The first example of a new type of acyclic, achiral nucleoside analogue: 1-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]thymine

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Various preparative routes to 1-(dihydroxyalk-1-enyl)thymines, which are acyclic, achiral nucleoside analogues, have been examined, and a successful synthesis of 1-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]thymine (**1**, B = T) has been devised.

Introduction

Nucleoside analogues are of considerable interest both as antiviral and antitumour agents,[1] and as monomer precursors for the generation of antisense oligonucleotides, which are short single-stranded nucleic acids that bind to a specific messenger RNA and thereby interfere with its translation to protein.[2] Well known examples of antiviral nucleoside analogues in clinical use are the acyclic herpes virus drug acyclovir[3] and the cyclic HIV drug 3′-azido-2′,3′-dideoxythymidine (AZT).[4] In the antisense field many nucleoside analogues have been examined, although so far only one deoxyoligonucleoside phosphorothioate, containing only unmodified nucleosides, has been approved as a drug.[5] Promising nucleoside analogues for antisense use which confer improved binding to RNA and high nuclease resistance on the oligonucleotide are the acyclic PNA,[6] the cyclic anhydrohexitol nucleosides,[7] and the recently introduced bicyclic LNA.[8] We became interested in nucleoside analogues **1** (Fig. 1), where the ribose sugar is substituted by a simple acyclic, achiral unit. According to Dreiding models this unit positions the nucleobase and the two hydroxy groups close to the positions of the nucleobases and the 3′- and 5′-OH groups in ribonucleosides in the North conformation prevailing in A-type double helices.[9] The double bond of **1** restricts the possible conformations of the acyclic unit, and oligonucleotides prepared from **1** might therefore be preorganised to form A-type double helices with RNA, which usually result in increased duplex stability.[10] In addition, compounds **1** might have interesting antiviral properties. To the best of our knowledge, no compounds with the structure **1** or substituted compounds **2** are known, apart from two acetal-protected compounds **3**[11] (Fig. 1). However, many structurally related non-sugar analogues with one or two hydroxy groups are known, *e.g.* the alk-1-enyl nucleobases **4**–**8**, the allenes **9** and **10**, the alk-2-enyl compounds **11** and **12**, the cyclopropane derivatives **13**–**16**, and the cyclobutane compounds **17** and **18**, analogues of the natural compound oxetanocin **19** (Fig. 2). Most of these compounds have been screened for antiviral properties, but we are only aware of antisense studies for compounds **17**–**19**.[26–28] We present here our synthetic efforts to prepare compounds with the structure **1** or **2**, including the successful preparation of 1-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]thymine (**1**, B = T).

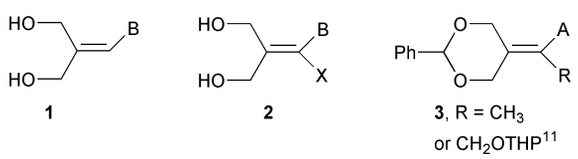

Fig. 1 Target molecules and known analogues.

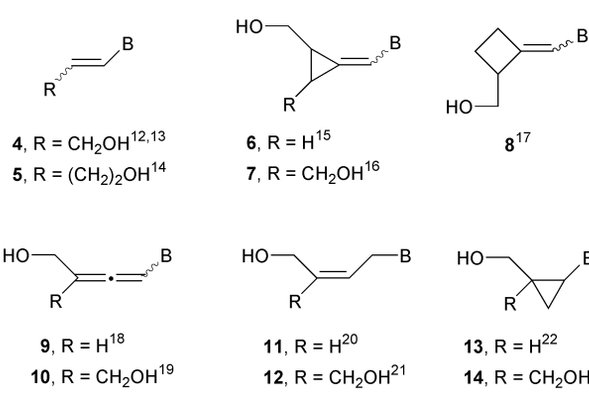

Fig. 2 Known nucleoside analogues structurally related to **1**.

Results and discussion

Many routes can be envisaged for the preparation of a trisubstituted alkene such as **1**. By analogy with methods published for the preparation of **4**–**7**,[12–16] early attempts were based on the alkylation of thymine with properly substituted alkyl halides. However, attempted reactions of the vicinal dibromide **20**[29]

