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SYNTHESIS OF BRIDGED DINUCLEOSIDES

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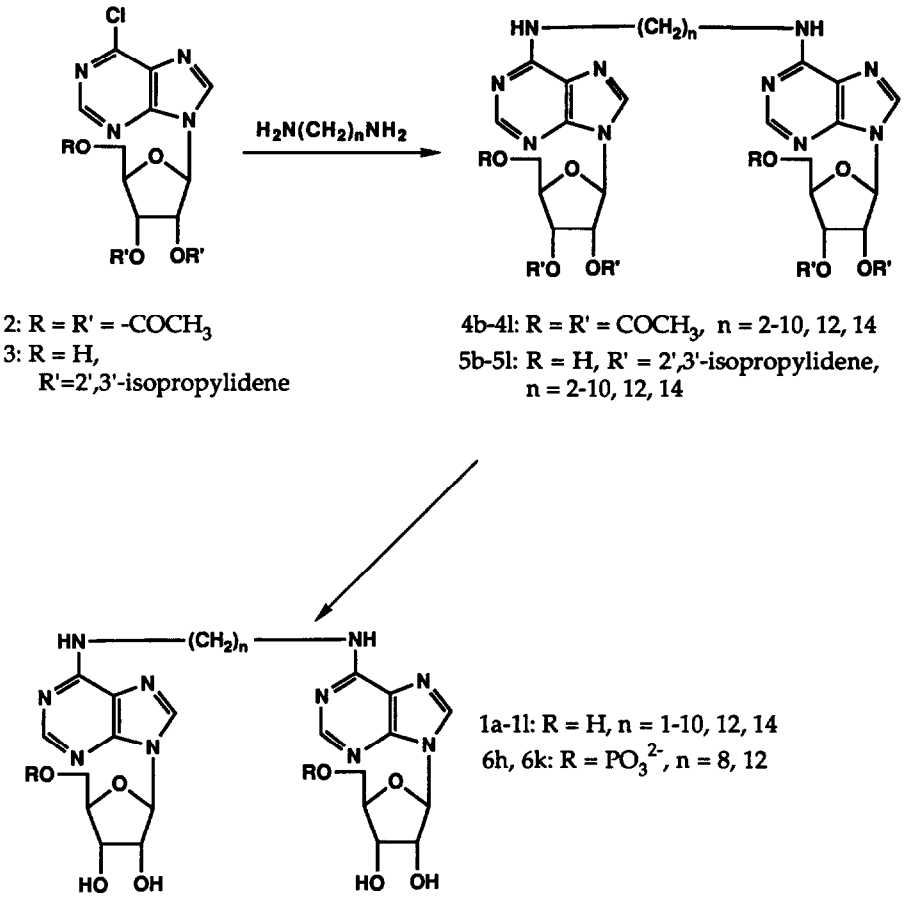
A series of di-(adenosin-N^6-yl) alkanes (1b) – (1l) has been prepared by reaction of the appropriate diaminoalkanes with 6-chloro-9-β-D-(2,3,5-tri-*O*-acetyl)-ribofuranosyl-purine (2) followed by ammonolysis. Alternatively, an analogous reaction with 6-chloro-9-β-D-(2,3-*O*-isopropylidene)-ribofuranosylpurine (3) produced an intermediate which could be phosphorylated at the 5'-position prior to hydrolytic removal of the isopropylidene group. 4-(1,2,4-Triazolo)-1-(β-D-2,3,5-tri-*O*-acetyl ribofuranosyl)-2(1*H*)-pyrimidone (7) was an effective intermediate for the preparation of di-(cytidin-N^4-yl) alkanes (8a), (8b), and (8c) from the appropriate diaminoalkanes. The longer chain di-(adenosin-N^6-yl) alkanes are very effective inhibitors of adenosine kinase. In addition an approach to the di-(adenosin-N^6-yl) alkanes is described which should allow tritium labelling of the alkyl chain.

Introduction

The reaction of formaldehyde with the exocyclic amino group of adenosine is well documented[2-4]. A rapid and reversible reaction converts the adenosine moiety into the hydroxymethyl adduct. In a slower reaction this adduct reacts with the amino group of another adenosine molecule to produce 1,1-di-(adenosin-N^6-yl) methane (1a). Analogous reactions have also been observed with nucleosides and nucleotides containing cytosine and guanine bases[2,3], and formaldehyde has been used to form crosslinks between these bases in RNA and DNA[4,5]. The mutagenic properties of formaldehyde have been the subject of much research[6], and although the mechanism of formaldehyde mutagenesis at a molecular level remains obscure, experiments indicate that an initial reaction between formaldehyde and adenosine-5'-monophosphate or adenosine is a prerequisite of mutagenicity[7,8]. More recently, circumstantial evidence has accumulated which indicates that formaldehyde may exert its mutagenic effects through a phosphorylated derivative of (1a)[9]. In addition, interesting antitumour activity has been reported for 1,2-di-(adenin-N^6-yl) ethane[10]. These reports suggest that purine nucleosides crosslinked through their bases may possess interesting biological activity.

