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Cyclic peptide-nucleotide hybrids (cPNH) with phosphoramidate bonds

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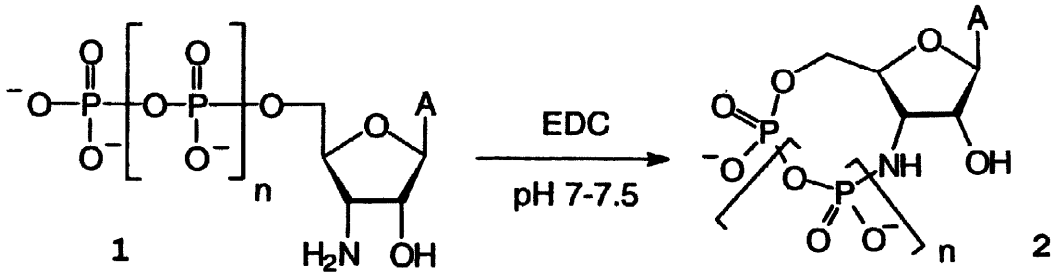
Abstract: The potential of the generally applicable intramolecular condensation of 3'-N-aminoacyl/peptidyl-5'-nucleotides with the water soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, for the production of cyclic peptide-nucleotide-hybrids has been extensively explored.

Keywords: Cyclisation; Nucleotides; Peptides and polypeptides; Phosphoramidates

Introduction

Recently we have shown that mono-[1,2], di- and triphosphates **1a-c** of 3'-amino-3'-deoxyadenosine **3a**[3] can be reacted with the water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), to give the cyclic phosphoramidates (3'-amido-3'-deoxyadenosine-3',5'-cyclo (mono-,di-,tri-) phosphates, **2a-c**) in good yield.[4]



	1	n	2
a	3'-NH_2-AMP	0	3'-NH-cAMP
b	3'-NH_2-ADP	1	3'-NH-cADP
c	3'-NH_2-ATP	2	3'-NH-cATP

As a continuation of this work we have considered ways of changing the size of the ring between the 3'- and 5'-positions of the nucleotide through the incorporation of amino acids and peptides as well as further phosphate groups. Here we report a number of interesting results. Initially, protected 3'-N-aminoacyl-, as well as peptidyl-nucleosides, were synthesized by known methods starting either from 3'-amino-3'-deoxy-nucleosides, that are found in nature for example in nucleosidic antibiotics[3] (lysyl aminoadenosine, homocitrullylaminoadenosine, puromycine), or from the 3'-N-peptidylnucleosides that originate from **3a**, 3'-amino-3'-deoxyguanosine **3b** [5] as well as 3'-amino-3'-deoxythymidine **3c**.[6] During our study we found that the N-hydroxysuccinimide ester (OSu-Ester) of N-Boc (Boc = t-butoxycarbonyl) or Z- (Z = benzyloxycarbonyl) protected amino acids or peptides in DMF was preferred for the conversion of the aminonucleosides **3a-c**.[7]

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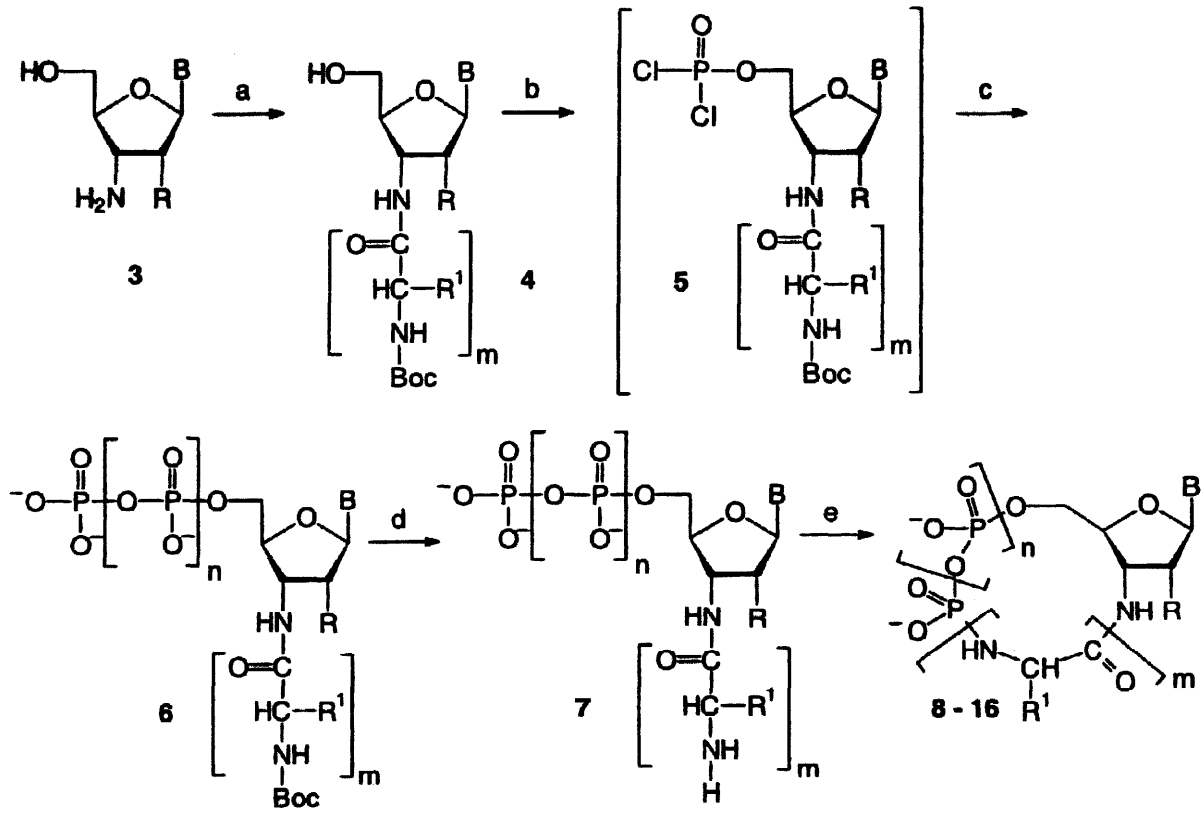