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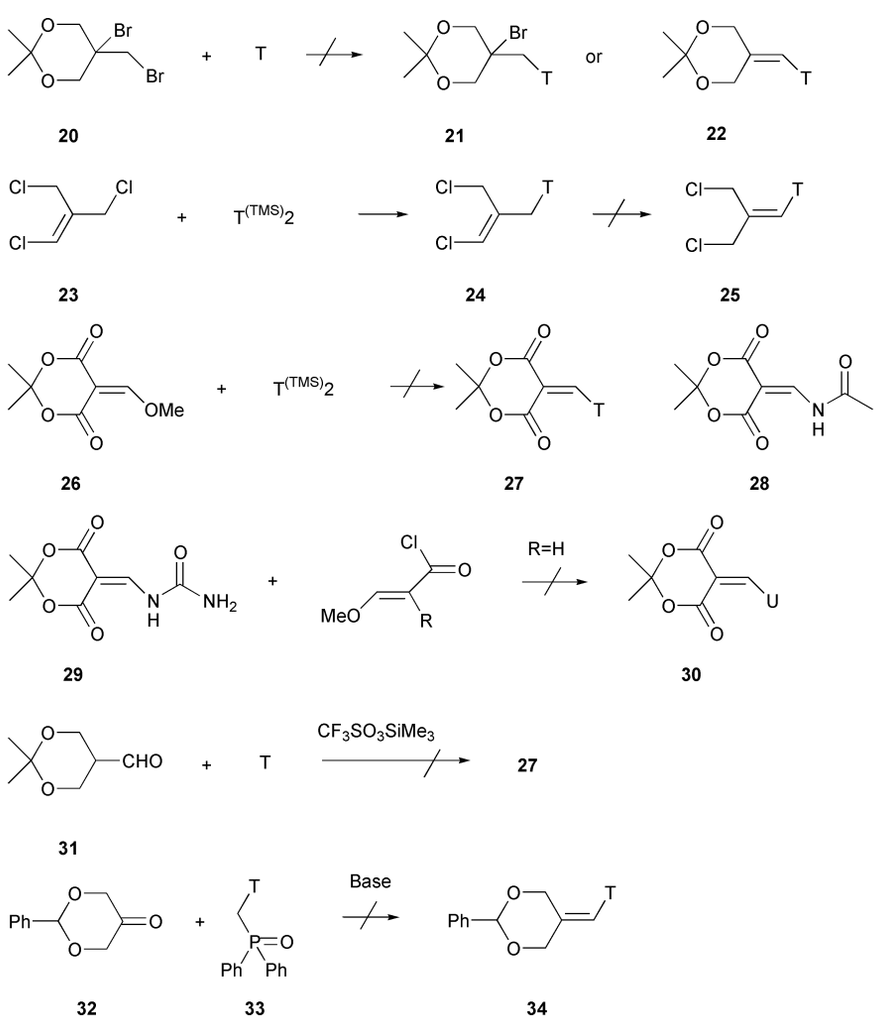
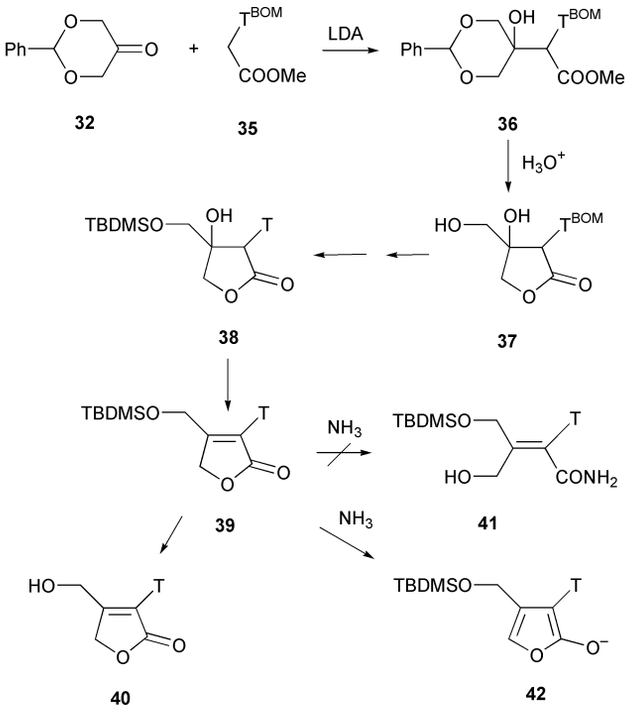
(Scheme 1) with thymine in the presence of K_2CO_3 in DMF, or with bis(trimethylsilyl)thymine in CH_3CN, failed to give any of either the alkylated product **21** or its eliminated counterpart **22** under various conditions. This is perhaps unsurprising given that **20** is a very hindered primary bromide (*cf*. neopentyl bromide); however, a similar reaction was possible on a cyclopropyl analogue.[15] Alkylation of either thymine or bis(trimethylsilyl)thymine using 2-chloromethyl-1,3-dichloropropene **23**[30] was slightly more successful and yielded small amounts of **24**. The tendency of thymine to undergo dialkylation and the ease with which both allylic chloro atoms of **23** underwent substitution undoubtedly contributed to the low yield of this reaction. In any event, this route was abandoned when all attempts to isomerise **24** to the target compound **25** failed. Another route to a potential precursor of **1**, *i.e*. addition of thymine or bis(trimethylsilyl)thymine to methoxymethylene Meldrum's acid **26**,[31] gave no substitution product **27**, even under forcing conditions. The low nucleophilicity of bis(trimethylsilyl)thymine towards **26** was evident from the isolation of the acetamido-substituted product **28** instead of **27** when bis(trimethylsilyl)thymine was prepared from bis(trimethylsilyl)acetamide (BSA). Attempts to build up the thymine base from the known urea derivative **29**[32] were aborted since **29** did not form the corresponding uracil derivative **30** with 3-methoxypropenoyl chloride. A recent method described by one of us[33] to prepare *N*-(alk-1-enyl)thymines from aldehydes and thymine in the presence of trimethylsilyl trifluoromethanesulfonate failed with the aldehyde **31**,[31] which self-condensed instead. Another recent method from our group to obtain *N*-(alk-1-enyl)thymines or adenines is by Horner or Horner–Wadsworth–Emmons reactions.[34] However, several hydroxy-protected dihydroxyacetone derivatives, *e.g*. **32**[35] and 1-(diphenylphosphorylmethyl)thymine **33**[34] (or the 3-benzoyl derivative), in the presence of NaH or LDA gave none of the desired alkene product **34** due to self-condensation of **32** under the reaction conditions. The high tendency of **32** to give self-aldol products has been observed earlier.[36] We thought that the Horner reaction failed because the anion of **33** is too strong a base, and we decided to study condensations of hydroxy-protected dihydroxyacetone derivatives with less basic anionic thymine reagents. Since we were unable to prepare 1-(triphenylphosphoniomethyl)thymine we turned to the known 1-(methoxycarbonylmethyl)thymine.[37]