Syntheses of 1,1-di-(adenosin-N^6-yl) methane (1a)[2], 1,2-di-(adenosin-N^6-yl) ethane (1b)[11,12], and

1,4-di-(adenosin-N^6-yl) butane (1d)[11] have been previously described, although di-(adenosin-N^6-yl) alkanes with longer alkyl bridges have to our knowledge not been prepared. The present paper describes the preparation of a number of novel dinucleoside alkanes derived from adenosine and cytidine. Phosphorylation of the di-(adenosin-N^6-yl) alkanes is also described. The results of biological studies on these compounds are reported elsewhere[13].



2: R = R' = -$COCH_3$
3: R = H,
R'=2',3'-isopropylidene

4b-4l: R = R' = $COCH_3$, n = 2-10, 12, 14
5b-5l: R = H, R' = 2',3'-isopropylidene,
n = 2-10, 12, 14

1a-1l: R = H, n = 1-10, 12, 14
6h, 6k: R = PO_3^{2-}, n = 8, 12

Scheme 1

Results and discussion

The synthesis of compounds (1b) – (1l) followed a general strategy which has been adopted for the preparation of N^6-alkylated adenosines and started from suitably protected derivatives of 6-chloro-9-(β-D-ribofuranosyl) purine (Scheme 1). Either 6-chloro-9-β-D-(2,3,5-tri-*O*-acetyl) ribofuranosylpurine (2) or 6-chloro-9-β-D-(2,3-*O*-isopropylidene) ribofuranosylpurine (3) was treated with a solution of the appropriate diaminoalkane (0.6 equiv.) in dry pyridine, and the resulting protected intermediates were isolated by column chromatography. These protected dinucleosides (4b) – (4l) and (5b) – (5l) were characterised by ^1H nmr and FAB^+ mass

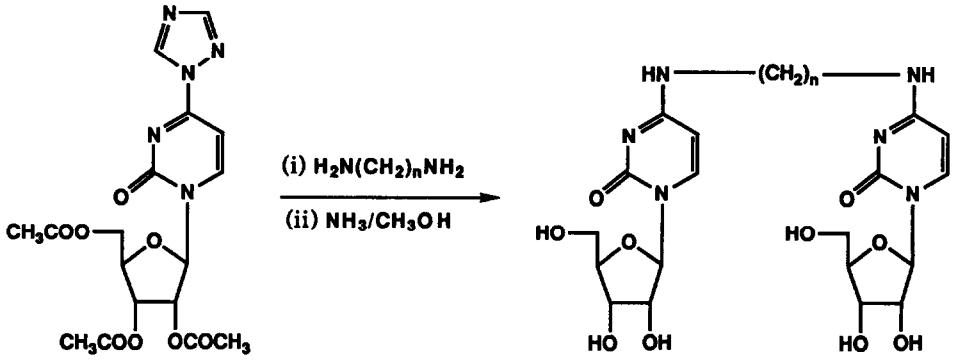
spectrometry. Compounds (1b) – (1l) were prepared from these intermediates by either deacetylation of (4b) – (4l) using methanolic ammonia solution (overall yield from (2), 5 – 30%) or by removal of the isopropylidene group from (5b) – (5l) employing acidic hydrolysis (overall yield from (3), 45 – 50%). In both deprotection procedures the di-(adenosin-N^6-yl) alkanes precipitated from the reaction mixtures and the products were generally in excess of 95% pure as determined by hplc analysis. Compounds which were not homogeneous were precipitated from ethanol or DMSO by the addition of water. The di-(adenosin-N^6-yl) alkanes were characterised chiefly by FAB^+ mass spectrometry (Table 1); $(M+H)^+$ ions were observed for compounds (1b) – (1l) together with $(M-264+H)^+$ fragments which result from the loss of both ribose sugars. ^1H Nmr spectra were obtained and fully assigned using decoupling experiments (see experimental section). Structural confirmation was also obtained from uv spectroscopy and analytical hplc[23].

The di-(adenosin-N^6-yl) alkanes were tested *in vitro* as inhibitors of rat liver adenosine kinase. The longer chain analogues in particular were very effective competitive inhibitors of this enzyme (K_i for 1k (n = 12) = 7.5×10^{-8} M)[13]. Interestingly, these compounds were also phosphorylated by the adenosine kinase although they were very much poorer substrates than adenosine. The longer chain analogues also showed pronounced cytostatic activity and since it was possible that the cytostatic activity of the di-(adenosin-N^6-yl) alkanes could result from the phosphorylated compounds, the synthesis of (6h) and (6k) was investigated. Phosphorylation of (5h) and (5k) was accomplished using phosphoryl chloride under standard Yoshikawa conditions[14], and the isopropylidene groups removed by hydrolysis at pH 1.5. After purification on reverse phase silica-gel the products appeared as single peaks by hplc, the ^{31}P nmr spectra showing singlets at about 4.0 ppm. Further structural confirmation was obtained from FAB^- (negative ion mode) mass spectrometry, both (6h) and (6k) giving $(M-H)^-$ ion signals. In addition (6h) and (6k) were both substrates for the enzyme alkaline phosphatase and were dephosphorylated to give (5h) and (5k) respectively. When (6k) was treated with a much lower concentration of enzyme (5×10^{-3} units/ml) the monophosphate (hplc retention time 12.8 min) was observed as an intermediate in the dephosphorylation.