Reaction conditions: a) BocHN-CH(R^1)OSu, Et_3N, DMF, 80-95%. b) $POCl_3$, $OP(OEt)_3$, 70-95%. c) $(Bu_3NH)_2$ $H_2P_2O_7$ / Bu_3N / DMF, O°C, 5 min; 1M $(Et_3NH)HCO_3$-buffer (pH 7.5); IC: DEAE-Sephadex (HCO_3^-), H_2O-$(Et_3NH)HCO_3$-buffer-gradient (pH 7.5), 40-60%. d) Boc-deprotection: TFA, 5 min, room temp.; Z-deprotection: H_2 (Pd/A); IC see c, 65-75%. e) EDC, pH 7-7.5, IC see c, 60-80%

Cyclic peptide-nucleotide hybrids. NMR data in tables 1 and 2.

Comp. No.	Base	R	R^1	m	n
8a-d	A	OH	H	1-3, 5	2
9	A	OH	H	5	1
10a, b	A	OH	H	3, 5	0
11a, b	A	OH	CH_2Ph	1, 2	2
12a-e*	A	OH	H; CH_2Ph; H	1	0-4
13	A	OH	$(CH_2)_4NH_2$	3	2
14	A	OH	CH_2Ph	1	methylene bisphosphonate
15	G	OH	H	1	2
16a, b§	T	H	H	1	2, 4

***12f-h** are the dimer, trimer and tetramers of **12a**, respectively. **§ 16c** is the γ-$POCH_3$ open-chain derivative of **16a**.

Results and discussion

Derivatives **4** were converted to the protected 3'-N-aminoacyl/peptidyl-3'-deoxynucleoside 5'-phosphorodichloridate intermediates **5** with phosphoryl chloride in triethyl phosphate (TEP) according to Kusashio and Yoshikawa.[8] Subsequent reaction with bis-(tri-n-butylammonium) diphosphoric acid following Ludwig's method[9] afforded good yields of 3'-N(AA-Boc/Z)-NTP (AA = amino acid, NTP = nucleoside triphosphate) **6** (n = 2), 3'-N-(AA-Boc/Z)-NMP **6** (n = 0) (hydrolysis product from **5**, NMP = nucleoside monophosphate) as well as a byproduct of 3'-(AA-Boc/Z)-NDP (NDP = nucleoside diphosphate) **6** (n = 1). After ion-exchange chromatography (IC) on DEAE-Sephadex A-25 (HCO_3^--form, water-triethylammonium hydrogen carbonate buffer-gradient, pH 7.5), cleavage of the N-Boc-protecting groups with trifluoroacetic acid (TFA) or the N-Z-protecting groups by hydrogenolysis and a further IC, the derivatives 7 were converted in good yield to the corresponding cyclic 3'-N-aminoacyl/peptidyl-nucleoside phosphoramidates **8 - 16** with EDC (5-10 fold excess, nucleotide concentration 1-2 mg mL^{-1}) at pH 7.0-7.5. After purification by IC the triethylammonium salts were converted to the sodium salts by ion exchange with Dowex-Na^+.

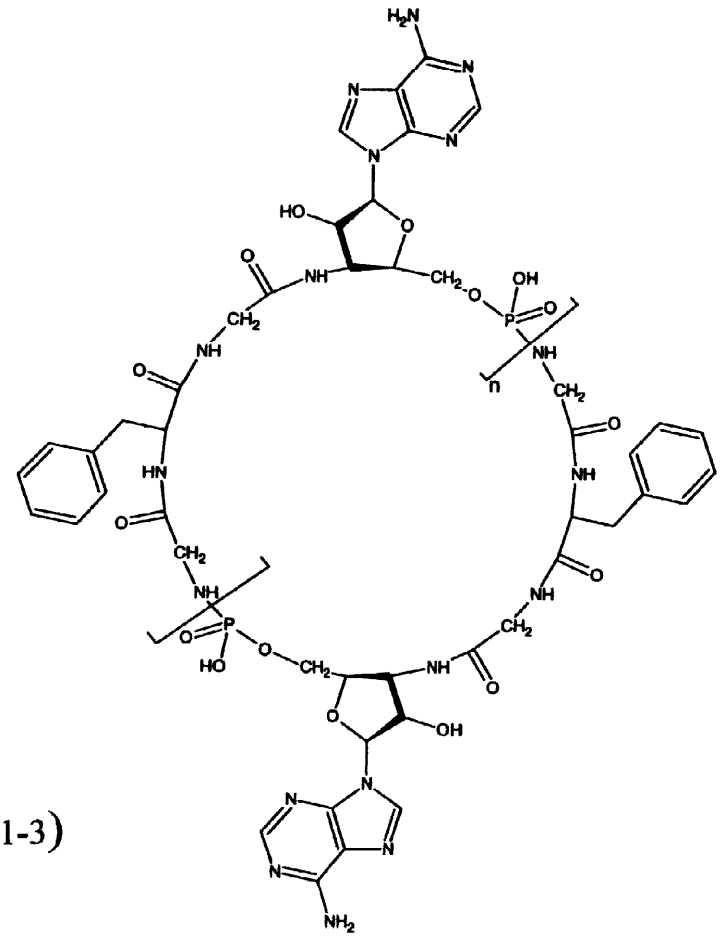
The procedure outlined above offers a large number of synthetic possibilities through variation of the base and ribose moieties, the phosphate substituent (mono/poly) at the 5'-position and the amino acid or peptide at the 3'-position. For instance a peptide library containing the 20 natural amino acids in a tetrapeptide would contain 160 000 (20^4) peptides. Hence the cyclisation method is demonstrated initially with a number of selected examples in which glycine or polyglycine (m = 2,3,5) are used to avoid the possibility of racemisation upon coupling of optically active amino acids at the 3'-NH-CO bond as well as at the P-N bond.