Attempted routes to 1 and 2 from 1-(methoxycarbonylmethyl)-thymine

The anion prepared from 3-benzyloxymethyl-1-(methoxycarbonylmethyl)thymine **35** and LDA at −78 °C and protected dihydroxyacetone **32** gave the addition product **36** in high yield (Scheme 2). Removal of benzaldehyde with aqueous acid was accompanied by lactonisation to give the lactone **37**, which, after removal of the benzyloxymethyl (BOM) group and protection of the primary hydroxy group with a *tert*-butyldimethylsilyl group, gave **38**. Elimination of water to give **39** occurred partly during silica column purification of **38**, and was complete after stirring with silica in chloroform for 48 h at rt. The compound **39** and the unprotected compound **40** were prepared as potential precursors of **2**, X = $CONH_2$. However, treatment of **39** with ammonia gave none of the desired **41**, but probably the furan **42**. Attempts to open the lactone ring of the dimethoxytrityl (DMT) protected analogue of **39**, or the unprotected lactone **40**, with ammonia or methylamine also



Scheme 1 Attempted routes to **1**.

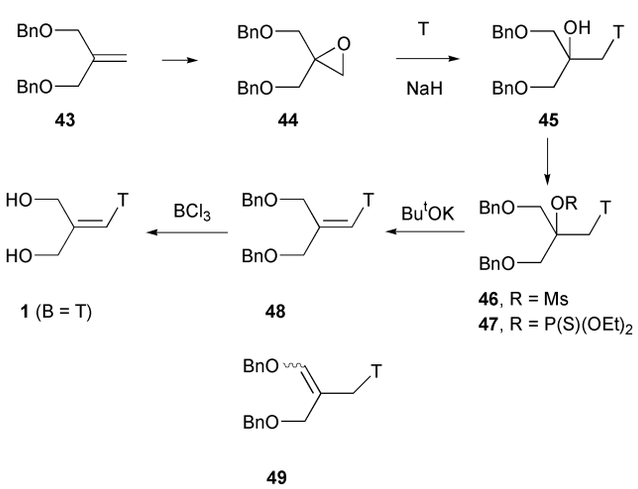


Scheme 2 Condensations with 3-benzyloxymethyl-1-(methoxycarbonylmethyl)thymine **35**.

failed. Treatment of **38** with ammonia likewise failed to give a ring-opened product, probably because elimination to **39** was faster than attack at the lactone carbon. The new compounds **36**–**40** might be valuable precursors of **2** with other X groups, *e.g.* after reduction of the ester group, and **36** after saponification should be able to eliminate CO_2 and OH^- to give protected **1**. Work is in progress to evaluate such routes to **1** and **2**.

The epoxide route to 1

Nucleobases or their anions have been shown to react with epoxides to give *N*-(2-hydroxyalkyl)nucleobase derivatives.[17,38,39] Subsequent mesylation of the 2-hydroxy group and treatment with potassium *tert*-butoxide was recently shown to give some *N*-(alk-1-enyl)adenine derivatives **8** in reasonable yields.[17] We decided to examine this route and found that the known epoxide **44**,[40] which we prepared from the easily obtainable alkene **43**,[23] and excess thymine in the presence of NaH gave a 70% yield of **45** (Scheme 3). Protected 2,2-bis(hydroxymethyl)-epoxides other than **44** were also tried, but with less success, due to partial removal of the protecting groups (acetyl) or



Scheme 3 The epoxide route to **1**.

subsequent elimination to unwanted regioisomeric alkenes analogous to **49** (benzaldehyde acetal). The tertiary alcohol group of **45** was slowly mesylated by methanesulfonyl chloride in pyridine to give **46** in 72% yield after 5 days at 5 °C. The corresponding diethyl thiophosphate **47** could be prepared in 85% yield much faster, *via* phosphitylation of **45** with diethyl chlorophosphite followed by oxidation with sulfur. The mesyl derivative **46** underwent elimination with potassium *tert*-butoxide in THF at rt to give a mixture of **48** and the regioisomers **49**, which isomerised to nearly pure **48** after standing for 2 days at rt. The thiophosphate **47** likewise gave **48** and **49**, but several unknown compounds were formed as well, which resulted in a lower yield of **48**. The last step, removal of the benzyl protecting groups from **48**, was expected to be troublesome because the ethers are allylic as well as benzylic. However, deprotection with boron trichloride in dichloromethane at −78 °C, following the procedure of Zemlicka,[17] gave **1** (B = T) in 42% yield after purification. That the thymine is alkylated at N-1 was verified on **48** by substantial NOE enhancements between the irradiated H-6 of thymine and both the alkene proton at 6.71 ppm and the CH_2C=CH protons at 4.02 ppm.

