexane 4 : 1 v/v). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.37–7.28 (5H, m, Ph), 4.93 (1H, d, *J* 6.6, OCH_AO), 4.80 (1H, d, *J* 6.6, OCH_BO), 4.60 (2H, m, CH_2-Ph), 4.16 (1H, d, *J* 17.2, NCH_A), 4.06 (1H, m, H-3), 4.02 (1H, d, *J* 17.2, NCH_B), 3.64 (3H, s, CH_3), 3.63–3.54 (3H, m, H-6 and N_3CH_2), 2.07–2.05 (2H, m, H-4 or H-5), 1.74–1.71 (2H, m, H-4 or H-5). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 169.9, 169.5, 138.2, 128.3, 127.7, 127.5, 93.7, 70.6, 69.0, 56.4, 52.0, 51.8, 46.8, 24.9, 21.9. FABMS *m*/*z* 363.2 (M + H).

(3*S*,6*R*)-6-*tert*-Butoxycarbonylaminomethyl-3-hydroxy-*N*-methoxycarbonylmethylpiperidin-2-one (8)

10% Pd/C (0.27 g) was added to a stirred solution of **7** (1.45 g, 4.0 mmol) and Boc_2O (1.74 g, 8.0 mmol) in AcOEt (40 ml) at 0 °C. The mixture was hydrogenated at 1 atm for 90 min at rt, and then passed through Celite. The solvent was evaporated off and the crude product (2.64 g) was purified by chromatography (AcOEt) to give 1.67 g (96%) of the Bom protected intermediate, pure on TLC, R_f 0.44 (AcOEt). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.37–7.27 (5H, m, Ph), 6.92 (1H, t, *J* 6.0, NH), 4.94 (1H, d, *J* 6.6, OCH_AO), 4.80 (1H, d, *J* 6.6, OCH_BO), 4.59 (2H, m, CH_2-Ph), 4.14 (1H, d, *J* 17.0, NCH_ACO), 4.02 (1H, m, H3), 3.90 (1H, d, *J* 17.0, NCH_BCO), 3.64 (3H, s, CH_3), 3.41 (1H, m, H-6), 3.10 (2H, m, BocN-CH_2), 2.00 (2H, m, H-4 or H-5), 1.68 (2H, m, H-4 or H-5), 1.37 (9H, s, Boc). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 169.7, 169.6, 155.9, 138.1, 128.2, 127.7, 127.5, 93.6, 78.0, 70.4, 68.9, 57.3, 51.8, 46.9, 41.5, 28.2, 24.8, 21.4. FABMS *m*/*z* 437.2 (M + H). This purified intermediate (1.67 g, 3.82 mmol) was dissolved in MeOH (57 ml), 10% $Pd(OH)_2$/C (0.22 g) was added and the mixture was hydrogenated overnight at 1 atm and rt and then passed through Celite. The solution was evaporated and the crude product (1.0 g) was purified by chromatography (a stepwise gradient of 5–10% MeOH in CH_2Cl_2). Yield 0.83 g of **8** as a white oil (68%), pure on TLC, R_f 0.32 (CH_2Cl_2–MeOH 9 : 1 v/v). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.88 (1H, t, *J* 5.6, BocNH), 5.08 (1H, d, *J* 3.7, OH), 4.15 (1H, d, *J* 17.3, NCH_ACO), 3.91 (1H, d, *J* 17.3, NCH_BCO), 3.83 (1H, m, H-3), 3.64 (3H, s, CH_3), 3.42 (1H, m, H-6), 3.14–3.07 (2H, m, BocN-CH_2), 2.02–1.94 (2H, m, H-4 or H-5), 1.68–1.42 (2H, m, H-4 or H-5), 1.37 (9H, s, Boc). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 173.7, 169.4, 155.6, 78.5, 66.7, 57.9, 51.5, 46.3, 41.7, 27.5, 26.1, 22.0. FABMS *m*/*z* 317.1 (M + H).

(3*R*,6*R*)-6-*tert*-Butoxycarbonylaminomethyl-3-hydroxy-*N*-methoxycarbonylmethylpiperidin-2-one (9)