The synthetic strategy was used to produce a homologous series of monoglycine (3'-N-Gly-cATP, **8a**) to pentagylcine derivates (3'-N-Gly_5-cATP, **8d**) in which the 3',5'-positions of the ribose were joined to give 13-(**8a**), 16-(**8b**), 19-(**8c**) and 25-membered (**8d**) ring systems. The synthesis of the decaglycine derivative failed at the phosphorylation step of the 3'-N-Gly_{10}-N-Boc-A-derivative as the compound is insoluble in TEP and the phosphorylation did not proceed in the heterogeneous phase with ultrasonic mixing. Cyclisation of the monophosphate derivatives (7, n = 0) afforded, depending on the reaction conditions (concentration, temperature), the cyclic monophosphoramidate together with cyclic oligomers (dimers, trimers and tetramers) that could be detected by ESI-MS and are of the form shown in the scheme below. Exemplary data for this type of compound are shown for the monomer and dimer of **12a** (3'-N-Gly-Phe-Gly-cAMP) in the tables.

In some cases the cyclic tetra- and pentaphosphoramidates (eg. **12d-e**) could be produced in addition to the cyclic mono-, di- and tri-derivatives. These arose from the presence of tetra- and pentaphosphates of 3'-N-Gly-Phe-Gly-A in the mono- and diphosphate mixture, which could not be separated by IC. Presumably these were produced from reaction of the reactive 5'-cyclic metatriphosphate in the preceeding reaction with phosphate and pyrophosphate. Similar condensation reactions in pyridine and DCC have been described by Smith and Khorana.[10] A further interesting compound was one in which the three negative charges of the cyclotriphosphate ring were neutralised by the incorporation of three lysine residues. The zwitterionic compound, 3'-N-Lys_3-cATP **13** was formed through reaction of **3a** consecutively with Boc-Lys(Z)-OSu to give the Z-protected 3'-N-[$Lys(Z)]_3$-cATP. Catalytic removal of the Z-protecting groups [hydrogen; palladium activated charcoal, (Pd/A)] afforded a good

yield of 3'-N-Lys_3-cATP, **13**, which could be eluted from DEAE-Sephadex with water.

7 (n = 0; m = GlyPheGly) ➡



12 f-h (n = 1-3)

Derivatisation of the 5'-substituted phosphomoiety was demonstrated through reaction of **4** ($R^1 = CH_2C_6H_5$) after tosylation of the 5'-position with p-tosylchloride in pyridine and 4-dimethylaminopyridine (DMAP) followed by nucleophilic exchanged of the tosyl group with tris-(tetra-n-butylammonium)methylenebisphosphonate according to Poulter et al.[11] Cleavage of the Boc-protecting group, cyclisation and IC gave a good yield of 3'-N-Phe-3'-deoxy-3',5'-cyclomethylene bisphosphonate **14**. The synthetic strategy was shown to be applicable to other nucleobases by the use of 3'-amino-3'-deoxyguanosine, **3b**, derivatives with 3'-N-glycyl in the 3'-position (AA = glycine) as well as a derivative of 3'-amino-3'-deoxythymidine, **3c** (AA = glycine). Cleavage of the nucleobase from 3'-AdA (**3a**) after trifluoroacetylation, acetylation and acetolysis yielded 1,2,5-tri-O-acetyl-3-trifluoroacetamido-β-D-ribofuranose. This was reacted with N^2-acetylguanine, according to Vorbrüggen and Bennua,[12] in acetonitrile in the presence of hexamethyldisilazane, trimethylchlorosilane and potassium perfluorobutane sulfonate. Cleavage of the protecting groups gave a 60 % yield of **3b** (25 % α and 75% β-anomers).[5] Synthesis of 3'-N-Gly-cGTP **15** proceeded under identical conditions to those for the A-derivative.

Catalytic reduction (H_2; Pd/A) of the azido group of AZT (longer reaction times caused further reduction of T to dihydro-T) afforded 3'-amino-3'-deoxythymidine (**3c**) which was derivatised with the OSu-ester of Z-

glycine. Subsequent phosphorylation of the isolated 3'-N-Gly(Z)-dT as above yielded the triphosphate. Finally the methanolic 3'-N-Gly(Z)-dTTP solution still containing $(Bu_3NH)_2H_2P_2O_7$ was hydrogenated (H_2; Pd/A). Surprisingly after IC not only was 3'-N-Gly-dTTP (ca. 25 %) isolated but also the γ-$POCH_3$ ester (ca. 50 %) of 3'-N-Gly-dTTP and 3'-N-Gly-dTPP (25 %). As with the A-derivatives the tri- and pentaphosphates were readily converted to the cyclic polyphosphoramidates **16a, b**. In contrast cyclisation of the γ-OCH_3 ester **16c** did not occur and afforded further proof of the γ-position of this substituent. The ^1H NMR chemical shift of the OCH_3 group at 3.64 ppm and $^3J(P,CH_3)$ of 11.3 Hz is identical with literature values.[13]

The formation of racemic products in varying amounts during the formation of the 3'-NH-aminoacyl-/peptidyl bond as well as the P-N-bond with optically active amino acids/peptides was detected by NMR spectroscopy for **11a-b** and **14**. The structures of all the new cyclic derivatives were established by ^1H, ^{13}C and ^{31}P NMR spectroscopy, fast-atom-bombardment (FAB)- and electrospray-ionisation mass spectrometry (ESI-MS). Several 2D COSY and ^1H-detected one-bond and multi-bond ^{13}C-^1H, together with internal comparisons, afforded unambiguous spectral assignments. Ring closure was accompanied by the observation of phosphorus couplings to Hα (AA-P in table 1) and CO (PNCHCO in table 2), and where appropriate Cβ (PNCHCH$_2$ in table 2), of the terminal amino acid moiety. The P-N-bond in all compounds was stable in the physiological pH-range.