Conclusion

Several preparative routes to the new nucleoside analogues **1** and **2** have been examined with thymine as the nucleobase. Aldol-type couplings between a protected dihydroxyacetone (*e.g.* **32**) and a protected thyminylacetic ester (*e.g.* **35**) gave promising precursors (**36**–**40**) to **1** and **2**, but this route has not yet led to the target compounds. A route *via* ring-opening of a properly substituted epoxide (*e.g.* **44**) with the anion of thymine was more successful and led to the first synthesis of **1** (B = T). Work is in progress to develop this route to prepare **1** (B = A, C, and G), and to evaluate **1** and **2** in an antisense and antiviral context.

Experimental

1-(Methoxycarbonylmethyl)thymine,[37] benzyloxymethyl chloride,[41] 2-phenyl-1,3-dioxan-5-one monohydrate (**32**),[35] and 1,1-bis(benzyloxymethyl)ethylene (**43**),[23] were prepared according to literature procedures. NaH was *ca.* 80% in mineral oil from Aldrich. Lithium diisopropylamide (LDA) was prepared fresh from diisopropylamine in dry THF and BuLi (1.4 M in hexane, Aldrich) at −78 °C. Other chemicals were 97–99% pure from Aldrich, Fluka, or Merck. Solvents were HPLC grade from LABSCAN, of which DMF and pyridine were dried over molecular sieves (Grace 4 Å) and THF was freshly distilled from Na–benzophenone to a water content below 30 ppm, measured on a Metrohm 652 KF-Coulometer. TLC was run on Merck 5554 silica 60 aluminium sheets, column chromatography on Merck 9385 silica 60 (0.040–0.063 mm). NMR spectra (reference tetramethylsilane for δ_H and δ_C, external 85% H_3PO_4 for δ_P, *J* values are given in Hz) were run on a Varian Mercury 300 MHz spectrometer, and FAB MS data obtained on a JEOL HX 110/110 Mass Spectrometer with *m*-nitrobenzyl alcohol (MNBA) as the matrix unless otherwise noted.

3-Benzyloxymethyl-1-(methoxycarbonylmethyl)thymine (35)

1-(Methoxycarbonylmethyl)thymine (2.00 g, 10.1 mmol) was dried by evaporation from dry pyridine (2 × 50 ml) and dry DMF (100 ml) was added under N_2. NaH (0.35 g, 70% in mineral oil, 10.1 mmol) was added and the reaction mixture stirred at rt for 20 min. Benzyloxymethyl chloride (1.60 ml, 11.5 mmol) was added and stirring continued at rt for 2.5 h. The reaction was quenched with H_2O (100 ml) and the mixture

was extracted with $CHCl_3$ (2 × 100 ml). The organic phase was dried ($MgSO_4$) and evaporated *in vacuo* to give a yellow oil that was purified by column chromatography, eluted with EtOAc–heptane (70 : 30 v/v). The fractions containing the product were concentrated *in vacuo* to give a clear oil that was crystallized from EtOAc–heptane to give pure **35** (2.44 g, 76%) as colourless crystals, mp 60–62 °C. TLC R_f 0.39 (EtOAc–heptane 80 : 20 v/v). NMR ($CDCl_3$): δ_H 7.38–7.23 (5H, m, Ph), 6.90 (1H, q, *J* 1.2, T-H6), 5.51 (2H, s, NCH_2O), 4.69 (2H, s, OCH_2Ph), 4.44 (2H, s, $CH_2CO_2CH_3$), 3.80 (3H, s, CO_2CH_3), 1.94 (3H, d, *J* 1.2, T-CH_3). δ_C 167.84, 163.40, 151.38, 138.78, 137.65, 128.04, 127.48, 127.42, 110.31, 71.92, 70.44, 52.57, 49.21, 12.76. FAB^+ MS: 319.0 (M + H^+ calc. 319.1) (Found: C, 60.4; H, 5.9; N, 8.8. Calc. for $C_{16}H_{18}N_2O_5$: C, 60.4; H, 5.7; N, 8.8%).

3-Benzyloxymethyl-1-[(5-hydroxy-2-phenyl-1,3-dioxan-5-yl)-(methoxycarbonyl)methyl]thymine (36)