The di-(cytidin-N^4-yl) alkanes (8a), (8b), and (8c) were prepared from 4-(1,2,4-triazolo)-1-(β-D-2,3,5-tri-*O*-acetylribofuranosyl)-2(1*H*)-pyrimidinone (7)[15] (Scheme 2). This pyrimidine nucleoside derivative had previously been identified as a product of a side reaction resulting from the use of arenesulphonyl derivatives of 1,2,4-triazole as activating agents in oligonucleotide synthesis[15,16]. More recently these triazole derivatives have been used as intermediates for the synthesis of a number of cytidine analogues[17,18]. The triazole derivative (7) was treated with a 0.5 mol equivalent of the appropriate diaminoalkane in dry dioxan for two days at room temperature. The protected derivatives were isolated in yields of about 35% following silica-gel chromatography. Deacetylation was accomplished using methanolic ammonia, and the resultant di-(cytidin-N^4-yl) alkanes characterised by FAB^+ mass spectrometry. Signals corresponding to $(M+H)^+$ ions were observed for (8a), (8b), and (8c), and fragments resulting from the loss of one ribosyl $(M-132+H)^+$ and two ribosyl $(M-264+H)^+$ moieties were

also detected. Confirmatory identification was obtained from ^1H nmr and hplc analysis.

The di-(cytidin-N^4-yl) alkanes showed no inhibitory effect on the adenosine kinase, indicating that the activity observed for the di-(adenosin-N^6-yl) alkanes results from a specific interaction attributable to the adenosine moieties rather than a non-specific effect of the alkyl chain[13].



7 8a, 8b, 8c: n = 4, 8, 12

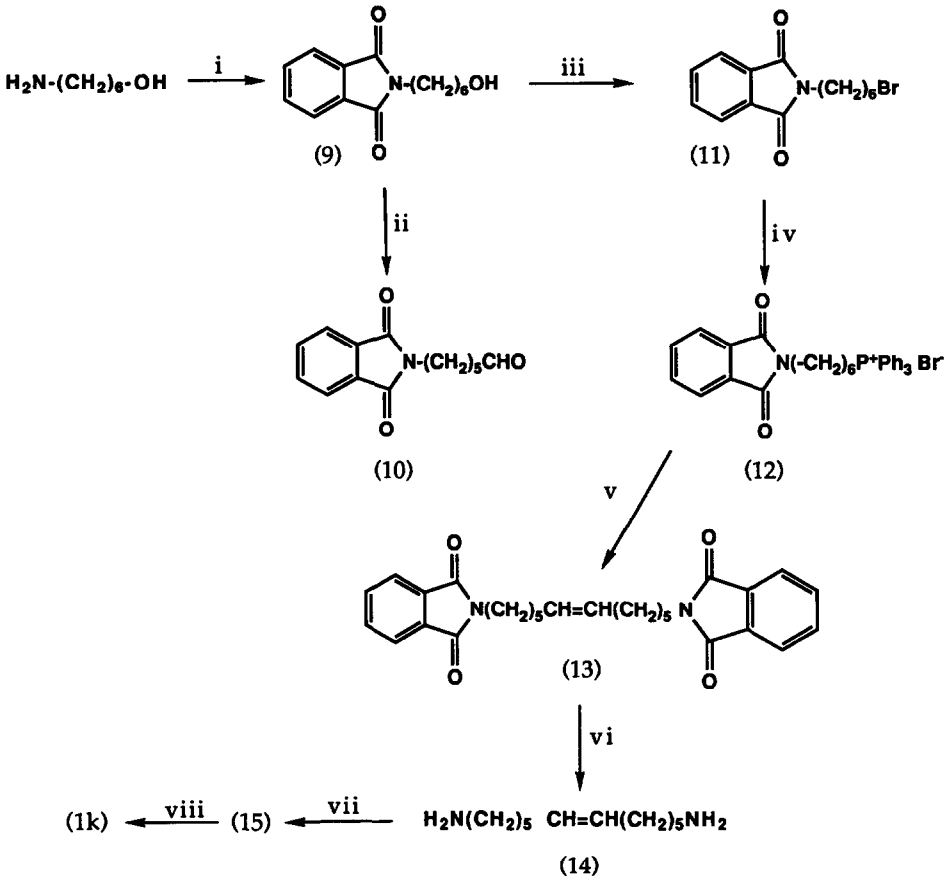
Scheme 2

In order to aid our investigations into the biological properties of the di-(adenosin-N^6-yl) alkanes it was necessary to develop a synthetic strategy that would enable these homologues to be prepared labelled with a radioisotope. To effect the labelling of (1k) we chose to adopt an approach in which the nucleoside units are initially linked through a diaminoalkene bridge which could be reduced by catalytic tritiation (Scheme 3). This strategy is attractive since the tritium is introduced in the last or next to last step and the handling of radioactive material is thereby reduced to a minimum.

6-Phthalimidohexan-1-ol (9) was prepared in 80% yield from 6-aminohexan-1-ol by fusion with phthalic anhydride according to the procedure of Turner[19]. Oxidation of the alcohol with pyridinium chlorochromate gave the 6-phthalimidohexanal (10) as an oily solid in 62% yield. Conversion of (9) to 6-phthalimido-1-bromohexane (11) was most effectively accomplished using carbon tetrabromide and triphenyl phosphine (75% yield). Yields were generally lower (65%) when phophorus tribromide was employed in this reaction. The spectroscopic data obtained for (11) is in accordance with that previously reported by Payne and Boger for a one-step conversion of 6-aminohexan-1-ol to (11)[20]. The phosphonium salt (12) was prepared by refluxing (11) in toluene with triphenylphosphine. Unfortunately the salt did not precipitate out of solution during the reaction and it could only be obtained in a solid form by evaporation of the solvent and trituration with diethyl ether.