Compound **8** (315 mg, 1.0 mmol) was dried by evaporation from CH_3CN (5 ml) and then redissolved in THF (5 ml). A solution of PPh_3 (786 mg, 3.0 mmol) in THF (2 ml) and a solution of benzoic acid (610 mg, 5.0 mmol) in toluene (10 ml) were successively added at 0 °C, and the mixture stirred for 2 min, before DEAD (0.79 ml, 5.0 mmol) was added dropwise. The clear yellow solution was allowed to warm to rt and stirred overnight. AcOEt (100 ml) was added and the mixture was extracted with 10% aq. citric acid (25 ml), brine (25 ml), half-saturated aq. $NaHCO_3$ (25 ml), and brine (25 ml). The organic phase was dried ($MgSO_4$) and evaporated. The crude product was purified by chromatography (AcOEt–hexane 2 : 1 v/v, then pure AcOEt). Yield 305 mg (73%) of the intermediate as a white foam, pure on TLC, R_f 0.25 (AcOEt–hexane 2 : 1 v/v). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.98 (2H, d, *J* 6.9, Ph), 7.47 (1H, m, Ph), 7.35 (2H, m, Ph), 5.65 (1H, t, *J* 5.7, BocNH), 5.36 (1H, m, H-3), 4.04 (2H, m, NCH_2CO), 3.64 (3H, s, CH_3), 3.44 (2H, m, BocN-CH_2), 3.16 (1H, m, H-6), 2.30–1.90 (4H, m, H-4 and H-5), 1.36 (9H, s, Boc). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 169.3, 167.6, 165.2, 155.9, 132.8, 129.4, 129.2, 127.9, 79.0, 69.0, 57.6, 51.8, 48.0, 41.6, 27.9, 22.8, 22.1. This intermediate (305 mg, 0.73 mmol) was dissolved in MeOH (5.3 ml) and cooled to 0 °C. NaOMe in methanol (1.0 M, 1.4 ml, 1.4 mmol) was added dropwise and the solution was stirred at 0 °C for 3 h. The reaction was quenched by addition of half-saturated aq. NH_4Cl (20 ml). The aqueous phase was extracted with AcOEt (4 × 40 ml). The organic phases were combined, dried over $MgSO_4$ and evaporated *in vacuo*. Chromatography (AcOEt, then CH_2Cl_2–MeOH 9 : 1 v/v) afforded **9** as a hygroscopic foam, yield: 195 mg (85%), pure on TLC, R_f 0.30 (CH_2Cl_2–MeOH 9 : 1 v/v). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.52 (1H, br s, BocNH), 4.04 (1H, d, *J* 17.2, NCH_ACO), 3.94–3.90 (2H, m, NCH_BCO and H-3), 3.61 (3H, s, CH_3), 3.37 (1H, m, BocN-CH_2 or H-6), 3.29 (1H, m, BocN-CH_2 or H-6), 2.98 (1H, m, BocN-CH_2 or H-6), 1.97 (1H, m, H-4 or H-5), 1.88 (2H, m, H-4 or H-5), 1.81 (1H, m, H-4 or H-5), 1.30 (9H, s, Boc). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 172.9, 169.3, 155.8, 79.2, 67.7, 57.6, 51.9, 47.9, 42.1, 27.9, 24.1, 22.2. FABMS *m*/*z* 317.2 (M + H).

(3*S*,6*R*)-3-[N^6-(Benzyloxycarbonyl)adenin-9-yl]-6-*tert*-butoxycarbonylaminomethyl-*N*-methoxycarbonylmethylpiperidin-2-one (10)

Compound **9** (369 mg, 1.17 mmol) was dried by co-evaporation from dry CH_3CN and redissolved in dry dioxane (24 ml). PPh_3 (0.660 g, 2.92 mmol) was added followed by adenine (0.789 g, 5.84 mmol). To this stirred suspension was slowly (during 20 min) added DEAD (0.36 ml, 2.29 mmol) at rt, and the suspension stirred at rt overnight. The solvent was evaporated off and the residue purified by chromatography (AcOEt then CH_2Cl_2–MeOH 9 : 1 v/v) to give an intermediate, yield 267 mg (53%), pure on TLC, R_f 0.33 (CH_2Cl_2–MeOH 9 : 1 v/v). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 169.7, 167.9, 156.2, 155.5, 152.3, 149.4, 139.7, 118.5, 79.4, 58.1, 53.9, 52.1, 49.6, 47.3, 42.1, 28.0, 25.6, 23.4. FABMS *m*/*z* 434.2 (M + H). The intermediate (245 mg, 0.57 mmol) was dissolved in dry CH_2Cl_2 (3.8 ml) and *N*-benzyloxycarbonyl-*N*′-methylimidazolium triflate (0.621 g, 1.70 mmol) was added, followed by stirring at rt overnight. Half-saturated aq. $NaHCO_3$ (25 ml) and CH_2Cl_2 (50 ml) were