In conclusion the new cyclic peptide-nucleotide-hybrids (cPNH) are new constructs offering a large variation potential and the possibility of interesting biological properties.

Experimental Section

TEP was from Schuchardt, DMF and tributylamine from Fluka, and tributylammonium pyrophosphate was prepared as described by Hoard and Ott.[14] Peptides and amino acid (AA)-derivatives were from Bachem: H-Gly Phe Gly-OH, H-Gly Gly Gly-OH, H-Gly Gly Gly Gly Gly-OH, Boc-Gly Gly-OH, Boc-Phe Phe-OH, Boc-Lys(Z)-OSu, Z-Gly-Osu and Boc-Gly-OH. The Boc-protecting group was introduced according to the procedure described by Moroder et al.[15] Thin layer chromatography (TLC) was carried out on precoated kieselgel 60 F_{254} aluminium sheets (E. Merck) with the solvent mixtures A: dichloromethane/methanol 8 : 2, B: isopropanol/H_2O/NH_3(conc.) 7:2:1 and C: isobutyric acid/H_2O/NH_3(conc.) 66 : 33 : 1. Products were visualised with UV light. Anion-exchange was performed on Pharmacia DEAE-Sephadex A-25 (HCO_3^-). NMR spectra were recorded on Bruker ARX 400 and DPX 300 NMR spectrometers locked to the deuterium resonance of the solvent D_2O. ESI- and FAB-MS were recorded on Finnigan TSQ-700 and MAT 8430 mass spectrometers, respectively.

Synthesis of 3'-N-aminoacyl/peptidyl nucleoside derivatives 4a-j. The procedure developed for the preparation of **4** involved the synthesis of N-protected amino acid/peptide-NHS esters in anhydrous dioxane or DMF with DCC as coupling agent followed by the reaction of the NHS esters *in situ* with a limiting amount of aminonucleotides **3a-c** (0.5 mmol scale) in DMF.[7] Compounds **4a-j** was purified by crystallisations from ethyl acetate/methanol or by TLC with solvent A. The predicted structure and the purity of all compounds were confirmed by their ESI-MS data and chromatographic properties.

Mass spectral data of **4a-j**. All compounds were the Boc derivatives except where stated. ESI-MS: **4a** (3'-N-Gly Boc-A): $C_{17}H_{24}N_7O_6$ calc. 423.4, found 422.3 $[M-H]^-$, 424.3 $[M+H]^+$; **4b** (3'-N-$(Gly)_2$ Boc-A): $C_{19}H_{28}N_8O_7$ calc. 480.5, found 479.3 $[M-H]^-$; **4c** (3'-N-$(Gly)_3$ Boc-A): $C_{21}H_{31}N_9O_8$ calc. 537.5, found 536.3 $[M-H]^-$, 572.4 $[M-Cl]^-$, 560.3 $[M+Na]^+$; **4d** (3'-N-$(Gly)_5$ Boc-A): $C_{20}H_{29}N_{11}O_8$ calc. 551.5, found 550.3 $[M-H]^-$; **4e** (3'-N-Phe Boc-A): $C_{24}H_{31}N_7O_6$ calc. 513.6, found 512.4 $[M-H]^-$, 536.2 $[M+Na]^+$; **4f** (3'-N-$(Phe)_2$ Boc-A): $C_{33}H_{40}N_8O_7$ calc. 660.7, found 659.4 $[M-H]^-$, 661.3 $[M+H]^+$, 683.3 $[M+Na]^+$; **4g** (3'-N-GlyPheGly Boc/Z-A): $C_{28}H_{37}N_9O_8$ calc. 627.7, found 626.4 $[M-H]^-$, 662.4 $[M-Cl]^-$, 650.3 $[M+Na]^+$; Z-deriv. $C_{31}H_{35}N_9O_8$ calc. 661.7, found 660.5 $[M-H]^-$, 684.3 $[M+Na]^+$; **4h** (3'-N-$(Lys\ Z)_3$ Boc-A): $C_{57}H_{76}N_{12}O_{14}$ calc. 1152.5, found 1153.4 $[M+H]^+$, 1175.5 $[M+Na]^+$, 1191.5 $[M+K]^+$; **4i** (3'-N-Gly Z-dT): $C_{20}H_{24}N_4O_7$ calc. 432.3, found 455.3 $[M+Na]^+$, 471.1 $[M+K]^+$; **4j** (3'-N-Gly Boc-G): $C_{17}H_{25}N_7O_7$ calc. 439.4, found 438.2 $[M-H]^-$, 462.1 $[M+Na]^+$. *Chromatographic data in solvent A and B* (R_f^A, R_f^B): **4a**:0.37, 0.77; **4b**: 0.18, 0.68; **4c**: 0.10, 0.69; **4d**:0.04, 0.66; **4e**: 0.57, 0.82; **4f**: 0.63, 0.83; **4g**: 0.37, 0.80; **4h**: 0.69, 0.88; **4i**: 0.59, 0.70; **4j**: 0.12, 0.63.