In the key Wittig reaction use of sodium hydride in thf at -78 °C to generate the ylid gave only

very low yields (< 5%) of the required alkene (13) together with several phosphorus-containing side products which were not identified. An improved, although still disappointing, yield (27%) was achieved by employing potassium tert-butoxide under analogous conditions. Unfortunately the symmetry of 1,12-diphthalimidododec-6-ene precluded assignment of stereochemistry about the carbon-carbon double bond through examination of nmr coupling constants. However, since the double bond was to be reduced at the end of the synthesis the assignment was not therefore absolutely necessary and no attempts were made to obtain this information. Removal of the phthaloyl group from (13) was accomplished by application of standard hydrazinolysis conditions to afford the diaminoalkene (14) in 39% yield.



Reagents: i, phthalic anhydride, 150 °C; ii, pyridinium chlorochromate; iii, triphenylphosphine, carbon tetrabromide; iv, triphenylphosphine; v, potassium *tert*-butoxide, (10); vi, hydrazine hydrate; vii, (2), *N,N*-dimethylaminopyridine; viii, hydrogen, palladium on carbon; methanolic ammonia

Scheme 3

Di-(2',3',5'-tri-*O*-acetyladenosin-N^6-yl) dodec-6-ene (15) was prepared from the diaminoalkene (14) and (2) under conditions analogous to those described for the synthesis of the di-(adenosin-N^6-yl) alkanes. Hydrogenation of (15) with 5% palladium on charcoal proceeded smoothly at 300 psi, hplc analysis after 40 hours showing that it had been fully converted into di-(2',3',5'-tri-*O*-acetyladenosin-N^6-yl) dodecane. Deacetylation with methanolic ammonia gave (1k); the hplc retention time and spectroscopic data for this compound were identical to that of a sample prepared from 1,12-diaminododecane.

In conclusion we have prepared and characterised a variety of base-linked dinucleosides. The longer chain di-(adenosin-N^6-yl) alkanes are very effective inhibitors of adenosine kinase. The results obtained from this study are currently being used to design inhibitors of other nucleoside kinases.

TABLE 1

Compound	Chain length (n)	M/z for $(MH)^+$	λ_{max} / nm[a]	Hplc retention time / min.[b]
1b	2	561	264.8	8.4
1c	3	575	263.7	8.9
1d	4	589	263.9	9.2
1e	5	603	265.0	9.6
1f	6	617	266.2	10.1
1g	7	631	266.8	10.7
1h	8	645	266.8	11.2
1i	9	659	267.0	12.4
1j	10	673	267.0	13.1
1k	12	701	266.8	14.2
1l	14	729	267.4	14.7

[a] Spectra recorded in 20 mM sodium borate, pH 8.0.

[b] Using elution conditions specified in the experimental section below.

Experimental

General

Analytical tlc was carried out on Alugram sil G/UV_{254} plates. Merck 9385 Kieselgel 60 was used for flash column chromatography. Reverse phase chromatography was carried out on Merck 9303 LiChroprep RP-18 silica-gel. Bovine intestinal alkaline phosphatase was purchased from Sigma.

Hplc was performed on a Varian 5000 liquid chromatograph operating with a UV50 uv detector.

Separations were accomplished on a column (250 x 4.6 mm) packed with 5μ LiChrosorb RP-18 and elution carried out with a 20 min linear gradient from 100% 50mM triethylammonium acetate to 100% acetonitrile at a flow rate of 1.5ml/min. ^1H nmr spectra were obtained on a Bruker WM250 spectrometer operating at 250 MHz. Chemical shifts are given in ppm downfield from an internal standard of tetramethylsilane. Proton decoupled ^{31}P nmr spectra were recorded on the same spectrometer operating at 101.2 MHz and signals referenced to an external standard of 85% H_3PO_4. FAB mass spectra were recorded on a VG Analytical 7070E mass spectrometer operating with a PDP 11/250 data system and an Ion Tech FAB ion gun working at 8 kv. 3-Nitrobenzyl alcohol was used as a matrix unless stated otherwise.

Pyridine was dried at reflux over calcium hydride for several hours prior to distillation. Dioxan was heated at reflux over sodium in the presence of benzophenone and then distilled.

1,14-Diaminotetradecane was prepared as previously described[21].

Di-(adenosin-N^6-yl) alkanes (1b) – (1l)

To a solution of (2)[22] (1.8g, 4.36 mmol) in dry pyridine (10 ml) the appropriate diaminoalkane (2.6 mmol) and dimethylaminopyridine (50 mg) were added and the resulting solution stirred overnight at room temperature. The pyridine was evaporated and the residue subjected to purification by flash column chromatography. Elution with chloroform followed by chloroform containing 1% methanol afforded the di-(2',3',5'-tri-*O*-acetyladenosin-N^6-yl) alkane. The intermediate was treated with a solution of methanolic ammonia (saturated at 0 °C) for about 16 hours at room temperature. The precipitated product was filtered, washed with a little methanol, and dried. The overall yield of (1b) – (1l) was usually in the range of 5 – 30%.