added and the aqueous phase extracted with CH_2Cl_2 (50 ml) and AcOEt (50 ml).The combined organic phases were dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by chromatography (AcOEt then AcOEt–MeOH 9 : 1 v/v) to give 191 mg (60%) of **10** as a white solid, pure on TLC, R_f 0.52 (CH_2Cl_2–MeOH 9 : 1 v/v), pure on RP-HPLC (RT 21.2 min). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.0 (1H, br s, ZNH), 8.62 (1H, s, H-8-adenine), 7.91 (1H, s, H-2-adenine), 7.30–7.19 (5H, m, Ph), 5.71 (1H, br s, BocNH), 5.18 (2H, m, OCH_2-Ph), 5.00 (1H, m, H-3), 4.13 (1H, d, *J* 17.2, NCH_ACO), 3.98 (1H, d, *J* 17.2, NCH_BCO), 3.61 (3H, s, CH_3), 3.58 (1H, m, BocN-CH_2 or H-6), 3.41 (1H, m, BocN-CH_2 or H-6), 3.11 (1H, m, BocN-CH_2 or H-6), 2.30 (1H, m, H-4 or H-5), 2.07 (2H, m, H-4 or H-5), 1.88 (1H, m, H-4 or H-5), 1.38 (9H, s, Boc). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 169.6, 167.4, 156.1, 152.0, 151.2, 149.2, 142.6, 135.2, 128.1, 128.0, 127.9, 121.4, 78.4, 67.1, 58.3, 54.2, 52.1, 47.3, 42.0, 28.0, 25.4, 23.4. FABMS *m*/*z* 568.0 (M + H).

(3*R*,6*R*)-3-[N^6-(Benzyloxycarbonyl)adenin-9-yl]-6-*tert*-butoxycarbonylaminomethyl-*N*-methoxycarbonylmethylpiperidin-2-one (11)

Compound **11** was prepared in a similar way to **10**. Starting from **8** (234 mg, 0.74 mmol), the intermediate was obtained in 137 mg (43%) yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.22 (1H, s, H-8-adenine), 7.81 (1H, s, H-2-adenine), 6.75 (2H, s, NH_2), 6.44 (1H, BocNH), 5.03 (1H, m, H-3), 4.23 (1H, d, *J* 17.1, NCH_ACO), 4.01 (1H, d, *J* 17.1, NCH_BCO), 3.64 (3H, s, CH_3), 3.60–3.35 (3H, m, NCH_2 and H-6), 2.72 (1H, m, H-4 or H-5), 2.20–2.03 (3H, m, H-4 and H-5), 1.37 (9H, s, Boc). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 169.2, 166.7, 156.1, 155.6, 152.4, 149.2, 140.3, 119.1, 79.4, 57.2, 54.4, 52.0, 48.5, 42.2, 28.1, 24.0, 23.9. FABMS *m*/*z* 434.1 (M + H). This intermediate (130 mg, 0.30 mmol) gave 66 mg (39%) of **11** as a white solid, R_f 0.52 (CH_2Cl_2–MeOH 9 : 1 v/v), 85% pure on RP-HPLC (RT 21.8 min). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 9.50 (1H, br s, Z-NH), 8.70 (1H, s, H-8-adenine), 7.95 (1H, s, H-2-adenine), 7.40–7.30 (5H, m, Ph), 5.91 (1H, br s, BocNH), 5.26 (2H, s, CH_2-Z), 4.95 (1H, m, H-3), 4.20 (1H, d, *J* 17.1, NCH_ACO), 4.05 (1H, d, *J* 17.1, NCH_BCO), 3.68 (3H, s, CH_3), 3.62–3.42 (3H, m, N-CH_2 and H-6), 2.79 (1H, m, H-4 or H-5), 2.15 (3H, m, H-4 or H-5), 1.41 (9H, s, Boc). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 169.2, 166.2, 156.0, 152.2, 151.0, 149.3, 143.0, 135.3, 128.4, 128.3, 128.1, 121.8, 79.6, 67.3, 57.5, 54.8, 54.8, 52.1, 48.7, 42.2, 28.1, 23.9. FABMS *m*/*z* 568.1 (M + H).

(3*S*,6*R*)-3-[N^6-(Benzyloxycarbonyl)adenin-9-yl]-6-*tert*-butoxycarbonylaminomethyl-*N*-carboxymethylpiperidin-2-one (12)