Compound **35** (2.00 g, 6.3 mmol) was dried by evaporation from dry pyridine (2 × 25 ml), dry THF (50 ml) was added under N_2 and the mixture cooled to −78 °C. Freshly prepared LDA (6.3 mmol in THF–hexane) was added to give a yellow solution which was stirred at −78 °C for 30 min. 2-Phenyl-1,3-dioxan-5-one monohydrate (**32**, 1.23 g, 6.3 mmol), dried by evaporation from dry pyridine (3 × 15 ml) and dissolved in dry THF (10 ml), was added dropwise and the reaction mixture stirred at −78 °C for 2 h. The reaction was quenched with sat. aq. NH_4Cl (10 ml) and transferred to a separation funnel with $CHCl_3$ (100 ml) and H_2O (100 ml), extracted with $CHCl_3$ (2 × 50 ml), and the combined organic phases washed with H_2O (100 ml) and dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a yellow solid (2.84 g, 91%, **36**, slightly contaminated with **35**). Recrystallization from EtOAc–heptane gave pure **36** (2.34 g, 75%), colourless crystals, mp 187.5–188 °C. TLC R_f 0.26 (heptane–EtOAc 40 : 60 v/v). ^1H NMR ($CDCl_3$): δ_H 7.75 (1H, q, *J* 1.1, T-H6), 7.52–7.14 (10H, m, Ph), 6.19 (1H, s, OH), 6.17 (1H, s, C*H*Ph), 5.56 (1H, s, C*H*CO_2CH_3), 5.39 (2H, s, NCH_2O), 4.55 (1H, dd, *J* 11.3 and 2.9, $OCH_2C(OH)$), 4.52 (2H, s, OCH_2Ph), 3.76 (1H, d, *J* 11.3, $OCH_2C(OH)$), 3.76 (3H, s, CO_2CH_3), 3.75 (2H, AB of ABX system, Δ 58.8 Hz, J_{AB} 11.5, J_{AX} 2.9, $OCH_2C(OH)$), 1.87 (3H, d, *J* 1.1, T-CH_3). ^{13}C NMR (DMSO-d_6): δ_C 168.8, 162.6, 151.5, 139.3, 137.9, 137.6, 129.0, 128.1, 128.0, 127.4, 127.2, 126.7, 108.1, 101.7, 72.2, 70.9, 70.5, 68.6, 57.8, 52.8, 13.0. FAB^+ MS: 496.9 (M + H^+ calc. 497.2) (Found: C, 63.0; H, 6.0; N, 5.7. Calc. for $C_{26}H_{28}N_2O_8$: C, 62.9; H, 5.7; N, 5.6%).

3-Benzyloxymethyl-1-[4-hydroxy-4-(hydroxymethyl)-2-oxotetrahydrofuran-3-yl]thymine (37)

A suspension of **36** (0.30 g, 0.60 mmol) in a mixture of MeOH (20 ml) and 0.5 M H_2SO_4 (8 ml, 8 mmol) was heated to reflux for 1 h to give a clear solution. The cooled reaction mixture was transferred to a separation funnel with H_2O (25 ml) and extracted with $CHCl_3$ (3 × 50 ml). The combined organic phases were dried (Na_2SO_4) and evaporated to give a colourless residue that was purified by column chromatography, eluted with EtOAc–heptane (9 : 1 v/v). The fractions containing the product were evaporated *in vacuo* to give pure **37** (0.18 g, 78%) as a colourless powder, mp 63–68 °C. TLC R_f 0.25 (EtOAc–heptane 9 : 1 v/v). NMR (DMSO-d_6): δ_H 7.37–7.26 (6H, m, Ph + T-H6), 5.92 (1H, br s, COH), 5.80 (1H, br s, CHC(O)), 5.36 (2H, s, NCH_2O), 5.13 (1H, br s, CH_2OH), 4.59 (2H, s, OCH_2Ph), 4.53 (1H, d, *J* 9.3, $C(O)OCH_2$), 4.23 (1H, d, *J* 9.3, $C(O)OCH_2$), 3.48 (2H, AB of ABX system, Δ = 20.5 Hz, J_{AB} 11.2, $J_{AX} = J_{BX}$ 5.2, CH_2OH), 1.83 (3H, s, T-CH_3). ^{13}C NMR ($CDCl_3$): δ_C 170.7, 163.2, 152.7, 138.9, 137.0, 128.3, 127.9, 127.7, 109.8, 78.3, 73.9, 72.3, 70.9, 62.7, 59.1, 12.9. FAB^+ MS: 377.1 (M + H^+ calc. 377.1) (Found: C, 57.4; H, 5.6; N, 7.1. Calc. for $C_{18}H_{20}N_2O_7$: C, 57.4; H, 5.4; N, 7.4%).

1-[4-(*tert*-Butyldimethylsilyloxymethyl)-4-hydroxy-2-oxotetrahydrofuran-3-yl]thymine (38)