Yields and nmr data for (1b)-(1l)

(1b). Yield 5%; $\delta_H((CD_3)_2SO)$ 8.37 (2H, s, H-8), 8.23 (2H, s, H-2), 8.04 (2H, broad, NH), 5.90 (2H, d, J 6.1 Hz, H-1'), 5.30 (3H, broad, OH-2',3', and 5'), 4.61 (2H, m, H-2'), 4.12 (2H, m, H-3'), 3.47 (2H, m, H-4'), and 3.58 - 3.77 (8H, m, H-5' and H-1 on alkyl chain).

(1c). Yield 10%; $\delta_H((CD_3)_2SO)$ 8.37 (2H, s, H-8), 8.22 (2H, s, H-2), 8.13 (2H, broad, NH), 5.84 (2H, d, J 6.2 Hz, H-1'), 5.46 (2H, s, OH-2'), 5.44 (2H, s, OH-5'), 5.19 (2H, d, J 4.5 Hz, OH-3'), 4.61 (2H, m, H-2'), 4.14 (2H, m, H-3'), 3.96 (2H, m, H-4'), 3.56 - 3.68 (8H, m, H-5' and N-CH_2), and 1.91 (2H, m, H-2 on alkyl chain).

(1d). Yield 9%; $\delta_H((CD_3)_2SO)$ 8.34 (2H, s, H-8), 8.20 (2H, s, H-2), 7.92 (2H, broad, NH), 5.88 (2H, d, J 6.2 Hz, H-1'), 5.45 (2H, s, OH-2'), 5.43 (2H, s, OH-5'), 5.19 (2H, d, J 4.3 Hz, OH-3'), 4.65 (2H, m, H-3'), 3.96 (2H, m, H-4'), 3.52 - 3.70 (8H, m, H-5' and H-1 on alkyl chain), and 1.65 (4H, broad, H-2 on alkyl chain).

(1e). Yield 27%; $\delta_H((CD_3)_2SO)$ 8.34 (2H, s, H-8), 8.19 (2H, s, H-2), 7.92 (2H, broad, NH), 5.87 (2H, d, J 6.2 Hz, H-1'), 5.47 (2H, s, OH-2'), 5.45 (2H, s, OH-5'), 5.20 (2H, d, J 4.3 Hz, OH-3'), 4.61 (2H,

m, H-2'), 4.31 (2H, s, H-3'), 3.96 (2H, m, H-4'), 3.47 - 3.70 (8H, m, H-5' and H-1 on alkyl chain), 1.62 (4H, m, H-2 on alkyl chain), and 1.30 (2H, m, H-3 on alkyl chain).

(1f). Yield 12%; $\delta_H((CD_3)_2SO)$ 8.32 (2H, s, H-8), 8.19 (2H, s, H-2), 7.85 (2H, broad, NH), 5.88 (2H, d, J 6.2 Hz, H-1'), 5.43 (2H, s, OH-2'), 5.42 (2H, s, OH-5'), 5.17 (2H, d, J 3.5 Hz, OH-3'), 4.60 (2H, m, H-2'), 4.14 (2H, s, H-3'), 3.96 (2H, m, H-4'), 3.54 - 3.69 (8H, m, H-5' and H-1 on alkyl chain), 1.59 (4H, m, H-2 on alkyl chain), and 1.35 (4H, broad, H-3 on alkyl chain).

(1g). Yield 31%; $\delta_H((CD_3)_2SO)$ 8.36 (2H, s, H-8), 8.22 (2H, s, H-2), 7.89 (2H, broad, NH), 5.91 (2H, d, J 5.8 Hz, H-1'), 5.50 (4H, broad, OH-2' and 5'), 5.23 (2H, broad, OH-3'), 4.64 (2H, m, H-2'), 4.18 (2H, m, H-3'), 4.01 (2H, m, H-4'), 3.58 - 3.68 (8H, m, H-5' and H-1 on alkyl chain), 1.59 (4H, m, H-2 on alkyl chain), and 1.33 (6H, broad, H-3 and H-4 on alkyl chain).

(1h). Yield 29%; $\delta_H((CD_3)_2SO)$ 8.34 (2H, s, H-8), 8.20 (2H, s, H-2), 7.91 (2H, broad, NH), 5.88 (2H, d, J 6.2 Hz, H-1'), 5.47 (2H, s, OH-2'), 5.45 (2H, s, OH-5'), 5.21 (2H, d, J 4.5 Hz, OH-3'), 4.14 (2H, m, H-3'), 3.97 (2H, m, H-4'), 3.45 - 3.72 (8H, m, H-5' and H-1 on alkyl chain), 1.58 (4H, broad, H-2 on alkyl chain), and 1.29 (8H, broad, H-3 and H-4 on alkyl chain).

(1i). Yield 12%; $\delta_H((CD_3)_2SO)$ 8.36 (2H, s, H-8), 8.22 (2H, s, H-2), 7.87 (2H, broad, NH), 5.91 (2H, d, J 5.7 Hz, H-1'), 5.50 (4H, broad, OH-2' and OH-5'), 5.24 (2H, broad, OH-3'), 4.64 (2H, d, H-2'), 4.18 (2H, m, H-3'), 4.00 (2H, m, H-4'), 3.58 - 3.89 (8H, m, H-5' and H-1 on alkyl chain), 1.58 (4H, broad, H-2 on alkyl chain), and 1.23 (10H, broad, H-3, H-4, and H-5 on alkyl chain).