LiOH (1 M, 0.82 ml, 0.82 mmol) was slowly added to a stirred solution of **10** (185 mg, 0.326 mmol) in THF (3.4 ml) at 0 °C, and the reaction was stirred at 0 °C. A small sample was removed after 2 min for TLC and acidified. TLC ($CHCl_3$–MeOH–HOAc 85 : 10 : 5), R_f 0.36 (product) and 0.74 (**10**). After 45 min H_2O (1 ml) was added and the THF was evaporated off. The product was precipitated by the slow addition of 2 M HCl (0.5 ml) at 0 °C. The white precipitate was spun down in a centrifuge tube and the supernatant removed. The white pellet was washed twice with H_2O (2 × 4 ml) and dried *in vacuo* to give the crude product (140 mg). TLC ($CHCl_3$–MeOH–HOAc 85 : 10 : 5 v/v/v) showed two spots of similar intensity, R_f 0.36 and 0.27. Attempts to purify the crude product by chromatography (CH_2Cl_2–MeOH–HOAc 85 : 10 : 5) failed, but preparative RP-HPLC purification gave **12** as a colourless solid, pure on TLC, R_f 0.36 ($CHCl_3$–MeOH–HOAc 85 : 10 : 5), R_f 0.53 ($CHCl_3$–EtOH–HOAc 80 : 15 : 5 v/v/v). Compound **12** was dried under high vacuum for two days, yield 6.1 mg (3%). FABMS *m*/*z* 554.2 (M + H). Electrospray MS: *m*/*z* 554.46 (M + H) (Found: C, 49.9; H, 4.9; N, 14.9. Calc. for $C_{26}H_{31}N_7O_7$·TFA: C, 50.4; H, 4.8; N, 14.7%).

(3*R*,6*R*)-3-[N^6-(Benzyloxycarbonyl)adenin-9-yl]-6-*tert*-butoxycarbonylaminomethyl-*N*-carboxymethylpiperidin-2-one (13)

Compound **13** was prepared in a similar way to **12**. From **11** (66 mg, 0.12 mmol) the crude product (50 mg) showed on TLC ($CHCl_3$–MeOH–HOAc 85 : 10 : 5 v/v/v) two spots of similar intensity, R_f 0.36 and 0.27. Preparative RP-HPLC gave **13** as a colourless solid, pure on TLC, R_f 0.27 ($CHCl_3$–MeOH–HOAc 85 : 10 : 5), R_f 0.36 ($CHCl_3$–EtOH–HOAc 80 : 15 : 5 v/v/v). Compound **13** was dried under high vacuum for two days, yield 2 mg (3%). HR FABMS *m*/*z* 554.2363 (M + H calc. 554.2363). ^{1}H-NMR (300 MHz, CD_3OD): δ 8.71 (1H, s, H-adenine), 8.61 (1H, s, H-adenine), 7.52 (2H, m, Ph), 7.42 (3H, m, Ph), 5.48 (1H, m, H-3), 5.40 (2H, s, CH_2-Ph), 4.40 (1H, d, *J* 17, NCH_ACO), 4.08 (1H, d, *J* 17, NCH_BCO), 3.69 (1H, m, H-6), 3.58 (2H, m, BocN-CH_2), 2.95 (1H, m, H-4 or H-5), 2.32 (2H, m, H-4 or H-5), 2.23 (1H, m, H-4 or H-5), 1.49 (9H, s, Boc).

Oligomer synthesis

H-TAC-TC(12)-TAC-TCT-LysNH$_2$. H-TAC-TCT-Lys-MBHA resin (24 mg, loading 0.12 mmol g^{-1}, dried in a desiccator) was transferred to an Eppendorf tube. Compound **12**·TFA (3.0 mg, 4.5 μmol), HBTU (2 mg, 5.3 μmol), and DIEA (4 μl, 23 μmol) were dissolved in DMF (92 μl) and, after preactivation for 2 min, added to the dry resin. Coupling was allowed to proceed overnight after which the resin and the coupling mixture were transferred (in DMF) to a glass reactor and the oligomerisation continued by the standard procedure. All Kaiser tests after couplings were yellow (indicating complete reaction). Cleavage and deprotection was carried out with TFA–trifluoromethanesulfonic acid–thioanisole–*m*–cresol 300 : 100 : 50 : 50 μl for 2 h at rt. The product was precipitated several times with ether and finally purified by RP-HPLC. Yield 1.1 mg, 88% pure on RP-HPLC, MALDI-TOF: 3332.8 (calc. 3333).

H-TAC-TC(13)-TAC-TCT-LysNH$_2$. This was prepared as above from **13** (1.5 mg). Yield 0.5 mg, pure on RP-HPLC, MALDI-TOF: 3332.0 (calc. 3333).

H-TAC-TC(3*R*,5*R* *pyr*-PNA)-TAC-TCT-LysNH$_2$. This was prepared as above from 3.0 mg of modified monomer. Yield: 1.0 mg, 93.4% pure on RP-HPLC. MALDI-TOF: 3321.8 (calc. 3319).

H-TAC-TC(3*S*,5*R* *pyr*-PNA)-TAC-TCT-LysNH$_2$. This was prepared as above from 3.0 mg of modified monomer. Yield: 1.3 mg, 93.2% pure on RP-HPLC. MALDI-TOF: 3321.8 (calc. 3319).

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