Synthesis of a hydrogen-bond-degenerate tricyclic pyrrolopyrimidine nucleoside and of its 5′-triphosphate

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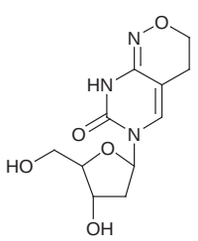
The syntheses of a number of 7-(2-deoxyribofuranosyl)pyrrolo[2,3-*d*]pyrimidine derivatives are described. Of these the 2-methylsulfanyl-4-hydroxy-5-acetoxyethyl derivative **7** was conveniently formed by condensation of 2-methylsulfanyl-4-hydroxy-6-aminopyrimidine with 2-chloro-4-acetoxybutanal **6**; it was then converted to the 4-chloro derivative **12** with phosphoryl trichloride and coupled to 3,5-di-*p*-toluoyl-2-deoxy-α-ribosyl chloride. Transformation to the 5-aminooxyethyl derivative and ring closure of the latter gave the tricyclic 2-(2-deoxyribosyl)-4-methylsulfonyl-7-oxa-2,3,5,6-tetraazabenz[*cd*]azulene. Hydrazinolysis of the methylsulfonyl residue, then mercury(II) oxide oxidation, led to the target nucleoside **4**. For its study as a polymerase substrate for incorporation into DNA the 5′-triphosphate was synthesised.

For some time there has been an interest in nucleoside analogues which possess ambivalent base-pairing properties, for example in duplex DNA. Since this property must depend, in the first place, on shifting the tautomeric constant of the heterocycle from the extreme of 10^4–10^5 shown by the natural bases, much emphasis has been put on derivatives carrying electronegative elements on the amino function, for example an oxyamino, alkoxyamino or hydrazino residue in place of the amino group.[1] Such a compound is **1**, a pyrimidooxazinone related to N^4-methoxy-2′-deoxycytidine.[2] In this pair, although both are mutagenic in prokaryotes the former is much more potent[3] and its 5′-triphosphate is an excellent polymerase substrate.[4] Since the N^4-methoxy group is predominately *syn* with respect to the hydrogen-bonding face, the *anti*-constrained analogue **1** was expected to have the desired ability to form Watson–Crick base-pairs more readily with both adenine and guanine, leading to enhancement of properties expected to flow from this. Much evidence was adduced in support of this.[5–8]



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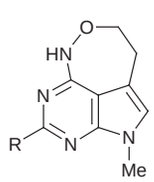
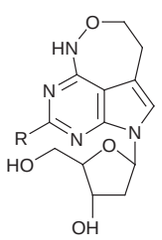


2 R = H
3 R = NH_2

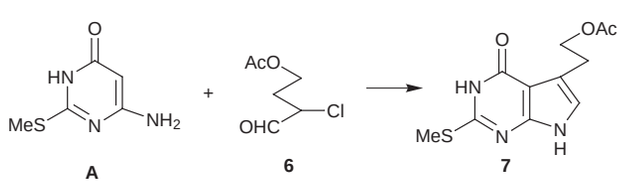


4 R = H
5 R = NH_2

Both N^6-methoxyadenine and 6-methoxyamino-2-aminopurine as the respective 2′-deoxynucleosides and their 5′-triphosphates show similar base-pair ambivalence[9] and it was therefore of interest to synthesise analogues where again the alkoxy function is restrained in the *anti*-configuration by incorporating it into a third ring. To this end model experiments provided a route to the tricyclic pyrrolopyrimidine **2**,[10] although the analogous ring closure to amine **3** could not be effected. In order to open the way to look further at routes to both the nucleosides **4** and **5**, three series of pyrrolopyrimidines, *viz*. **7**, **8** and **9** and the corresponding nucleosides derived from them were synthesised. In the event the series derived from sulfide **7** was followed through since the 2-methylsulfanyl residue potentially provided more flexibility to reach both the 2-H and 2-NH_2 tricyclic nucleoside analogues. In the present paper we describe the synthesis of the 2′-deoxyribonucleoside **4** together with its 5′-triphosphate.

Results and discussion

In earlier work the synthetic route to the pyrrolopyrimidines **7**–**9**[10] route was characterised by disappointing yields. An alternative route (Scheme 1) to compound **7** was



A **6** **7**

Scheme 1

devised based on the known procedure for the conversion of 2-methylsulfanyl-4-hydroxy-6-aminopyrimidine to 2-methylsulfanyl-4-hydroxypyrrolo[2,3-*d*]pyrimidine.[11] To this end monoacetylation of butane-1,4-diol, oxidation of the fractionated product to the aldehyde by PCC and chlorination with sulfuryl dichloride gave 2-chloro-4-acetoxybutanal **6**. Condensation of **6** with the pyrimidine **A** in aq. sodium acetate gave the desired product **7** in moderate yield. Conversion of lactams **7**–**9** to the 4-chloro derivatives **10**–**12** was achieved by the well known method using phosphoryl trichloride in the presence of dimethylaniline.[12] This less than satisfactory method, we have found, can be replaced in the case of substrate **7** by use of phosphoryl trichloride alone under carefully controlled conditions (Scheme 2). The 4-chloro compound **12** is obtained in 54% yield with recoverable unchanged starting material. Subsequently we found that hydrolysis of the acetyl protecting group on the C-5 2-hydroxyethyl side chain of the ditoluoyl-protected nucleosides was not selective and a mixture of partially and fully deprotected nucleoside was obtained. Consequently, we chose the C-5 TBDMS ether as an orthogonal protecting group which could be removed selectively in the

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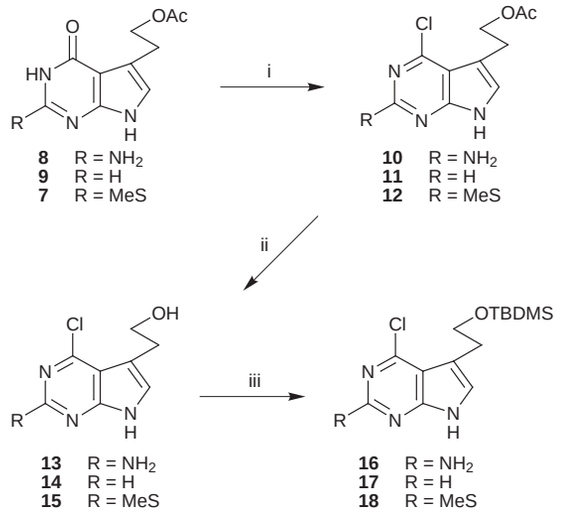