A solution of **37** (2.86 g, 7.6 mmol) in MeOH (50 ml) and 20% $Pd(OH)_2/C$ (0.5 g) was stirred under H_2 at 1 atm and rt for 24 h. The solution was filtered through Celite, the Celite washed with MeOH (100 ml), and the solvent removed *in vacuo*. The residue was purified on a column, eluted with CH_2Cl_2–MeOH 9 : 1 v/v, to give 1-[4-hydroxy-4-(hydroxymethyl)-2-oxotetrahydrofuran-3-yl]thymine (0.76 g, 39%). To this intermediate (0.76 g, 3.0 mmol) in dry DMF (5 ml) was added imidazole (0.41 g, 6 mmol) and *tert*-butyldimethylsilyl chloride (0.55 g, 3.6 mmol) and the mixture was stirred for 20 h. Evaporation *in vacuo* gave a yellow syrup that was purified by column chromatography, eluted with EtOAc–heptane (6 : 4 v/v) to give **38** (R_f = 0.22), contaminated with *ca*. 10% of the elimination product **39** (R_f = 0.44) (1.08 g, 75%, 29% from **37**). NMR (DMSO-d_6) for **38**: δ_H 11.46 (1H, s, NH), 7.19 (1H, s, T-H6), 5.89 (1H, s, OH), 5.7 (1H, br s, NCHC(O)O), 4.34 (2H, AB system, Δ = 73.4 Hz, J_{AB} 9.2, $CH_2OC(O)$), 3.60 (2H, AB system, Δ = 22.1 Hz, J_{AB} 10.3, $SiOCH_2$), 1.76 (3H, s, T-CH_3), 0.86 (9H, s, t-Bu), 0.05 (6H, s, Si-CH_3). δ_C 172.4, 163.9, 151.4, 140.5, 108.2, 77.9, 77.0, 74.4, 64.1, 57.5, 25.6, 18.0, 12.2. FAB^+ MS: 371.1 (M + H^+ calc. 371.2) (Found: C, 52.5; H, 6.8; N, 7.5. Calc. for $C_{16}H_{26}N_2O_6Si$: C, 51.9; H, 7.1; N, 7.6%).

1-[4-(*tert*-Butyldimethylsilyloxymethyl)-2-oxo-2,5-dihydrofuran-3-yl]thymine (39)

A suspension of **38** (0.37 g, 1 mmol, contaminated with *ca*. 10% of **39**) and silica (Merck 9385 silica 60, 30 g) in $CHCl_3$ (150 ml) was stirred at rt for 48 h. The suspension was filtered, the silica washed with CH_2Cl_2–MeOH (9 : 1 v/v) (2 × 50 ml), and the combined solutions evaporated *in vacuo* to a colourless solid residue, which was nearly pure **39** (0.22 g, 62%). NMR (DMSO-d_6): δ_H 11.54 (1H, s, NH), 7.44 (1H, q, *J* 1.2, T-H6), 5.07 (2H, s, $CH_2OC(O)$), 4.66 (2H, s, $SiOCH_2$), 1.77 (3H, d, *J* 1.2, T-CH_3), 0.83 (9H, s, t-Bu), 0.04 (6H, s, Si-CH_3). FAB^+ MS: 353.1 (M + H^+ calc. 353.2) (Found: C, 55.0; H, 7.1; N, 8.0. Calc. for $C_{16}H_{24}N_2O_5Si$: C, 54.5; H, 6.9; N, 7.95%).

1-[4-(Hydroxymethyl)-2-oxo-2,5-dihydrofuran-3-yl]thymine (40)

To a solution of **39** (0.18 g, 0.5 mmol) in dry THF (15 ml) was added $Et_3N{\cdot}3HF$ (0.40 ml, 2.5 mmol) and the mixture stirred at rt for 3 h. The residue after evaporation *in vacuo* was purified on a column, eluted with CH_2Cl_2–MeOH (9 : 1 v/v), to give **40** (0.11 g, 92%) as a colourless solid. NMR (DMSO-d_6): δ_H 11.54 (1H, s, NH), 7.43 (1H, s, T-H6), 5.45 (1H, t, *J* 5.3, OH), 5.09 (2H, s, $CH_2OC(O)$), 4.43 (2H, d, *J* 5.3, $HOCH_2$), 1.78 (3H, s, T-CH_3). δ_C 169.0, 164.1, 163.5, 149.1, 139.6, 120.9, 109.5, 69.7, 56.6, 11.9. FAB^+ MS (matrix DMSO): 239.2 (M + H^+ calc. 239.1) (Found: C, 48.6; H, 4.1; N, 11.0. Calc. for $C_{10}H_{10}N_2O_5$+0.5 H_2O: C, 48.6; H, 4.5; N, 11.3%).

2,2-Bis(benzyloxymethyl)oxirane (44)

A mixture of 1,1-bis(benzyloxymethyl)ethylene (**43**, 7.00 g, 26.1 mmol) and *m*-chloroperbenzoic acid (70%, 7.71 g, 31.3 mmol) in $CHCl_3$ (200 ml) was refluxed for 90 min. After cooling to rt 10% aq. $Na_2S_2O_5$ (60 ml) and more $CHCl_3$ (100 ml) were added, and the mixture was extracted with sat. aq. $NaHCO_3$ (4 × 100 ml) and brine (150 ml). The organic phase was dried (Na_2SO_4), and the solvent removed *in vacuo* to give a yellow oil that was purified by column chromatography, eluted with heptane–EtOAc (7 : 3 v/v), to give pure **44** (4.45 g, 60%) as a colourless oil. TLC R_f 0.35 (heptane–EtOAc 7 : 3 v/v). NMR ($CDCl_3$): δ_H 7.40–7.33 (10H, m, Ph), 4.60 (4H, AB system, Δ = 8.4 Hz, J_{AB} 12.0, OCH_2-oxirane), 3.73 (4H, s, $PhCH_2$), 2.83 (2H, s, oxirane). ^{13}C NMR: δ_C 137.7, 128.2, 127.5, 73.2, 69.9, 57.5, 48.5. FAB^+ MS: 285.3 (M + H^+ calc. 285.1) (Found: C, 75.95; H, 7.0. Calc. for $C_{18}H_{20}O_3$: C, 76.0; H, 7.1%).