Synthesis of 3'-N-aminoacyl/peptidyl nucleoside 5'phosphates 7a-j. *General Procedure*: $POCl_3$ (55 µl, 0.6 mmol) was added to a solution of **4a-j** (~0.3 mmol) in triethyl phosphate (TEP, 1-3 ml). The mixture was stirred at 0°C and was monitored by TLC until the phosphorylation was complete. Upon completion a mixture of 0.5 M bis(tributylammonium) pyrophosphate (fresh prepared) in anh. DMF (3 ml) and Bu_3N (0.3 ml) was injected in to the vessel with rigorous stirring at 4°. After 5 min. an aq. 1 M (Et_3NH) HCO_3 soln. (20 ml) was added. The soln. was evaporated and the residue dissolved in H_2O. The aq. phosphate soln. was deposited on to a DEAE-Sephadex A-25 column (2.5 x 45 cm, HCO_3^-) and eluted with a linear gradient of $(Et_3NH)HCO_3$ (0.05-1M). The nucleotide fractions were evaporated to dryness and repeatedly co-evaporated with MeOH to remove buffer. The resulting protected nucleotide **7** weas deprotected with either TFA (5 min., room temp.) for Boc or by catalytic hydrogenation (H_2, Pd/A) in MeOH and the product was isolated by IC as above.
Variation without the 1st. chromatographic step: After evaporation the product was dissolved in MeOH and hydrogenated using H_2, Pd/A under DC control. The products were purified as above.

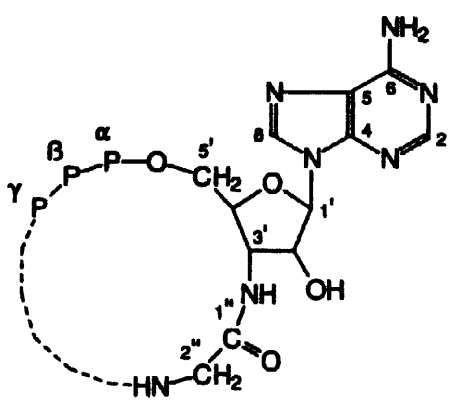
Synthesis of cyclic 3'-N-aminoacyl/peptidyl-5'-phosphoramidate derivatives of 7
Cyclisation procedures: Variation A for 5'-monophosphate derivatives: Compound **7** [n=0, 3'-N-Gly_n (n=3,5), 3'-N-GlyPheGly] was dissolved in water at a concentration of 1 mg ml^{-1} and the pH of the soln. was adjusted to 7.5 by addition of triethylamine. Freshly prepared EDC (free base) was then added with stirring. The pH was adjusted to 7.5 if necessary by bubbling CO_2 through the soln. The reaction proceeded at 40° and was monitored by TLC. After completion of the reaction (12-15 h) the mixture was diluted and applied on to a column packed with DEAE-Sephadex A 25 (HCO_3^-, 2.5 x 40 cm). The compound (free from Et_3NHCl) was eluted with a linear gradient between 1000 ml water and 1000 ml 0.2 M TEAB-buffer. For the isolation of the cyclic oligomers a gradient 0.05 M to 1 M TEAB-buffer was used. The effluent was monitored at 254 nm and collected in fractions. Fractions containing **10a, b** and **12 a** were combined, concentrated and repeatedly co-evaporated with MeOH.

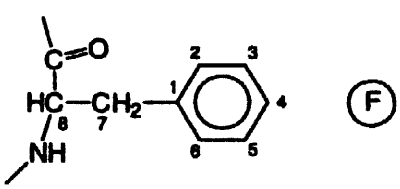
Variation B for 5'-polyphosphate derivatives **7** (n=1-5): This used the same procedure as above with EDC·HCl for the cyclisation reaction. The gradient for IC was 0.05 M to 1 M TEAB-buffer. 3'-N-Aminoacyl-/peptidyl-cyclo adenosine polyphosphates wer converted into the sodium salt by passage through a column (25 x 1 cm) of Dowex 50 W cation - exchange resin (100-200 mesh, sodium form). The resulting aqueous solution was

lyophilised and the derivatives 7 were isolated as white powders.

Mass spectral data of cyclic derivatives. All compounds were calculated as the free acids. FAB-MS (glycerol): **8a**: $C_{12}H_{18}N_7O_{12}P_3$ calc. 545.2, found 544 $[M-H]^-$; **8b**: $C_{14}H_{21}N_8O_{13}P_3$ calc. 602.3, found 601 $[M-H]^-$; ESI-MS: **8c**: $C_{16}H_{24}N_9O_{14}P_3$ calc. 659.3, found 658.3 $[M-H]^-$; **8d**: $C_{20}H_{30}N_{11}O_{16}P_3$ calc. 773.4, found 772.4 $[M-H]^-$; **9**: $C_{20}H_{29}N_{11}O_{13}P_2$ calc. 693.5, found 692.4 $[M-H]^-$; **10a**: $C_{16}H_{22}O_9P_8$ calc. 499.4, found 498.3 $[M-H]^-$; **10b**: $C_{20}H_{28}N_{11}O_{10}P$ calc. 613.5, found 612.4 $[M-H]^-$; **11a**:$C_{19}H_{24}N_7O_{12}P_3$ calc. 635.4, found 634.3 $[M-H]^-$; **11b**: $C_{28}H_{33}N_8O_{13}P_3$ calc. 782.5, found 781.4 $[M-H]^-$; **12a**: $C_{23}H_{28}N_9O_8P$ calc. 589.5, found 588.4 $[M-H]^-$; **12b**: $C_{23}H_{29}N_9O_{11}P_2$ calc. 669.5, found 668.1 $[M-H]^-$; **12c**: $C_{23}H_{30}N_9O_{14}P_3$ calc. 749.1, found 748.1 $[M-H]^-$; **12d**: $C_{23}H_{31}N_9O_{17}P_4$ calc. 829.4, found 828.1 $[M-H]^-$; **12e**: $C_{23}H_{32}N_9O_{20}P_5$ calc. 909.4, found 908.2 $[M-H]^-$; **12f**: $C_{46}H_{56}N_{18}O_{16}P_2$ calc. 1178.4, found 1177.4 $[M-H]^-$, 588.2 $[M-2H]^{2-}$; **12g**: $C_{69}H_{84}N_{27}O_{24}P_3$ calc. 1768.5, found 1767.0 $[M-H]^-$, 883.2 $[M-2H]^{2-}$, 588.5 $[M-3H]^{3-}$;**12h**: $C_{92}H_{112}N_{36}O_{32}P_4$ calc. 2358.0, found (by MALDI-MS) 2423 $[M+3Na]^{3+}$, 2468 $[M+5Na]^{5+}$; **13**: $C_{28}H_{51}N_{12}O_{14}P_3$ calc. 872.7, found 871.3 $[M-H]^-$; **14**: $C_{20}H_{25}N_7O_8P_2$ calc. 553.4, found 552.0 $[M-H]^-$; **15**: $C_{12}H_{18}N_7O_{13}P_3$ calc. 561.3, found 560.1 $[M-H]^-$; **16a**: $C_{12}H_{19}N_4O_{13}P_3$ calc. 520.2, found 519.2 $[M-H]^-$; **16b**: $C_{12}H_{21}N_4O_{19}P_5$ calc. 680.0, found 679.0 $[M-H]^-$; **16c**: $C_{13}H_{23}N_4O_{14}P_3$ calc. 552.3, found 551.2 $[M-H]^-$. *Chromatographic data in solvent B and C* (R_f^B , R_f^C): **8a**: 0.1, 0.25; **8b**: 0.17, 0.19; **8c**: 0.18, 0.20; **8d**: 0.04, 0.17; **9**: 0.1, 0.25; **10a**: 0.36, 0.38; **10b**: 0.51, 0.35; **11a**: 0.24, 0.48; **11b**: 0.25, 0.59; **12a**: 0.58, 0.52; **12b**: 0.52, 0.55; **12c**: 0.37, 0.43; **12d**: 0.14, 0.36; **12e**: 0.04, 0.28; **12f**: 0.66, 0.62; **12g**: 0.63, 0.61; **12h**: 0.57, 0.60; **13**: 0.01, 0.14; **14**: 0.43, 0.45; **15**: 0.06, 0.1; **16a**: 0.12, 0.13; **16b**: 0.01, 0.05; **16c**: 0.11, 0.09.