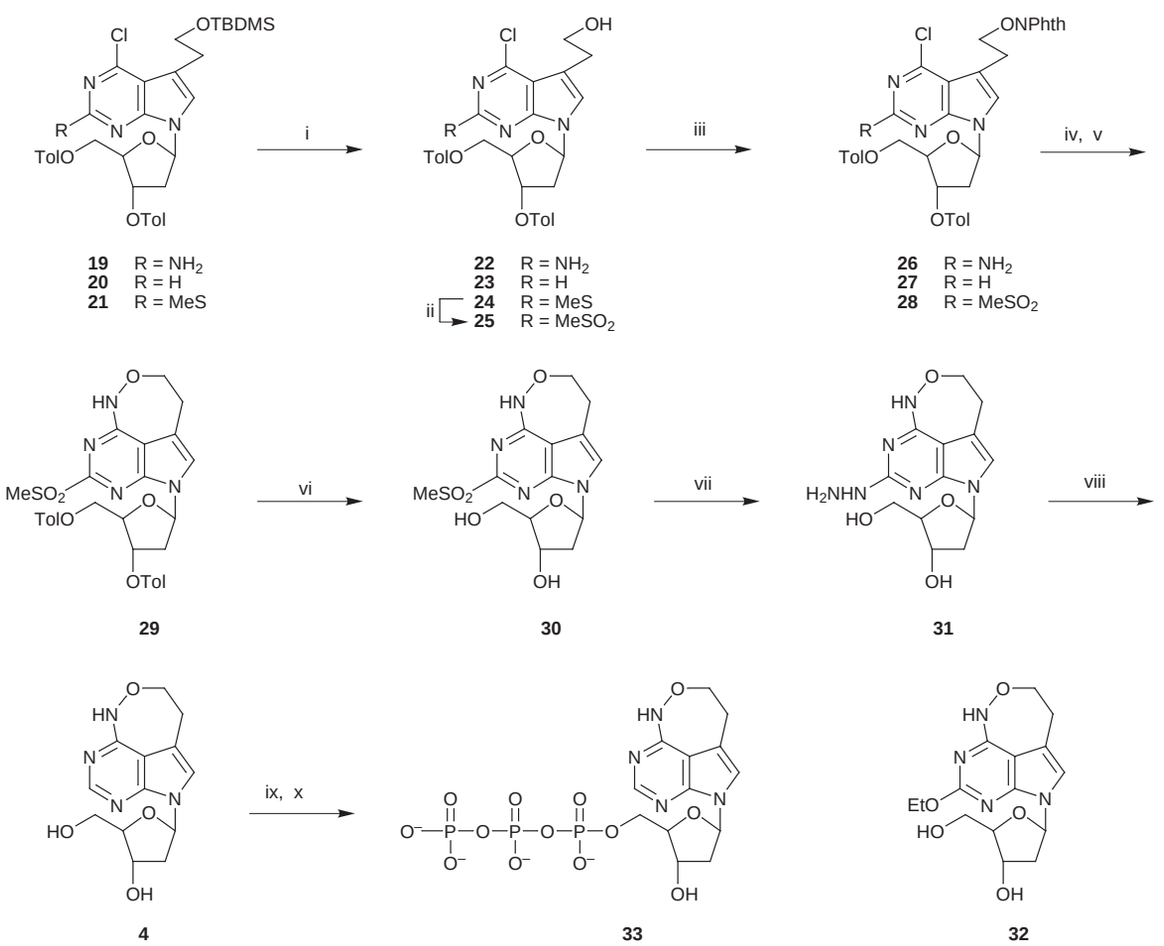
Scheme 2 *Reagents and conditions*: i, $POCl_3$, 50 °C; ii, NH_4OH, MeOH; iii, TBMSCl, imidazole.

presence of the toluoyl ester groups of the sugar following glycosylation.

Deacetylation of the pyrrolopyrimidines **10**–**12** to give the alcohols **13**–**15**, then protection of the hydroxy function by TBDMSCl, gave siloxanes **16**–**18** from which the nucleosides **19**–**21** were formed by coupling of the sodium hydride-derived anions with 3,5-di-*O*-*p*-toluoyl-2-deoxy-α-ribosyl chloride.[13] With pure crystalline sugar the β-glycosides were formed with no contamination from α-anomers. Removal of the TBDMS group with ammonium fluoride in methanol gave the alcohols **22**–**24** in high yield (Scheme 3). The methylsulfanyl derivative **24** was cleanly oxidised by magnesium monoperoxyphthalate to the methyl sulfone **25**. In one experiment underoxidation yielded the corresponding sulfoxide. Each of the hydroxyethyl nucleosides **22**, **23** and **25** underwent Mitsunobu coupling with *N*-hydroxyphthalimide to give the corresponding 5-(phthalimidooxyethyl) nucleosides **26**–**28**.

At this time, whilst working in the N^7-methylpyrrolopyrimidine series,[10] we found that the 2-H series cyclised in only 2% yield, whilst the 2-amino series failed to cyclise under a variety of conditions. Further work was pursued solely with the methylsulfonyl derivative **28**, which was treated with 1,4-dioxane–ammonia or, preferably, by short treatment with hydrazine to remove the phthaloyl group. In the latter case filtration off of the phthaloyl hydrazide and removal of the solvent gave a product running slowly on TLC, evidently the free aminooxy intermediate, which was characterised as its crystalline oxime with acetone. It had been assumed that the methylsulfonyl group would increase the reactivity of the 4-halogeno function and, in itself, more readily undergo nucleophilic displacement. This indeed was the case although the activation was not great. Dissolution of the aminooxy compound in ethanol and heating overnight at 75 °C led to ring closure to give the tricyclic hydroxylamine **29** in moderate yield. This was then deacylated with methanolic ammonia to form the 2-methylsulfonyl nucleoside **30**.

Treatment of sulfone **30** with hot hydrazine afforded the 2-hydrazino derivative **31** in high yield, and from the latter mercury(II) oxide oxidation[14] in water gave the desired nucleoside **4** (R = H). The yield in this latter step was disappointingly low. This was due in part to adsorption of the product on the solid residues; treatment with hydrogen sulfide significantly increased the yield. When the oxidation was carried out in ethanol, the solvent commonly used for this reaction, the major



Scheme 3 *Reagents and conditions*: i, NH_4F; ii, magnesium monoperphthalate; iii, *N*-hydroxyphthalimide, PPh_3, DEAD; iv, H_2NNH_2, MeCN; v, EtOH, 75 °C; vi, MeOH, NH_3; vii, H_2NNH_2, EtOH; viii, HgO, water; ix, $POCl_3$, Me_3PO_4; x, $H_2P_2O_7(Bu^n_3NH)_2$, Bu^n_3N.

product isolable was the 2-ethoxy derivative **32**. The corresponding compound was also observed, albeit as a minor product, for the reaction in the N^7-methyl series.[10]

In attempts to synthesise the required tricyclic nucleoside **5** (R = NH_2) we found that in this case displacement of the methylsulfonyl group by ammonia could not be effected. Nor did it react with acetamide–sodium hydride. This we believe is due to the acidifying effect of the oxygen in the third ring on the NH proton, since this reaction we have found is effective in 2-methylsulfonyl-9-methyladenine.