1-[3-Benzyloxy-2-(benzyloxymethyl)-2-hydroxypropyl]thymine (45)

To a mixture of thymine (8.86 g, 70 mmol) and NaH (70% in oil, 0.68 g, 20 mmol) in dry DMF (250 ml) under N_2 was added a solution of **44** (4.00 g, 14.1 mmol) in dry DMF (25 ml), and the mixture was stirred at 110 °C for 48 h. After cooling to rt sat. aq. NH_4Cl (50 ml) was added, followed by H_2O (500 ml), and $CHCl_3$ (250 ml). The aqueous phase was separated and extracted with $CHCl_3$ (3 × 300 ml), and the combined organic phases were washed with brine, dried (Na_2SO_4) and evaporated *in vacuo* to give a light brown oil. Purification by column chromatography, eluted with CH_2Cl_2–MeOH (95 : 5 v/v), followed by recrystallisation from EtOAc–heptane gave **45** (4.05 g, 70%) as colourless crystals, mp 110–111 °C. TLC R_f 0.40 (CH_2Cl_2–MeOH 9 : 1 v/v). NMR ($CDCl_3$): δ_H 8.6 (1H, s, NH), 7.40–7.25 (10H, m, Ph), 7.11 (1H, q, *J* 1.2, T-H6), 4.52 (4H, s, PhCH_2), 3.94 (2H, s, NCH_2), 3.48 (4H, s, OCH_2C(OH)), 1.84 (3H, d, *J* 1.2, T-CH_3). δ_C 163.9, 152.0, 142.1, 137.4, 128.3, 127.8, 127.6, 109.8, 74.3, 73.5, 71.7, 51.3, 12.1. FAB$^+$ MS: 411.2 (M + H$^+$ calc. 411.2) (Found: C, 67.3; H, 6.3; N, 6.8. Calc. for $C_{23}H_{26}N_2O_5$: C, 67.3; H, 6.4; N, 6.8%).

1-[3-Benzyloxy-2-(benzyloxymethyl)-2-(methylsulfonyloxy)-propyl]thymine (46)

To a stirred solution of **45** (3.70 g, 9.0 mmol) in dry pyridine (45 ml) under N_2 at 0 °C was added dropwise methanesulfonyl chloride (3.5 ml, 45 mmol), and the solution kept in a refrigerator at 5 °C for 5 days. Excess methanesulfonyl chloride was hydrolysed by dropwise addition of MeOH–H_2O (2 : 1 v/v, 10 ml) at 0 °C, followed by stirring for 1 h at rt before removal of the solvents *in vacuo*. The product was extracted from the residue with boiling EtOAc (3 × 100 ml), followed by purification on a column, eluted with CH_2Cl_2–MeOH (9 : 1 v/v), to give **46** (3.15 g, 72%). An analytical sample was obtained by recrystallisation from EtOAc–hexane, mp 114–115 °C. TLC R_f 0.44 (CH_2Cl_2–MeOH 9 : 1 v/v). NMR ($CDCl_3$): δ_H 10 (1H, br s, NH), 7.36–7.26 (10H, m, Ph), 7.18 (1H, q, *J* 1.2, T-H6), 4.53 (4H, s, PhCH_2), 4.22 (2H, s, NCH_2), 3.90 (4H, AB system, Δ = 27.3 Hz, J_{AB} 10.6, OCH_2C(OMs)), 3.03 (3H, s, Ms), 1.84 (3H, d, *J* 1.2, T-CH_3). δ_C 164.0, 151.4, 141.1, 136.8, 128.3, 127.9, 127.7, 110.2, 91.3, 73.6, 69.2, 50.0, 40.2, 12.0. FAB$^+$ MS: 489.2 (M + H$^+$ calc. 489.2) (Found: C, 58.8; H, 5.8; N, 5.9; S, 6.5. Calc. for $C_{24}H_{28}N_2O_7S$: C, 59.0; H, 5.8; N, 5.7; S, 6.6%).

1-[3-Benzyloxy-2-(benzyloxymethyl)-2-(diethoxythiophosphoryloxy)propyl]thymine (47)

To a stirred solution of **45** (4.50 g, 11.0 mmol) in dry pyridine (50 ml) under N_2 at rt was added dropwise diethyl chlorophosphite (1.96 ml, 11.5 mmol). After 45 min S_8 (0.38 g, 12 mmol S) was added, and the mixture was stirred for 1.5 h, followed by evaporation *in vacuo*. The residue was dissolved in $CHCl_3$ (400 ml) and the solution extracted with sat. aq. $NaHCO_3$ (2 × 150 ml), brine (80 ml), dried (Na_2SO_4), and evaporated *in vacuo*. Crystallisation of the residue from EtOAc–heptane gave pure **47** (5.25 g, 85%) as colourless crystals, mp 88.5–89.5 °C. NMR (DMSO-d_6): δ_H 11.26 (1H, s, NH), 7.32–7.26 (11H, m, Ph + T-H6), 4.49 (4H, s, PhCH_2), 4.13 (2H, s, NCH_2), 4.01–3.91 (4H, m, Et), 3.81 (4H, AB system, Δ = 19.1 Hz, J_{AB} 10.3, OCH_2C(OP)), 1.68 (3H, s, T-CH_3), 1.10 (6H, t, *J* 7.0, Et). δ_C 164.0, 151.4, 141.9, 137.7, 128.1, 127.5, 108.0, 86.2 (d, *J* 10), 72.6, 69.3, 63.9 (d, *J* 6), 49.2, 15.5 (d, *J* 8), 12.0. δ_P 58.6. FAB$^+$ MS: 563.4 (M + H$^+$ calc. 563.2) (Found: C, 57.6; H, 6.35; N, 5.1; S, 5.3. Calc. for $C_{27}H_{35}N_2O_7PS$: C, 57.6; H, 6.3; N, 5.0; S, 5.7%).