(1j). Yield 10%; $\delta_H((CD_3)_2SO)$ 8.35 (2H, s, H-8), 8.21 (2H, s, H-2), 7.88 (2H, broad, NH), 5.89 (2H, d, J 5.8 Hz, H-1'), 5.47 (2H, s, OH-2'), 5.45 (2H, s, OH-5'), 5.21 (2H, d, J 3.6 Hz, OH-3'), 4.63 (2H, m, H-2'), 4.16 (2H, m, H-3'), 3.99 (2H, m, H-4'), 3.47 - 3.67 (8H, m, H-5' and H-1 on alkyl chain), 1.58 (4H, broad, H-2 on alkyl chain), and 1.27 (12H, broad, H-3, H-4, and H-5 on alkyl chain).

(1k). Yield 12%; $\delta_H((CD_3)_2SO)$ 8.33 (2H, s, H-8), 8.19 (2H, s, H-2), 7.87 (2H, broad, NH), 5.87 (2H, d 6.2 Hz, H-1'), 5.44 (2H, s, OH-2'), 5.42 (2H, s, OH-5'), 5.18 (2H, d, J 4.4 Hz, OH-3'), 4.61 (2H, m, H-2'), 4.14 (2H, m, H-3'), 3.96 (2H, m, H-4'), 3.46 - 3.70 (8H, m, H-5' and H-1 on alkyl chain), 1.57 (4H, broad, H-2 on alkyl chain), and 1.23 (16H, broad, H-3, H-4, H-5, and H-6 on alkyl chain).

(1l). Yield 25%; $\delta_H((CD_3)_2SO)$ 8.34 (2H, s, H-8), 8.20 (2H, s, H-2), 7.87 (2H, broad, NH), 5.89 (2H, d, J 6.0 Hz, H-1'), 5.46 (2H, s, OH-2'), 5.44 (2H, s, OH-5'), 5.20 (2H, d, J 3.4 Hz, OH-3'), 4.62 (2H, m, H-2'), 4.15 (2H, m, H-3'), 3.93 (2H, m, H-4'), 3.46 - 3.71 (8H, m, H-5' and H-1 on alkyl chain), 1.58 (4H, broad, H-2 on alkyl chain), and 1.23 (20H, broad, H-3, H-4, H-5, H-6, and H-7 on alkyl chain).

Phosphorylation of di-(adenosin-N^6-yl) alkanes (6h) and (6k)

Into a stirred, ice cold mixture of freshly distilled phosphoryl chloride (2ml, 22 mmol) and triethylphosphate (10ml) either (5h) or (5k) (0.5 mmol) was added. After 2.5 hours at room

temperature the solution was poured into anhydrous ether (50ml) and the precipitate collected by centrifugation. The precipitate was dissolved in ice water (5 - 10 ml), the pH of the solution adjusted to 1.5 by the addition of 1M HCl, and the reaction mixture heated at 70 °C for 1 hour. The solution was neutralised by the addition of solid sodium bicarbonate and concentrated to a small volume. The product was isolated by chromatography on a reverse phase silica-gel column (14 x 2.5cm) with a stepwise gradient of 0 - 15% acetonitrile in water as eluant. Compounds (6h) and (6k) were isolated in yields of about 35%. For (6h): δ_P (D_2O) 4.07 ppm; m/z (FAB^-) (glycerol matrix) 803 $(M-H)^-$. Hplc retention time 10.9 min. For (6k): δ_P (D_2O) 3.87 ppm; m/z (FAB^-) (glycerol matrix) 859 $(M-H)^-$. Hplc retention time 11.7 min.

Enzymatic dephosphorylation

The dinucleotide (2 - 3 A_{260} units) was dissolved in 300ml of 50mM tris-(hydroxymethyl) methylamine-HCl (pH 7.3) containing 5mM magnesium chloride and bovine intestinal alkaline phosphatase (10 units). The reactions were analysed by hplc after 1 hour at room temperature.

Synthesis of di-(cytidin-N^4-yl) alkanes (8a), (8b), and (8c)

A solution of (7) (1.0g, 2.37 mmol) in dry dioxan was stirred with the appropriate diaminoalkane for 2 days at room temperature. After removal of the dioxan under reduced pressure the residue was subjected to flash chromatography. Elution with chloroform followed by chloroform containing 2% methanol afforded the di-(2',3',5',-tri-O-acetylcytidin-N^4-yl) alkane. Removal of the acetyl group was accomplished by overnight treatment with methanolic ammonia solution. The resulting solution was concentrated under reduced pressure and the crude di-(cytidin-N^4-yl) alkane precipitated from ethanol by the addition of water. The pure products were obtained in yields ranging from 30 - 35%.

(8a). Yield 34%; δ_H ($(CD_3)_2SO$) 7.78 (2H, d, J 7.3 Hz, H-5), 7.77 (2H, broad, NH), 5.75 (4H, m, H-6 and H-1'), 5.30 (2H, d, J 3.8 Hz, HO-3'), 5.05 (2H, t, J 4.9 Hz, HO-5'), 4.98 (2H, d, J 4.2 Hz, HO-2'), 3.93 (2H, m, H-3'), 3.81 (2H, m, H-4'), 3.67 - 3.51 (4H, m, H-5'), 3.27 (4H, m, H-3, H-4, and H-1 on alkyl chain), and 1.52 (4H, m, H-2 on alkyl chain); m/z (FAB^+) 541 $(M+H)^+$. Hplc retention time 8.5 min.