The NMR spectrum of compound **4** in [$^2H_6$]DMSO showed only one NH resonance, at δ_H 10.65 ppm. There was no evidence of coupling with H-2. We draw the conclusion that this compound is essentially in the adenine-like aminooxy tautomeric form, but we are making a closer study of this question by more sensitive techniques. It is anticipated, however, by comparison with compound **1**, which also shows only one NH resonance, that the compound will show ambivalent base-pairing. To this end the nucleoside has been converted to its 5′-triphosphate **33**.[15] Its substrate properties with various polymerases will be communicated elsewhere.

Experimental

^{1}H NMR spectra were obtained on a Bruker DRX-300 spectrometer for samples in [$^2H_6$]DMSO. *J*-Values are in Hz. Mass spectra were recorded on a Kratos MS890 instrument. UV spectra were recorded on a Perkin-Elmer Lambda 2 spectrophotometer for samples in 10% aq. methanol unless otherwise stated. TLC was carried out on pre-coated F_{254} silica plates, and column chromatography with Merck Kieselgel 60. Mps were measured on a Gallenkamp melting point apparatus (Fisons) and are uncorrected. Reactions were worked up as follows, unless otherwise stated: Reaction mixtures were evaporated to dryness, the product was dissolved in chloroform and the solution was washed with saturated aq. sodium hydrogen carbonate. The organic fractions were combined and dried over anhydrous sodium sulfate, filtered and then evaporated to dryness. Ether refers to diethyl ether.

4-Acetoxy-2-chlorobutanal 6

To a solution of butane-1,4-diol (135 g, 1.5 mol) in pyridine (79 g, 1 mol) at 0 °C was added dropwise acetic anhydride (102 g, 1 mol), and the solution was stirred at rt overnight before being evaporated. The residue was dissolved in chloroform, and the solution was washed with saturated aq. sodium hydrogen carbonate, dried and evaporated to give a clear liquid, which was distilled to give the monoacetate (76–84 °C, 11 mbar‡) (126 g, 64%). Product still contained some diacetate. For characterisation a portion was further purified by column chromatography ($CHCl_3$–5% MeOH); δ_H([$^2H_6$]DMSO) 1.38–1.47 (2H, m, CH_2), 1.53–1.62 (2H, m, CH_2), 1.98 (3H, s, OCH_3), 3.33–3.42 (2H, m, CH_2OH), 3.98 (2H, t, *J* 6.5, CH_2OAc) and 4.41 (1H, t, *J* 5.1, OH).

To a solution of PCC (24 g, 1.5 equiv.) in dichloromethane (200 cm^3) was added dropwise a solution of the monoalcohol acetate (9.8 g) in dichloromethane (20 cm^3) and the solution was stirred at rt for 1.5 h. Ether (200 cm^3) was added, the solution was filtered through Florisil, and the residues were washed with ether (3 × 200 cm^3). The ether solution was evaporated and the residue was distilled to give the aldehyde as a liquid (48–52 °C, 11 mbar) (7.2 g, 75%); δ_H([$^2H_6$]DMSO) 1.76–1.86 (2H, m, CH_2), 1.98 (3H, s, OCH_3), 2.50 (2H, t, *J* 7.1, CH_2), 3.98 (2H, t, *J* 6.4, CH_2O) and 9.66 (1H, s, CHO).

To a solution of the aldehyde (8.15 g) in dry chloroform (100 cm^3) was added dropwise a solution sulfuryl dichloride (1.1 equiv., 5.5 cm^3) in chloroform (25 cm^3) and the solution was stirred at rt overnight before being evaporated, and the residue was then co-evaporated with toluene. Crude yield 10.06 g (98%). The chloro aldehyde was used without further purification. For characterisation a portion of the solution was distilled to give the chloro aldehyde **6** as a liquid (69–74 °C, 11 mbar); δ_H([$^2H_6$]DMSO) 1.60 (2H, m, CH_2), 1.98 (3H, s, OCH_3), 2.76–2.80 (1H, m, CHCl), 4.00 (2H, m, CH_2O) and 9.41 and 9.49 (1H, 2 × s, CHO).

5-(2-Acetoxyethyl)-2-methylsulfanyl-3,7-dihydropyrrolo[2,3-*d*]-pyrimidin-4-one 7

To a suspension of 2-methylsulfanyl-6-aminopyrimidin-4(3*H*)-one[16] **A** (3 g, 19 mmol) and sodium acetate (3.3 g, 40 mmol) in water (100 cm^3) was added freshly prepared chloro aldehyde **6** (4.7 g, 28.6 mmol) in three aliquots at two-hourly intervals at 85 °C and then heating was continued overnight. The solution was cooled, the aqueous layer was decanted, and the product was chromatographed ($CHCl_3$–5% MeOH) to give the product as a pale yellow solid (2.35 g, 46%). The ^{1}H NMR spectrum was as described.[10] λ_{max}/nm 287 (ε 13 000 dm^3 mol^{-1} cm^{-1}) and 224 (18 100); λ_{min} 245; pH 12 λ_{max} 281 (12 300).

5-(2-Acetoxyethyl)-4-chloro-2-methylsulfanyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine 12

The above pyrrolopyrimidinone **7** (3.7 g, 13.8 mmol) was suspended in phosphoryl trichloride (20 cm^3) and the solution was heated at 45–50 °C overnight before being cooled, poured onto ice, stirred for 10 min and then neutralised with conc. aq. ammonia. The solid was filtered off, and recrystallised from methanol to give a yellow-brown solid, which was chromatographed ($CHCl_3$–2% MeOH) to give the product **12** as a pale brown solid (1.3 g). Unchanged starting material was eluted from the column in $CHCl_3$–5% MeOH (1.44 g). Overall yield 54%. The ^{1}H NMR spectrum was as described;[10] λ_{max}/nm 314 (4800), 254 (28 300) and 222 (13 000); pH 12 λ_{max}/nm 291 (5100) and 257 (26 700). Accurate mass measurement *m*/*z*, 285.0339. $C_{11}H_{12}ClN_3O_2S$ requires *M*, 285.0356.