Numbering Scheme for tables 1 and 2 below:





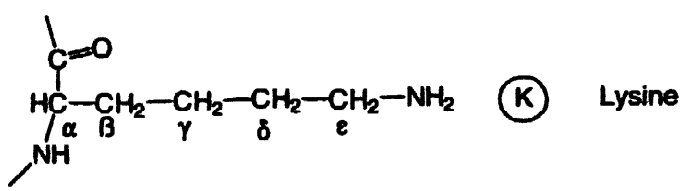


Table 1. ^{1}H NMR data for **8a-d, 9, 10a-b, 11a-b, 12a, 12e-f, 13, 14, 15, 16a** in D_2O. The compound numbering is shown above.

Chemical shifts (ppm)

	2	8	1'	2'	3'	4'	5'A	5'B	2"A	2"B	4"A	4"B	6"A	6"B	8"A/B	10"A/B
8a	8.22	8.33	6.18	4.73	4.61	4.71	4.37	4.21	3.68							
b	8.22	8.34	6.17	4.78	4.48	4.79	4.30	4.30	4.31	3.85	--3.75[a]--					
c	8.29	8.41	6.24	4.78	4.62	4.72	4.37	4.29	4.21	3.95	--4.10[b]--		--3.80--			
d[b]	8.29	8.48	6.23	4.79	4.66	4.58	4.37	4.26	4.08	4.00	--4.06--		--4.08--		4.08	3.73
9[b]	8.33	8.52	6.27	4.77	4.77	4.56	4.38	4.23	4.16	4.01	4.16	4.08	--4.09--		4.10	3.72
10a	8.32	8.38	6.27	4.88	4.42	4.88	--4.10---		4.22	3.94	4.06	3.93	3.89	3.74		
b[b]	8.33	8.56	6.28	4.76	4.83	4.51	4.30	4.12	4.27	3.91	4.20	4.02	4.08	3.99	4.08	3.64/3.62
13	8.33	8.38	6.27	4.93	4.31	4.80	4.39	4.20	Others Kα 4.43, 4.35, 3.99(PN) Kβ-δ 2.10-1.40 Kε 3.14-3.05							
15	----	8.00	6.03	4.76	4.82	4.69	4.38	4.24	3.75							

		2	8	1'	2'	3'	4'	5'A	5'B	F8	F7A	F7B	F2/6	F3/5	F4	G2A/2B (NCO)	G2A/2B (PN)
11a[f]	A	8.28	8.34	6.17	4.69	4.63	4.68	4.34	4.22	4.15	3.15	3.08	7.33	7.40	7.34		
	B	8.34	8.46	5.90	2.34	3.57	4.15	4.49	4.49	4.41	3.28	2.70	7.18	6.60	6.31		
b[e]	A	8.14	8.24	6.12	4.70	4.27	4.95	4.18	4.09	4.52	2.88	2.59	7.09	7.35 - 7.21			
										4.10	3.16	2.99	7.19	7.35 - 7.21			
	B	8.11	8.30	6.03	4.34	4.58	4.47	4.28	4.07	4.36	3.00	2.81	6.95	7.35 - 7.21			
										4.09	2.89	2.78	7.01	7.35 - 7.21			
12a		8.31	8.39	6.26	4.80	4.44	4.70	4.10	4.05	4.45	3.27	3.17	7.33	7.45	7.38	4.28/3.61	3.68/3.60
12e		8.32	8.51	6.30	---4.75-4.66----			4.44	4.34	4.70	3.45	3.15	---7.50-7.36---			4.15/4.05	3.74/3.59
12f		8.14	8.43	6.12	4.66	4.61	4.45	4.22	4.03	4.60	3.13	2.93	7.20	7.30	7.33	4.01/3.87	3.58/3.50
14	A	8.32	8.33	6.25	4.72	4.62	4.62	4.52	3.95	4.01	3.19	3.06	---7.45-7.34---				
	B	8.33	8.42	5.98	2.82	3.58	4.20	4.44	4.30	4.45	3.23	2.77	7.16	6.70	6.57		