(8b). Yield 30%; δ_H ($(CD_3)_2SO$) 7.78 (2H, d, J 7.8 Hz, H-5), 7.70 (2H, broad, NH), 5.75 (4H, m, H-6 and H-1'), 5.31 (2H, broad, HO-3'), 5.06 (2H, t, J 5.0 Hz, HO-5'), 5.01 (2H, broad, HO-2'), 3.93 (2H, m, H-3'), 3.83 (2H, m, H-4'), 3.67 - 3.51 (4H, m, H-5'), 3.23 (4H, m, H-1 on alkyl chain), 1.48 (4H, m, H-2 on alkyl chain), and 1.29 (8H, broad, H-3 and H-4 on alkyl chain); m/z (FAB^+) 597 $(M+H)^+$. Hplc retention time 10.7 min.

(8c). Yield 33%; δ_H ($(CD_3)_2SO$) 7.79 (2H, d, J 7.8 Hz, H-5), 7.73 (2H, broad, NH), 5.78 - 5.73 (4H, m, H-6 and H-1'), 5.31 (2H, d, J 4.3 Hz, HO-3'), 5.07 (2H, t, J 5.0 Hz, HO-5'), 5.01 (2H, d, J 4.2 Hz, HO-2'), 3.94 (2H, m, H-3'), 3.83 (2H, m, H-4'), 3.65 - 3.52 (4H, m, H-5'), 3.23 (4H, m, H-1 on alkyl chain), 1.48 (4H, m, H-2 on alkyl chain), and 1.25 (16H, broad, H-3, H-4, H-5, and H-6 on alkyl chain); m/z (FAB^+) 653 $(M+H)^+$. Hplc retention time 12.3 min.

6-Phthalimidohexan-1-ol (9)

6-Aminohexan-1-ol (25g, 0.21 mol) and phthalic anhydride (31.64g, 0.21 mol) were fused at 150 - 160 °C for about 15 minutes to give a very viscous syrup which solidified on cooling. The crude residue was recrystallized from petroleum ether (60 - 80 °C) to give (9) (42.2g, 80%), m.p. 43 - 45 °C. $\nu_{max.}$ 1760 and 1700 cm^{-1}; δ_H ($CDCl_3$) 7.83 (2H, m, Ar), 7.71 (2H, m, Ar), 3.67 (4H, m), 1.85 (1H, broad, OH), 1.70 (2H, m), 1.56 (2H, m), and 1.41 (2H, m); m/z (CI, NH_3) 248 $(m+H)^+$.

6-Phthalimidohexanal (10)

In a 500 ml round-bottomed flask fitted with a reflux condenser was suspended 18 g (0.08 mol) of pyridinium chlorochromate in anhydrous dichloromethane (100 ml). 6-Phthalimidohexan-1-ol (10 g, 0.04 mol) in dichloromethane (20 ml) was added in one portion to the stirred solution. After 2 hours dry ether (100 ml) was added and the supernatant liquid decanted from the black gum. The insoluble residue was washed thoroughly three times with 50 ml portions of anhydrous ether whereupon it became a black granular solid. The combined organic solution was passed through a short pad of silica-gel and the solvent was removed under vacuum. Flash column chromatography of the crude oily residue gave the product (6.15 g, 62.1%) as an oily solid; $\nu_{max.}$ 1760 and 1700 cm^{-1};δ_H ($CDCl_3$), 9.76 (1H, s, CHO), 7.81 (2H, m, Ar), 7.73 (2H, m, Ar), 3.68 (2H, t, J 5.3 Hz, NCH_2), 2.44 (2H, t, J 6.6 Hz, CH_2CO), 1.68 (4H, m), and 1.41 (2H, m); m/z (CI, NH_3) 246 $(M+H)^+$ and 263 $(M+H+NH_3)^+$.

6-Phthalimido-1-bromohexane (11)

To a solution of 6-phthalimidohexan-1-ol (10 g, 0.04 mol) and triphenylphosphine (10.6 g, 0.04 mol) in dry acetonitrile (200 ml) was added anhydrous carbon tetrabromide (10 g, 0.03 mol) and the solution was magnetically stirred for 1 hour at room temperature. The solvent was evaporated and the crude solid was purified by flash chromatography to give the product (9.34 g, 74.4%), m.p. 56 - 57 °C. $\nu_{max.}$ 1760 and 1700 cm^{-1}; δ_H ($CDCl_3$), 7.85 (2H, m, Ar), 7.75 (2H, m, Ar), 3.75 (2H, t, J 6.2 Hz, N-CH_2), 3.40 (2H, t, J 6.8 Hz, CH_2Br), 1.88 (2H, m), 1.72 (2H, m), and 1.45 (2H, m); m/z (CI, NH_3), 327 $(M+H+NH_3)^+$, 100%, 329 $(M+H+NH_3)^+$, 98%.

Triphenyl-6-phthalimidohexylphosphonium bromide (12)

Triphenylphosphine (10 g, 0.038 mol) was added to a solution of 6-phthalimido-1-bromohexane (8 g, 0.026 mol) in dry toluene (100 ml). The clear solution was refluxed for about 15 hours and the solvent was removed under reduced presssure to give a glassy material. The latter was triturated several times with ether to remove the excess triphenylphosphine allowing isolation of the salt (13.37 g, 90.6%). M/z (FAB^+) 492 (M^+).