4-Chloro-5-(2-hydroxyethyl)-2-methylsulfanyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine 15

The above pyrrolopyrimidine **12** (2.14 g, 7.5 mmol) was suspended in 20 cm^3 methanol, 0.880 ammonia (10 cm^3) was added and the solution was stirred at 40 °C overnight. The solvent was removed, the product was suspended in methanol and the mixture was filtered to give a pale yellow solid (0.71 g). A further crop of 1.05 g was isolated by concentration of the mother liquors. Overall yield 1.76 g (96%); δ_H([$^2H_6$]DMSO) 2.52 (3H, s, SCH_3), 2.91 (2H, t, *J* 7.1, OCH_2CH_2), 3.65 (2H, t, *J* 6.9, OCH_2), 4.68 (1H, t, *J* 5.4, OH), 7.27 (1H, s, H-6) and 12.12 (1H, s, NH); *m*/*z* 244 (M + H)$^+$. Accurate mass measurement *m*/*z* 244.03160. $C_{12}H_{11}ClN_3OS$ requires *m*/*z* 244.03113 (deviation 1.9 ppm).

2-Amino-5-[2-(*tert*-butyldimethylsiloxy)ethyl]-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine 16

5-(2-Acetoxyethyl)-2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine **10**[10] (1.3 g, 5.74 mmol) was stirred in methanol–0.880 ammonia (1 : 1; 150 cm^3) for 6 h at rt. The solution was then evaporated and the residue was dried by evaporation of dry pyridine (3 × 50 cm^3). The hydroxyethyl derivative **13** was then dissolved in dry pyridine (130 cm^3), and TBDMSCl (1.3 g, 8.64 mmol) was added. The solution was stirred overnight, evaporated, and residual pyridine was removed by co-evaporation with water (2 × 20 cm^3). The reaction mixture was worked up as described and the crude product was purified by silica gel chomatography; elution with a gradient of 0–1% methanol in dichloromethane gave a cream-coloured solid (1.34 g, 71%). Crystallisation from chloroform gave compound

‡ 1 bar = 10^5 Pa.

16 as plates (Found: C, 51.4; H, 7.2; N, 17.4. $C_{14}H_{23}ClN_4OSi$ requires C, 51.45; H, 7.04; N, 17.1%); $\delta_H([^2H_6]DMSO)$ −0.03 (6H, s, 2 × CH_3), 0.83 (9H, s, 3 × CH_3), 2.86 (2H, t, *J* 7.1, OCH_2CH_2), 3.78 (2H, t, *J* 7.1, OCH_2), 6.44 (2H, br s, NH_2), 6.88 (1H, s, H-6) and 11.22 (1H, br s, NH).

5-[2-(*tert*-Butyldimethylsiloxy)ethyl]-4-chloro-7*H*-pyrrolo-[2,3-*d*]pyrimidine 17

5-(2-Acetoxyethyl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine **11**[10] (350 mg, 1.66 mmol) was deprotected with methanol–aq. ammonia as in the preparation of compound **16**, and the product was dried by evaporation of dry pyridine. The residue **14** was dissolved in dry pyridine (80 cm^3) and TBDMSCl (1.7 g, 11.3 mmol) was added. The solution was stirred overnight, evaporated, and residual pyridine was removed by co-evaporation with water (2 × 20 cm^3). The reaction product was worked up as described and chromatographed (CH_2Cl_2) to give a cream-coloured solid (2.65 g, 96%). Crystallisation from cyclohexane gave needles, mp 161–162 °C (Found: C, 54.0; H, 7.3; N, 13.6. $C_{14}H_{22}ClN_3OSi$ requires C, 53.9; H, 7.1; N, 13.5%); $\delta_H([^2H_6]DMSO)$ −0.05 (6H, s, 2 × CH_3), 0.83 (9H, s, 3 × CH_3), 3.02 (2H, t, *J* 7.0, OCH_2CH_2), 3.85 (2H, t, *J* 7.0, OCH_2), 7.46 (1H, s, 6-H), 8.50 (1H, s, 2-H) and 12.3 (1H, br s, NH).

5-[2-(*tert*-Butyldimethylsiloxy)ethyl]-4-chloro-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 18

The hydroxyethylpyrrolopyrimidine **15** (1.91 g, 7.8 mmol) was dissolved in DMF (25 cm^3), TBDMSCl (1.77 g, 11.7 mmol) and imidazole (1.6 g, 23.5 mmol) were added and the solution was stirred at rt for 6 h. The solvent was removed and the product was dissolved in chloroform, the solution was washed with water and the solvent was evaporated. The product was precipitated by trituration with methanol, filtered off and dried to give an off-white solid. A second fraction was obtained by concentration of the mother liquors. Yield 2.51 g (89%); $\delta_H([^2H_6]DMSO)$ −0.07 (6H, s, 2 × CH_3), 0.80 [9H, s, $C(CH_3)_3$], 2.52 (3H, s, SCH_3), 2.94 (2H, t, *J* 7, OCH_2CH_2), 3.78 (2H, t, *J* 7, OCH_2), 7.29 (1H, s, H-6) and 12.10 (1H, s, NH); *m*/*z* 358.1 $(M + H)^+$. Accurate mass measurement *m*/*z*, 358.11920. $C_{15}H_{25}ClN_3OSSi$ requires *m*/*z*, 358.11761 (deviation 4.5 ppm).

5-[2-(*tert*-Butyldimethylsiloxy)ethyl]-4-chloro-7-(3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 21

The above pyrrolopyrimidine **18** (3.3 g, 9.2 mmol) was suspended in acetonitrile (50 cm^3), sodium hydride was added (60%; 0.45 g, 11.2 mmol) and the solution was stirred at rt for 30 min. To this was then added 3,5-di-*O*-*p*-toluoyl-2-deoxy-α-ribosyl chloride (4.3 g, 11 mmol) and the solution was stirred for a further 1.5 h. The solution was evaporated, the residue was worked up as usual and the product was chromatographed (hexane–ether, 3:1) to give a foam (5.56 g, 88%); $\delta_H([^2H_6]$-DMSO) −0.13 (6H, s, 2 × $SiCH_3$), 0.74 [9H, s, $C(CH_3)_3$], 2.33 (3H, s, tol-CH_3), 2.37 (3H, s, tol-CH_3), 2.55 (3H, s, SCH_3), 2.72–2.75 (1H, m, H-2′), 2.84 (2H, t, *J* 6.6, OCH_2CH_2), 2.98–3.29 (1H, m, H′-2′), 3.71 (2H, t, *J* 6.7, OCH_2), 4.45–4.61 (3H, m, H-4′, H-5′), 5.71–5.73 (1H, m, H-3′), 6.67 (1H, t, *J* 7, H-1′), 7.24–7.33 (4H, m, tol-H), 7.46 (1H, s, H-6), and 7.80–7.93 (4H, m, tol-H); *m*/*z* (FAB) 710.6 $(M + H)^+$. Accurate mass measurement *m*/*z*, 710.24720. $C_{36}H_{45}ClN_3O_6SiS$ requires *m*/*z*, 710.24573 (deviation 2.1 ppm).