	6	5-Me	1'	2'A	2'B	3'	4'	5'A	5'B
16a	7.63	1.98	6.32	2.69 - 2.60		4.51	4.48	4.32	4.19

Coupling constants (Hz)

		1'-2'	2'-3'	3'-4'	4'-5'A	4'-5'B	5'A-5'B	5'A-P	5'B-P
8a		<2	5.2	10.2	4.6	4.6	11.4	11.4	10.3
b		1.3	5.6	10.4	n.a.	n.a.	n.a.	n.a.	n.a.
c		<2	5.4	10.2	n.a.	n.a.	n.a.	n.a.	n.a.
d		2.3	5.4	8.9	2.5	4.9	11.9	6.2	6.5
9		<2	n.a.	n.a.	d	5.0	11.8	d	5.0
10a		<2	5.3	10.7	n.a.	n.a.	n.a.	n.a.	n.a.
b		1.8	5.5	9.2	c	n.a.	11.6	c	n.a.
11a	A	1.0	5.4	10.2	5.2	4.0	12.0	10.5	11.1
	B	<1	4.4	10.1	n.a.	n.a.	n.a.	n.a.	n.a.
b	A	<2	5.9	10.6	2.2	8.2	~12	~11	n.a.
	B	<2	5.2	10.3	n.a.	n.a.	n.a.	n.a.	n.a.

Coupling Constants (Hz) cont.

12a		1.3	~4.8	9.9	3.4	6.4	11.8	7.3	6.4
12e		<2	----------	----------	----------	second-order system	----------	----------	----------
12f		2.4	5.7	8.3	2.4	4.7	11.9	4.7	4.7
13		1.8	5.9	10.2	n.a.	6.9	11.4	n.a.	7.9
14	A	<1	n.a	n.a.	n.a.	n.a	n.a.	n.a.	n.a.
	B	<1	4.4	9.9	5.9	3.1	10.8	n.a.	6.3
15		<2	5.6	10.2	4.9	4.8	11.6	11.8	11.4

	1'-2'A	1'-2'B	2'A-2'B	2'A-3'	2'B-3'	3'-4'	4'-5'A	4'-5'B	5'A-5'B
16a	3.8	7.5	----	----	1111	----	3.3	3.0	11.7

		AA-P	F8-F7A	F8-F7B	F7A-F7B	Others			
8a		15.4							
b		15.4				(2"A-2"B) 17.0			
c		14.2				(2"A-2"B) 16.8			
d		13.5				(2"A-2"B) 17.2			
9		12.7				(2"A-2"B) 17.3	(4"A-4"B) 16.9		
10a		11.5, 14.7				(2"A-2"B) 17.1	(4"A-4"B) 15.8	(6"A-6"B) 17.0	
b		10.4, 11.9				(2"A-2"B) 17.0	(4"A-4"B) 17.2	(6"A-6"B) 17.1	
						(10"A-10"B) 18.1			
11a	A	9.4	6.9	5.7	13.9				
	B	8.6	4.3	11.5	12.9				
b[c]	A	e	7.4	7.8	13.8				
		e	5.0	9.8	14.2				
	B	e	5.1	6.3	13.8				
		e	~5	8.0	13.3				
12a		12.2, 12.8	6.8	9.1	13.6	(2A-2B) 17.5 (PN)	(2A-2B)16.1 (NCO)		
12e		11.3, 11.6	4.3	10.7	13.8	(2A-2B) 17.8 (PN)	(2A-2B)16.9 (NCO)		
12f		11.7, 12.1	6.3	8.5	13.9	17.9	17.0		
13		9.3				(2-3) 4.3/9.3 (PN)			
14	A	n.a.	6.6	8.2	13.5				
	B	n.a.	3.9	11.9	12.5				
15		15.3							

	6-5Me	3'-P	5'A-P	5'B-P
16a	1.0	10.2	10.5	5.5

Footnotes:

a AB system with small shift difference that is not well defined, b It is assumed that the AB system with the largest shift difference is nearest the asymmetric centre, c 2.8 or 4 Hz, d 2.6 or 5.5 Hz, e Racemization at one asymmetric centre causes the appearance of four distinct sets of signals for the phenylalanine systems of almost equal intensity that could not be unambiguously assigned to either isomer A or B. Overlap of the signals at 4.10 and 4.09 ppm did not allow the determination of the ^{31}P-^{1}H coupling constants, although the form of the cross-peaks in the COSY spectrum indicated these were present, f Note the large shifts attributable to intramolecular interactions in isomer B. The unambiguous assignments follow from COSY and long-range ^{13}C-^{1}H correlations.

Table 2. ^{31}P and ^{13}C NMR data for **8a-d, 9, 10a-b, 11a-b, 12a-f, 13, 14, 15, 16a-b** in D_2O. The compound numbering scheme is shown prior to table 1.

^{31}P		Chemical shifts (ppm)					Coupling constants (Hz)			
		α	β	γ	δ	ε	α-β	β-γ	γ-δ	δ-ε
8a		-9.1	-20.3	-0.7			18.3	16.8		
b		-9.4	-20.5	-0.5			17.3	19.7		
c		-10.2	-21.3	-1.0			19.9	20.3		
d		-10.1	-21.2	-1.2			19.4	20.1		
9		-9.9	-1.7				21.4			
10a		+9.0								
b		+9.1								
11a[a]	A	-9.7	-20.8	-3.0			20.4	16.5		
	B	-10.7	-21.3	-3.0			23.8	16.5		
b	A	-9.5	-20.5	-3.1			16.9	22.6		
	B	-9.7	-20.1	-2.7			14.8	18.2		
12a		+8.6								
b		-8.8	-0.4				21.4			
c		-10.3	-20.9	-1.2			20.0	19.9		
d		-10.1	-21.7	-21.1	-1.1		18.7	18.5	19.9	
e		-9.9	-20.8	-21.3	-21.3	-1.1	19.0	----	----	20.0
f		+9.0								
13		-10.3	-21.5	-3.7			19.6	21.8		
14[a]		+21.2,+20.5,+16.4,+16.2					11.4,11.6			
15		-9.8	-21.1	-1.4			18.8	16.1		
16a		-9.9	-21.2	-1.6			19.4	16.2		
b		-10.1	-21.3	-20.9	-20.56	-1.2	19.7	13.7	13.7	19.3
c		-10.2	-21.5	-8.3			18.8	18.7		