1-[3-Benzyloxy-2-(benzyloxymethyl)prop-1-enyl]thymine (48)

Method A. To a stirred solution of **46** (1.95 g, 4.0 mmol) in dry THF (75 ml) under N_2 at 0 °C was added t-BuOK (1.12 g, 10 mmol). After stirring for 20 min at 0 °C the solution was kept at rt for 3 days, followed by neutralisation with 4 M aq. HCl at 0 °C and removal of the solvents and t-BuOH *in vacuo*. The residue in $CHCl_3$ (100 ml) was extracted with sat. aq. $NaHCO_3$ (2 × 50 ml), brine (50 ml), and the organic phase dried ($MgSO_4$), followed by removal of the solvent *in vacuo*. Crystallisation of the residue from EtOAc–hexane gave pure **48** (0.80 g, 51%) as colourless crystals, mp 93–93.5 °C.

Method B. Starting from **47** (4.50 g, 8.0 mmol) and t-BuOK (2.24 g, 20.0 mmol) in dry THF (150 ml), the same procedure as above gave a less pure crude product. Purification by column chromatography, eluted with CH_2Cl_2–EtOAc (6 : 4 v/v), followed by crystallisation from EtOAc–heptane, gave **48** (1.26 g, 40%) as colourless crystals, mp 92–94 °C. TLC R_f = 0.35 (CH_2Cl_2–EtOAc 6 : 4 v/v). NMR ($CDCl_3$): δ_H 8.6 (1H, s, NH), 7.36–7.26 (10H, m, Ph), 7.23 (1H, q, *J* 1.1, T-H6), 6.71 (1H, br s, N-CH=C), 4.55 and 4.50 (2 × 2H, 2 × s, PhCH_2), 4.16 (2H, d, *J* 1.2, CH_2C=CH), 4.02 (2H, s, CH_2C=CH), 1.86 (3H, d, *J* 1.1, T-CH_3). δ_C 164.0, 150.1, 140.8, 138.0, 137.5, 132.2, 128.8, 128.7, 128.3, 128.13, 128.10, 128.05, 126.2, 110.6, 73.7, 72.7, 70.1, 64.8, 12.5. FAB$^+$ MS: 392.9 (M + H$^+$ calc. 393.2) (Found: C, 69.65; H, 6.3; N, 7.3. Calc. for $C_{23}H_{24}N_2O_4$: C, 70.4; H, 6.2; N, 7.1%).

1-[3-Hydroxy-2-(hydroxymethyl)prop-1-enyl]thymine (1, B = T)

To a stirred solution of **48** (0.39 g, 1.0 mmol) in dry CH_2Cl_2 (35 ml) at −78 °C under N_2 was added dropwise BCl_3 (1 M in CH_2Cl_2, 6.0 ml, 6 mmol) during 5 min. Stirring was continued at −78 °C for 4 h, followed by dropwise addition of MeOH–CH_2Cl_2 (1 : 1 v/v, 8 ml) at −78 °C. The cooling bath was removed and the solvents removed *in vacuo* to give a residue that was stirred for 2 h with a mixture of MeOH (15 ml) and solid $NaHCO_3$ (0.40 g, 6 mmol). The solids were removed by filtration and washed with MeOH–CH_2Cl_2 (1 : 1 v/v, 2 × 10 ml). The combined filtrates were concentrated *in vacuo* and the residue purified by column chromatography, eluted with CH_2Cl_2–MeOH (9 : 1 v/v), to give pure **1**, B = T, (89 mg, 42%) as colourless crystals, mp 166–169 °C. TLC R_f 0.10 (CH_2Cl_2–MeOH 9 : 1 v/v). NMR (DMSO-d_6): δ_H 11.3 (1H, s, NH), 7.41 (1H, s, T-H6), 6.41 (1H, s, N-CH=C), 5.01 and 4.94 (2 × 1H, 2 × t, *J* 5 and 5, OH), 4.08 and 3.92 (2 × 2H, 2 × d, *J* 5 and 5, CH_2), 1.76 (3H, s, T-CH_3). δ_C 164.1, 150.1, 141.1, 137.8, 121.6, 108.4, 60.5, 55.8, 11.9. FAB$^+$ MS: 213.0, FAB$^-$ MS: 211.1 (M calc. 212.1) (Found: C, 50.8; H, 5.7; N, 12.9. Calc. for $C_9H_{12}N_2O_4$: C, 50.9; H, 5.7; N, 13.2%).

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