2-Amino-4-chloro-7-[3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl]-5-(2-hydroxyethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 22

A stirred suspension of compound **16** (1.06 g, 3.23 mmol) in dry acetonitrile (70 cm^3) under argon was treated with sodium hydride (60% in oil; 142 mg, 3.55 mmol). After 30 min, 3,5-di-*O*-*p*-toluoyl-2-deoxy-α-ribosyl chloride (1.57 g, 4.04 mmol) was added and stirring was continued for a further 30 min. The solution was evaporated *in vacuo* and the residue was partitioned between chloroform (600 cm^3) and water (60 cm^3). The organic phase was evaporated and the crude was product purified by silica gel chomatography and elution with a gradient of 0–1% methanol in dichloromethane to afford the TBDMS-protected nucleoside **19** as a pale yellow foam. This was then heated overnight at 60 °C in methanol (175 cm^3) containing ammonium fluoride (1.30 g, 35 mmol). The solution was then evaporated, the residue was worked up as described, and the crude product was purified by silica gel chomatography and elution with chloroform to give a solid (1.47 g, 81%), mp 171.5–172.5 °C; $\delta_H([^2H_6]DMSO)$ 2.39 (3H, s, tol-CH_3), 2.41 (3H, s, tol-CH_3), 2.61 (1H, m, H-2′), 2.78 (2H, t, *J* 7.1, OCH_2CH_2), 2.87 (1H, m, H′-2′), 3.56 (2H, m, CH_2O), 4.51 (2H, m, H_2-5′), 4.60 (1H, m, H-4′), 4.65 (1H, t, *J* 5.0, OH), 5.66 (1H, m, H-3′), 6.52 (1H, t, *J* 5.9, H-1′), 6.73 (2H, s, NH_2), 7.06 (1H, s, H-6), 7.35 (2H, d, *J* 8.2, 2 × tol-H), 7.38 (2H, d, *J* 8.2, 2 × tol-H), 7.90 (2H, d, *J* 8.2, 2 × tol-H) and 7.93 (2H, d, *J* 8.2, 2 × tol-H); *m*/*z* (FAB) 565.2 $(M + H)^+$. Accurate mass measurement *m*/*z*, 565.1841. $C_{29}H_{30}ClN_4O_6$ requires *m*/*z*, 565.1745 (deviation 17 ppm).

4-Chloro-7-[3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl]-5-(2-hydroxyethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 23

Compound **17** (397 mg, 1.27 mmol) as a solution in dry acetonitrile (25 cm^3) was treated with sodium hydride (60% in oil; 62 mg, 1.54 mmol) and the chlorohalogenose (680 mg, 1.75 mmol) as for the preparation of compound **22**. The solution was then evaporated and the product was worked up as described. The majority of nonpolar impurities were removed by silica gel chromatography (elution with dichloromethane) to afford the TBDMS-protected nucleoside **20** as a pale yellow foam. This was then desilylated as for the preparation of compound **22**, with ammonium fluoride (518 mg, 14 mmol) in methanol (70 cm^3). Purification by silica gel chomatography and elution with chloroform gave a foam (419 mg, 60%); $\delta_H([^2H_6]DMSO)$ 2.37 (3H, s, tol-CH_3), 2.40 (3H, s, tol-CH_3), 2.70–2.74 (1H, m, H-2′), 2.93 (2H, t, *J* 6.8, OCH_2CH_2), 3.01–3.10 (1H, m, H-2′), 3.62 (2H, m, CH_2O), 4.51–4.59 (3H, m, H-4′, H_2-5′), 4.64 (1H, t, *J* 5.0, OH), 5.75 (1H, m, H-3′), 6.74 (1H, dd *J* 7.0, H-1′), 7.31 (2H, d, *J* 8.2, 2 × tol-H), 7.36 (2H, d, *J* 8.2, 2 × tol-H), 7.67 (1H, s, H-6), 7.86 (2H, d, *J* 8.2, 2 × tol-H), 7.94 (2H, d, *J* 8.2, 2 × tol-H) and 8.57 (1H, s, H-2); *m*/*z* (FAB) 550.2 $(M + H)^+$. Accurate mass measurement *m*/*z*, 550.1786. $C_{29}H_{29}ClN_3O_6$ requires *m*/*z*, 550.1745 (deviation 7.5 ppm).

4-Chloro-7-[3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl]-5-(2-hydroxyethyl)-2-methylsulfanyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 24

Compound **18** (5.86 g, 16.4 mmol) as a solution in dry acetonitrile (200 cm^3) was treated with sodium hydride (60% in oil; 720 mg, 18 mmol) and the chlorohalogenose (8.94 g, 23 mmol). Purification by silica gel chomatography and elution with dichloromethane gave the TBDMS-protected nucleoside as a foam. This was then desilylated as for the preparation of compound **22**, with ammonium fluoride (6.07 g, 164 mmol) in methanol (500 cm^3). Purification by silica gel chomatography and elution with chloroform gave a foam (7.15 g, 73%); (Found: C, 60.3; H, 4.9; N, 7.0. $C_{30}H_{30}ClN_3O_6S$ requires C, 60.4; H, 5.1; N, 7.0%); $\delta_H([^2H_6]DMSO)$ 2.36 (3H, s, tol-CH_3), 2.39 (3H, s, tol-CH_3), 2.78–2.86 (1H, m, H-2′), 2.96 (2H, t, *J* 6.9, OCH_2CH_2), 3.07–3.17 (1H, m, H′-2′), 3.43 (3H, s, CH_3SO_2), 3.63 (2H, m, CH_2O), 4.55–4.59 (2H, m, H_2-5′), 4.63 (1H, t, *J* 5.0, OH), 4.77–4.71 (1H, m, H-4′), 5.74 (1H, m, H-3′), 6.77 (1H, t, *J* 7.0, H-1′), 7.23 (1H, s, H-6), 7.26 (2H, d, *J* 8.2, 2 × tol-H), 7.29 (2H, d, *J* 8.2, 2 × tol-H), 7.93 (2H, d, *J* 8.2, 2 × tol-H) and 7.98 (2H, d, *J* 8.2, 2 × tol-H); *m*/*z* (FAB) 596.2 $(M + H)^+$. Accurate mass

measurement *m*/*z*, 596.1650. $C_{30}H_{31}ClN_3O_6S$ requires *m*/*z*, 596.1622 (deviation 4.7 ppm).