^{13}C Chemical shifts (ppm)

		2	4	5	6	8	1'	2'	3'	4'
8a		153.5	149.0	119.6	156.3	140.4	91.1	74.3	53.3	79.8
b[b]		153.2	148.7	119.3	155.9	140.2	90.8	73.8	52.7	79.8
c		153.2	148.6	119.3	155.9	140.1	90.7	73.7	52.3	79.9
d		153.2	148.9	119.3	156.1	140.2	89.9	73.8	51.8	80.7
9		not measured								
10a[c]		153.1	148.5	119.1	155.7	140.0	90.7	73.3	53.0	80.1
b										
11a	A	152.9	148.4	119.1	155.7	139.9	90.9	73.8	52.4	80.0
	B	152.9	147.6	119.5	155.9	138.9	90.2	74.7	50.9	80.5
b[a]	A	153.1	148.5	119.7	155.7	140.1	91.1	73.9	d	79.3
	B	153.0	148.2	119.2	155.7	139.7	90.7	74.1	d	80.2
12e		152.4	148.0	118.5	155.1	139.4	89.5	73.3	54.9	79.6
13		153.3	n.a.	n.a.	156.0	140.2	90.9	73.7	55.0	79.6
14[a]		153.2,153.1	148.6,147.9	119.8,119.2	156.1,155.9	139.9,139.0	91.4,90.5	74.6,74.2	56.1,54.5	79.0,77.2
15		154.3	151.4	117.0	159.5	138.1	91.0	73.9	53.2	79.5

^{13}C Chemical shifts (ppm) cont.

		5'	PNCHCO	PNCH	NCHCO	NCH	Others
8a		66.1	176.1	45.9			
b[b]		65.8	176.4	45.3	173.1	43.0	
c		66.1	177.0	45.5	172.4, 173.0	43.3, 42.8	
d		65.5	176.8	45.4	173.3, 173.0	43.1, 43.1	
					172.7, 171.9	43.1, 42.7	
9		not measured					
10a[c]		65.7	176.0	44.9	173.2, 172.4	44.0, 43.2	
b		not measured					
11a	A	66.2	177.1	58.3			F1: 137.1, F2/F6: 130.0, F3/F5: 129.0
							F4: 127.3, F7: 39.9
	B	62.3	177.8	52.7			F1: 136.6, F2/F6: 130.0, F3/F5: 128.5
							F4: 126.4, F7: 42.4
b	A	67.0	176.6	d	175.0	d	F1: (A) 136.8, 136.2 (B) 137.1, 137.0
	B	64.6	175.2	d	173.3	d	F2/6,F3/F5: 129.9,129.6,129.3,129.0,128.9
							F4: (A) 127.7, 127.5 (B) 127.4, 127.3
							F7:[c] (A) 39.5, 36.9 (B) 41.4, 36.7
12a		64.7	175.1	44.7	173.8, 171.8	42.7, 50.7	F1: 137.0 F2/6:129.0
							F3/5: 128.4 F4: 126.7
13		67.1	K(CO):179.2, 174.7, 174.2; α: 56.2, 54.3, 53.4; β: 32.8, 31.0, 30.1; γ: 22.8, 22.4, 22.3;				
			δ: 26.5 x2, 26.2; e: 39.6, 39.5 x2				
14[e]		63.5,63.1	177.8,177.6	59.0,52.9			F1: 137.1,137.0, F2/F6: 129.9,129.7,
							F3/F5: 129.0, 128.5, F4: 127.4,126.5
							F7: 43.7,40.6
15		66.0	175.7	45.5			
16a			175.7	45.7			

	2	4	5	6	5-Me	1'	2'	3'	4'	5'
16a	152.1	167.2	112.0	138.3	11.9	85.5	35.9	50.2	80.4	65.8

Coupling constants (Hz)

		P-4'	P-5'	PNCHCO	$PNCHCH_2$			P-4'	P-5'	PNCHCO	$PNCHCH_2$
8a		5.6	5.7			11b	A	5.4	~6	>0	9.0
b		6.6	5.4	1.9			B	7.4	>0	2.6	7.8
c		8.7	5.4	4.1		12a		8.7	5.1	7.3	
d		7.1	5.1	4.8		13		8.0	5.1	>0	
9		not measured				14[e]		10.4,13.7	6.1,5.1	7.2,>0	>0,6.1
10a		7.2	5.1	2.9		15		5.3	5.9	>0	
b		not measured				16a		5.0	6.0	1.5	
11a	A	5.1	5.6	-	7.7						
	B	>0	5.0	5.8	6.3						

Footnotes to table 2.

a The intensity of the signals gave 75% **11aA** and 25% **11aB**, 57% **11bA** and 43% **11bB**, b The assignment was taken from a long-range ^{13}C-^{1}H correlation,c The shift of C-1' was adjusted to 90.7 ppm, d The signals for C-3'and methine carbons of the two enantiomers could not be unambiguously assigned and were at 57.3, 56.8, 56.7, 55.0, 53.0 and 50.6, e The signals of the two enantiomers could not be unambiguously distinguished. Signals at 30.1 and 29.8 ppm belong to the phosphorus-bound methylene carbon with couplings of 115.8 and 133.5, and 115.0 and 127.7 Hz, respectively. The signal at 52.9 showed a coupling of 3 Hz, f In each case the carbon showing coupling to phosphorus is given first.

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