1,12-Diphthalimidododec-6-ene (13)

To a suspension of the above phosphonium salt (5.72 g, 0.01 mol) in dry thf (80 ml) at -78 °C, potassium tert-butoxide (2.0 g, 0.018 mol) was added, followed by 6-phthalimidohexanal (3.0 g, 0.012 mol). The yellowish suspension was stirred magnetically for about 7 hours at -78 °C and

was then allowed to reach room temperature overnight. The solvent was evaporated and to the crude residue water (50 ml) was added. The aqueous solution was extracted several times with chloroform and the combined extract dried over magnesium sulphate. The crude material obtained after evaporation of the solvent was purified by flash column chromatography to give the product (1.23 g, 27%), m.p. 48-50 °C. δ_H ($CDCl_3$) 7.82 (4H, m, Ar), 7.71 (4H, m, Ar), 5.32 (2H, t, J 4.5 Hz, CH=CH), 3.66 (4H, t, J 7.3 Hz, N-CH_2), and 1.99 (4H, m, N-C-CH_2), 1.67 (12H, m); m/z (CI, NH_3) 459 $(M+H)^+$, 476 $(M+H+NH_3)^+$.

1,12-Diaminododec-6-ene (14)

Finely powdered (13) (1.0 g, 2.2 mmol) was suspended in ethanol (30 – 40 ml) and an excess of hydrazine hydrate (4 ml, 0.08 mol) was added. The suspended material dissolved after about 15 minutes and the clear solution was left stirred magnetically overnight. The resultant white gelatinous precipitate was filtered, and the filtrate treated with 1M HCl (15 ml). The solution was concentrated and the resulting precipitate was once more filtered. The aqueous acidic solution was made alkaline by means of potassium hydroxide pellets, and then extracted several times with ether/chloroform. The combined extract was dried over magnesium sulphate and after evaporation of the solvents a crystalline solid was obtained (0.17 g, 39%). The HCl salt was prepared by bubbling HCl gas into a solution of (14) in ether. For the salt: m.p. 205 – 209 °C. δ_H($(CD_3)_2SO$) 5.35 (2H, t, J 4.6 Hz, CH=CH), 2.73 (4H, broad, N-CH_2), 1.99 (4H, broad), and 1.30 - 1.14 (12H, broad); m/z (FAB^+) 199 $(M+H)^+$.

Di-(-2',3',5'-tri-*O*-acetyladenosin-N^6-yl) dodec-6-ene (15)

To a solution of 6-chloro-9-β-D-(2,3,5-tri-*O*-acetyl)-ribofuranosylpurine (2) (0.64 g, 1.55 mmol) in dry pyridine (15 ml), (14) (0.16 g, 0.81 mmol) and a catalytic amount of dimethylaminopyridine were added and the resulting solution stirred overnight at room temperature. The pyridine was evaporated under reduced pressure and the residue purified by flash column chromatography. Elution with chloroform followed by chloroform containing 1% methanol afforded the product (0.29 g, 20%). M/z (FAB^+) 951 $(M+H)^+$. Hplc retention time 18.9 min.

Di-(2',3',5'-tri-O-acetyladenosin-N^6-yl) dodecane from (15)

Di-(2',3',5'-tri-*O*-acetyladenosin-N^6-yl) dodec-6-ene (15) (10 mg, 0.01 mmol) was dissolved in hplc grade methanol (30 ml) and hydrogenated in a sealed and stirred chamber at 300 psi in the presence of 5% palladium on charcoal (10 mg). Hplc analysis after 40 hours indicated that the reaction was complete, the catalyst was then removed by filtration through celite, and the filtrate evaporated to yield the product (8 mg, 80%). This product was identical to that previously prepared.

Di-(adenosin-N^6-yl) dodec-6-ene from (15)

Treatment of di-(2',3',5'-tri-*O*-acetyladenosin-N^6-yl) dodec-6-ene (15) (20 mg, 0.02 mmol) with methanolic ammonia as previously described gave the product (9 mg, 64%). δ_H($(CD_3)_2SO$) 8.34 (2H, s, H-8), 8.19 (2H, s, H-2), 7.89 (2H, broad, NH), 5.87 (2H, d, J 6.2 Hz, H-1'), 5.46 (2H, s, OH-2'),

5.43 (2H, s, OH-5'), 5.31 (2H, t, J 4.8 Hz, CH=CH), 5.19 (2H, d, J 4.5 Hz, OH-3'), 4.61 (2H, m, H-2'), 4.13 (2H, m, H-3'), 3.96 (2H, m, H-4'), 3.44 - 3.69 (8H, m, H-5' and H-1 on alkyl chain), 1.97 (4H, m, H-5 on alkyl chain), 1.57 (4H, m, H-2 on alkyl chain), and 1.31 (8H, broad, H-3 and H-4 on alkyl chain); m/z (FAB$^+$) 699 $(M+H)^+$. Hplc retention time 15.4 min.

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References

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23. We have been unable to recrystallise the crosslinked nucleosides due to their very poor solubility and we are therefore unable to report acceptable combustion analysis data. The involatility of the compounds has also prevented us from obtaining accurate mass data using EI or CI techniques.