4-Chloro-7-(3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-(2-hydroxyethyl)-2-methylsulfonyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 25

The methylsulfanyl derivative **24** (3.9 g, 6.5 mmol) was suspended in ethanol (300 cm^3); to this was added aq. magnesium monoperoxyphthalate (6.5 g, 13 mmol in 100 cm^3) and the solution was stirred at 50 °C for 4 h before being concentrated and worked up as usual to give a foam, which was chromatographed ($CHCl_3$–1% MeOH) to give compound **25** as a foam (3.90 g, 95%), mp 157–158 °C; $\delta_H([^2H_6]DMSO)$ 2.36 (3H, s, tol-CH_3), 2.39 (3H, s, tol-CH_3), 2.78–2.86 (1H, m, H-2′), 2.95 (2H, t, *J* 6.8, OCH_2CH_2), 3.11–3.16 (1H, m, H′-2′), 3.43 (3H, s, SO_2CH_3), 3.62 (2H, t, *J* 6.8, OCH_2), 4.49–4.68 (3H, m, H-4′, H_2-5′), 4.76 (1H, t, *J* 5.4, OH), 5.76–5.78 (1H, m, H-3′), 6.77 (1H, t, *J* 6.8, H-1′), 7.26–7.37 (4H, m, tol-H), 7.96 (1H, s, H-6) and 7.79–8.31 (4H, m, tol-H); λ_{max}/nm 247 (42 000); λ_{min}/nm 220; pH 1 λ_{max}/nm 249 (36 500); pH 12 λ_{max}/nm 243 (41 300); *m*/*z* (FAB) 628.3 $(M + H)^+$. Accurate mass measurement *m*/*z*, 628.15800. $C_{30}H_{31}ClN_3O_8$ requires *m*/*z*, 628.16398 (deviation −9.5 ppm).

On one occasion the oxidation did not go to completion and the sulfoxide 4-chloro-7-(3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-(2-hydroxyethyl)-2-methylsulfinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine was isolated during the chromatography; $\delta_H([^2H_6]DMSO)$ (Most peaks show diastereoisomeric pairs) 2.36 (3H, s, tol-CH_3), 2.39 (3H, s, tol-CH_3), 2.79–2.85 (1H, m, H-2′), 2.92 (3H, s, SCH_3), 2.94 (2H, t, *J* 6.9, OCH_2CH_2), 3.07–3.11 (1H, m, H′-2′), 3.63 (2H, t, *J* 6.9, OCH_2), 4.51–4.67 (3H, m, H-4′, H_2-5′), 4.74 (1H, t, OH), 5.76–5.79 (1H, m, H-3′), 6.73–6.79 (1H, t, *J* 6.8, H-1′), 7.28–7.38 (4H, m, tol-H), 7.79–7.98 (4H, m, tol-H) and 7.82 (1H, s, H-6); λ_{max}/nm 244 (34 000); λ_{min}/nm 220; pH 12 λ_{max}/nm 240 (38 400); *m*/*z* (FAB) 612.2 $(M + H)^+$. Accurate mass measurement *m*/*z*, 612.16020. $C_{30}H_{31}ClN_3O_7S$ requires *m*/*z*, 612.16329 (deviation −5.1 ppm).

2-Amino-4-chloro-7-[3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl]-5-(2-phthalimidooxyethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 26

Compound **22** (362 mg, 0.64 mmol) was suspended in dry THF (15 cm^3) containing triphenylphosphine (211 mg, 0.8 mmol) and *N*-hydroxyphthalimide (132 mg, 0.8 mmol). DEAD (126 mm^3, 0.8 mmol) was then added and the solution was stirred for 2 h at room temp. The mixture was then evaporated off and the product was worked up as usual. Purification by silica gel chomatography and elution with chloroform gave a foam. Crystallisation from ethanol gave yellow needles of *compound* **26** (278 mg, 61%), mp 186–187 °C (Found: C, 62.2; H, 4.4; N, 9.8. $C_{37}H_{32}ClN_5O_8$ requires C, 62.6; H, 4.5; N, 9.9%); $\delta_H([^2H_6]DMSO)$ 2.34 (3H, s, tol-CH_3), 2.43 (3H, s, tol-CH_3), 2.62–2.69 (1H, m, H-2′), 2.93–3.02 (1H, m, H′-2′), 3.24 (2H, m, OCH_2CH_2), 4.43 (2H, m, CH_2O), 4.56–4.65 (2H, m, H_2-5′), 4.77 (1H, m, H-4′), 5.79 (1H, m, H-3′), 6.57 (2H, br s, NH_2), 6.63 (1H, dd, *J* 5.9, H-1′), 7.16 (2H, d, *J* 8.2, 2 × tol-H), 7.27 (2H, d, *J* 8.2, 2 × tol-H), 7.42 (1H, s, H-6), 7.72–7.79 (2H, m, 2 × Phth-H), 7.84–7.80 (2H, m, 2 × Phth-H), 7.97 (2H, d, *J* 8.2, 2 × tol-H), 7.99 (2H, d, *J* 8.2, 2 × tol-H).

4-Chloro-7-[3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl]-5-(2-phthalimidooxyethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 27

Compound **23** (318 mg, 0.58 mmol) was suspended in dry THF (12 cm^3) containing triphenylphosphine (158 mg, 0.6 mmol) and *N*-hydroxyphthalimide (98 mg, 0.6 mmol). DEAD (95 mm^3, 0.6 mmol) was then added and the solution was stirred for 2 h at rt. After this time the product was worked up as for compound **26**. The product was purified by silica gel chomatography and eluted with chloroform to give a foam. Crystallisation from ethanol gave needles (284 mg, 71%), mp 148–149 °C (Found: C, 62.1; H, 4.2; N, 7.8. $C_{37}H_{31}ClN_4O_8 \cdot H_2O$ requires C, 62.3; H, 4.6; N, 7.9%); $\delta_H([^2H_6]DMSO)$ 2.36 (3 H, s, tol-CH_3), 2.39 (3 H, s, tol-CH_3), 2.78–2.86 (1 H, m, H-2′), 3.07–3.17 (1 H, m, H-2′), 3.25 (2 H, t, *J* 6.7, OCH_2CH_2), 4.38 (2 H, m, CH_2O), 4.50–4.67 (3 H, m, H-4′, H_2-5′), 5.80 (1 H, m, H-3′), 6.79 (1 H, t, *J* 6.9, H-1′), 7.31 (2 H, d, *J* 8.2, 2 × tol-H), 7.37 (2 H, d, *J* 8.2, 2 × tol-H), 7.70 (1 H, s, H-6), 7.75 (2 H, d, *J* 8.2, 2 × tol-H), 7.86 (4 H, s, 4 × Phth H), 7.96 (2 H, d, *J* 8.2, 2 × tol-H) and 8.62 (1 H, s, H-2).

4-Chloro-5-(2-phthalimidooxyethyl)-2-methylsulfonyl-7-(3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine 28

To a solution of the nucleoside **25** (3.67 g, 5.9 mmol), triphenylphosphine (2.3 g, 8.8 mmol) and *N*-hydroxyphthalimide (1.43 g, 8.8 mmol) in THF (50 cm^3) was added DEAD (1.54 g, 8.8 mmol) and the solution was stirred at rt overnight. The solution was evaporated, the residue was worked up as usual, and the product was chromatographed ($CHCl_3$–1%, MeOH) to give a product, which was recrystallised from ethanol to give a solid (2.79 g, 62%); $\delta_H([^2H_6]DMSO)$ 2.28 (3H, s, tol-CH_3), 2.38 (3H, s, tol-CH_3), 2.85–2.91 (1H, m, H-2′), 3.06–3.14 (1H, m, H′-2′), 3.27 (2H, t, *J* 6.6, OCH_2CH_2), 3.45 (3H, s, SO_2CH_3), 4.40 (2H, t, *J* 6.6, OCH_2), 4.51–4.56 (1H, m, H-4′), 4.61–4.69 (2H, m, H_2-5′), 5.78–5.80 (1H, m, H-3′), 6.79 (1H, t, *J* 6.7, H-1′), 7.16–7.36 (4H, m, tol-H), 7.72–8.00 (4H, m, tol-H), 7.85 (4H, br s, phthaloyl) and 8.24 (1H, s, H-6); *m*/*z* (FAB) 773.7 $(M + H)^+$. Accurate mass measurement *m*/*z*, 773.17330. $C_{38}H_{34}ClN_4O_{10}S$ requires *m*/*z*, 773.17820 (deviation −6.4 ppm).

2-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-4-methylsulfonyl-2,6,8,9-tetrahydro-7-oxa-2,3,5,6-tetraazabenz[*cd*]azulene 29

To a solution of the phthalimido derivative **28** (2.4 g, 3.1 mmol) in acetonitrile (50 cm^3) was added anhydrous hydrazine (107 mm^3, 3.4 mmol) and the solution was stirred at rt for 1 h. The phthalic hydrazide was filtered off and washed with acetonitrile and then the solution was evaporated to dryness. The slow-running product on TLC was dissolved in ethanol (50 cm^3) and the solution was heated at 75 °C overnight before being evaporated; the product was worked up as usual and chromatographed (ethyl acetate–hexane, 1 : 1) to give a solid (0.63 g, 33%), mp 173–174.5 °C (Found: C, 58.4; H, 4.7; N, 8.9. $C_{30}H_{30}N_4O_8S \cdot 0.5 H_2O$ requires C, 58.3; H, 5.1; N, 9.2%); $\delta_H([^2H_6]DMSO)$ 2.36 (3H, s, tol-CH_3), 2.39 (3H, s, tol-CH_3), 2.73–2.79 (1H, m, H-2′), 2.90 (2H, m, OCH_2CH_2), 3.03–3.09 (1H, m, H′-2′), 3.31 (3H, s, SO_2CH_3), 4.33 (2H, m, OCH_2), 4.47–4.56 (2H, m, H_2-5′), 4.62–4.66 (1H, m, H-4′), 5.74–5.76 (1H, m, H-3′), 6.69 (1H, t, *J* 6.8, H-1′), 7.29–7.38 (4H, m, tol-H), 7.57 (1H, s, H-6§), 7.84–7.96 (4H, m, tol-H) and 11.52 (1H, NH); λ_{max}/nm 309 (8200) and 238 (43 000), λ_{min}/nm 225; pH 12 λ_{max}/nm 240, 287 (10 300) and 339 (4100); *m*/*z* (FAB) 607.4 $(M + H)^+$. Accurate mass measurement *m*/*z*, 607.18860. $C_{30}H_{31}N_4O_8S$ requires *m*/*z*, 607.19096 (deviation −3.9 ppm).

To characterise the slow-running material on TLC its *acetone oxime* was prepared: 250 mg of the crude oxyamino derivative (derived from deprotection of the phthalimido compound with hydrazine) was treated with acetone in ethanol and the mixture was stirred at rt overnight. The solvent was evaporated off and the product was chromatographed ($CHCl_3$–1% MeOH) to give the oxime as a solid, $\delta_H([^2H_6]DMSO)$ 1.72 (3H, s, N=CCH_3), 1.77 (3H, s, N=CCH_3), 2.36 (3H, s, tol-CH_3), 2.39 (3H, s, tol-CH_3), 2.80–2.86 (1H, m, H-2′), 3.05–3.13 (3H, m, H′-2′, OCH_2CH_2), 3.43 (3H, s, SO_2CH_3), 4.15 (2H, t, *J* 6.7, OCH_2),

§ NMR locants for compounds **29**–**33** and **4** are kept the same as for the bicyclic-nucleobase compounds **7**–**28** for consistency.

4.48–4.52 (1H, m, H-4′), 4.59–4.68 (2H, m, H_2-5′), 5.76–5.78 (1H, m, H-3′), 6.78 (1H, t, *J* 6.8, H-1′), 7.27–7.38 (4H, m, tol-H), 7.94 (1H, s, H-6) and 7.80–7.99 (4H, m, tol-H); mp 123–124 °C; λ_{max}/nm 243 (43 100); λ_{min}/nm 225; pH 1 λ_{max}/nm 251 (41 250); pH 12 λ_{max}/nm 250 (43 900); *m*/*z* (FAB) 683.4 $(M + H)^+$. Accurate mass measurement *m*/*z*, 683.18870. $C_{33}H_{36}ClN_4O_8S$ requires *m*/*z*, 683.18318 (deviation 8.1 ppm).

2-(2-Deoxy-β-D-ribofuranosyl)-4-methylsulfonyl-2,6,8,9-tetrahydro-7-oxa-2,3,5,6-tetraazabenz[*cd*]azulene 30

A solution of the nucleoside **29** (0.63 g, 1.04 mmol) in methanolic ammonia (25 cm^3) was stirred at rt overnight. The solvent was removed, and the product was chromatographed ($CHCl_3$–10% MeOH) to give the *product* **30** as a foam (0.28 g, 80%); $\delta_H([^2H_6]DMSO)$ 2.20–2.34 (1H, m, H-2′), 2.49–2.54 (1H, m, H′-2′), 2.97–3.00 (2H, m, OCH_2CH_2), 3.29 (3H, s, SO_2CH_3), 3.47–3.55 (2H, m, OCH_2), 3.82–3.83 (1H, m, H-4′), 4.35 (3H, br m, H-3′, H_2-5′), 4.89 (1H, t, 5′-OH), 5.33 (1H, d, 3′-OH), 6.57 (1H, t, *J* 7, H-1′), 7.62 (1H, s, H-6) and 11.43 (1H, NH); λ_{max}/nm 313 (5300), 232 (18 800) and 280 (sh); λ_{min}/nm 255; pH 12 λ_{max}/nm 232, 287 (5100) and 313 (5400); pH 1 λ_{max}/nm 289 (8900) and 338 (3500); *m*/*z* (FAB) 371.1 $(M + H)^+$. Accurate mass measurement (M + H), 371.10330. $C_{15}H_{19}N_4O_6S$ requires *m*/*z*, 371.10252 (deviation 2.1 ppm).

2-(2-Deoxy-β-D-ribofuranosyl)- 2,6,8,9-tetrahydro-7-oxa-2,3,5,6-tetraazabenz[*cd*]azulene 4

The deprotected tricyclic nucleoside **30** (270 mg, 0.8 mmol) was dissolved in ethanol (15 cm^3) and to this was added hydrazine (0.5 cm^3) and the solution was heated at 80 °C under nitrogen in a sealed bottle for 24 h. The solvent was then removed, the sole product **31** was re-dissolved in water (10 cm^3), yellow mercury(II) oxide (0.86 g, 4 mmol) was added in 4 portions over a period of 1 h at 95 °C, and then the mixture was heated at reflux for a further 2 h before being cooled, diluted with water and hydrogen sulfide gas was bubbled into the solution for 2 min. The mixture was then filtered through Celite, the filtrate was evaporated, and the residue was chromatographed ($CHCl_3$–10% MeOH) to give the *title product* **4** as a pale yellow solid (31 mg, 13%); $\delta_H([^2H_6]DMSO)$ 2.10–2.19 (1H, m, H-2′), 2.43–2.55 (1H, m, H′-2′), 2.90–2.99 (2H, m, OCH_2CH_2), 3.41–3.57 (2H, m, H_2-5′), 3.79–3.82 (1H, m, H-4′), 4.29–4.34 (3H, m, H-3′, OCH_2), 4.99 (1H, t, 5′-OH), 5.27 (1H, d, 3′-OH), 6.55 (1H, t, *J* 7, H-1′), 7.36 (1H, s, H-6), 8.20 (1H, s, H-2) and 10.65 (1H, s, NH); λ_{max}/nm 286 (5000); λ_{min}/nm 255; pH 1 λ_{max}/nm 290 (5850) and 290 (9800); λ_{min}/nm 260; pH 12 λ_{max}/nm 290 (8700); λ_{min}/nm 250; ε_{260} (μM) 2.9, ε_{280} (μM) 4.7; *m*/*z* (FAB) 292.1 (M^+). Accurate mass measurement M^+, 292.1179. $C_{13}H_{16}N_4O_4$ requires *M*, 292.1171 (deviation 2.7 ppm).

2-(2-Deoxy-β-D-ribofuranosyl)-4-ethoxy-2,6,8,9-tetrahydro-7-oxa-2,3,5,6-tetraazabenz[*cd*]azulene 32

Compound **29** (607 mg, 1 mmol) was heated in a sealed bottle with anhydrous hydrazine (2 cm^3) in dry ethanol (25 cm^3) at 100 °C for 24 h. The solution was then evaporated and residual hydrazine was removed by co-evaporation with water to give the crude hydrazino derivative (streaks from baseline on silica TLC). The residue was then heated to reflux in 25% aq. ethanol (30 cm^3) and yellow mercury(II) oxide (2.17 mg, 10 mmol) was added in four portions over a period of 1 h. After refluxing for a further 2 h, the reaction mixture was cooled and hydrogen sulfide gas was bubbled into it for 5 min. The mixture was then filtered though Celite and the solid was washed with hot ethanol (100 cm^3). The combined filtrates were evaporated and the residue was purified twice by silica gel chomatography and elution with a gradient of 0–3% methanol in chloroform to give compound **32** as a pale brown solid (73 mg, 25%); $\delta_H([^2H_6]DMSO)$ 1.27 (3H, t, CH_3), 2.09–2.17 (1H, m, H-2′), 2.42–2.48 (1H, m, H′-2′), 2.88 (2H, m, OCH_2CH_2), 3.77 (2H, m, CH_2O), 3.44–3.54 (3H, m, H-4′, H_2-5′), 4.26 (2H, q, CH_3CH_2O), 4.30 (1H, m, H-3′), 4.92 (1H, m, 5′-OH), 5.28 (1H, m, 3′-OH), 6.41 (1H, t, *J* 6.9, H-1′), 7.11 (1H, s, H-6) and 10.59 (1H, s, NH); *m*/*z* (EI) 336.1 (M^+). Accurate mass measurement M^+, 336.1419. $C_{15}H_{20}N_4O_5$ requires *M*, 336.1434 (deviation −4.5 ppm).

2-(2-Deoxy-β-D-ribofuranosyl)-2,6,8,9-tetrahydro-7-oxa-2,3,5,6-tetraazabenz[*cd*]azulene 5′-triphosphate 33

To a solution of the nucleoside **4** (48.5 mg, 165 μmol) in trimethyl phosphate (0.5 cm^3) at 0 °C was added phosphoryl trichloride (19 mm^3, 200 μmol), and the solution was stirred at 0 °C for 2 h. To this were then added 0.5 M bis-*n*-tributylammonium pyrophosphate in DMF (825 mm^3, 413 μmol) and tri-*n*-butylamine (100 mm^3, 420 μmol) in DMF (200 mm^3) and the solution was stirred at rt for 10 min. The solution was then neutralised with 1.0 M tetraethylammonium bromide (TEAB) and stirred at rt for 3 h. The crude triphosphate was applied to a Sephadex A25 column and eluted with a linear gradient of 0.05 to 1.0 M TEAB (pH 7), the triphosphate eluting between 0.48 and 0.53 M TEAB. The solution was lyophilised to give the title product as a pale yellow solid, yield 245 A_{282} units (52 μmol, 32%); $\delta_H(^2H_2O)$ −5.85 (d, γ-P), −10.71 (d, α-P) and −22.04 (t, β-P).

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