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SYNTHESIS OF SOME ANALOGUES OF NUCLEOSIDE 5'-TRIPHOSPHATES

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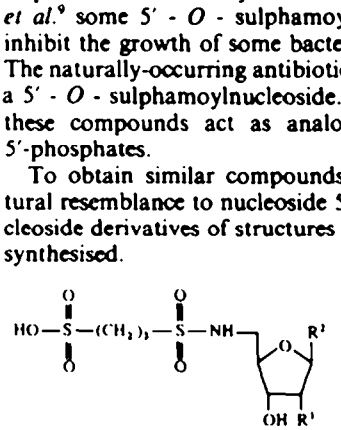
Abstract—Reaction of propane - 1,3 - disulphonyl chloride with the appropriate 5' - amino - 5' - deoxyribonucleoside gave 5' - deoxy - 5' - (3 - sulphopropanesulphonylamino)thymidine (**1**), 5' - deoxy - 5' - (3 - sulphopropanesulphonylamino)uridine (**2**), 5' - deoxy - 5' - (3 - sulphopropanesulphonylamino)adenosine (**3**), and (*E*) - 5 - (2 - bromovinyl) - 2',5' - dideoxy - 5' - (3 - sulphopropanesulphonylamino)uridine (**4**).

5' - Deoxy - 5' - p - sulphaminobenzenesulphonylaminothymidine (**8**) was obtained by treatment of 5' - p - aminobenzenesulphonylamino - 5' - deoxythymidine (**10**) with SO_3-triethylamine. This reaction gave also some 5' - p - aminobenzenesulphonylamino - 5' - deoxy - 3' - *O* - sulphothymidine (**12**). Compounds **2** and **3** were inactive when tested for inhibitory activity against *E.coli* RNA polymerase holoenzyme. Compounds **1** and **8** inhibited *E.coli* DNA polymerase I non-competitively at relatively high concentrations. Compound **4** was without significant inhibitory activity against HSV-1 DNA polymerase.

Nucleoside 5'-triphosphates play a vital role in a wide range of metabolic processes including energy transfer and the biosynthesis of intermediates and macromolecules. Many nucleoside analogues which act as antimetabolites (e.g. antiviral agents) exert their inhibitory action by virtue of the fact that they can be converted in the cell into their neucleoside 5'-triphosphates which are the actual antimetabolites.[1-5] Nucleoside analogue 5'-triphosphates themselves cannot be used, however, because of their inability to penetrate into cells. This disability also applies to analogues which contain modified phosphate groups such as phosphorothioates[6] and phosphonates.[7] The present work was undertaken in order to obtain compounds which resemble the structure of nucleoside 5'-triphosphates and which might penetrate cells. Sulphonic acid and sulphamid derivatives of nucleosides are a group of such compounds. Mungall *et al.*[8] have prepared a number of 5' - sulphamino - 5' - deoxyribonucleosides and Shuman *et al.*[9] some 5' - *O* - sulphamoylnucleosides. These inhibit the growth of some bacteria and L1210 cells. The naturally-occurring antibiotic, nucleocidin is also a 5' - *O* - sulphamoylnucleoside.[10] It is thought that these compounds act as analogues of nucleoside 5'-phosphates.

To obtain similar compounds, but having structural resemblance to nucleoside 5'-triphosphates, nucleoside derivatives of structures **1-4** and **8** have been synthesised.



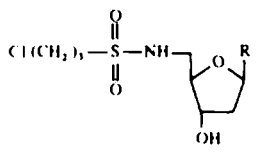
1 R^1 = H; R^2 = thymin-1-yl- 3 R^1 = OH, R^2 = adenin-9-yl-
2 R^1 = OH; R^2 = uracil-1-yl- 4 R^1 = H; R^2 = (*E*)-5-(2-bromovinyl) uracil-1-yl-

5' - Amino - 5' - deoxythymidine was treated with propane - 1,3 - disulphonyl chloride under carefully controlled conditions and the product hydrolysed to give 5' - (3 - sulphopropanesulphonylamino) - 5' - deoxythymidine (**1**) as the sodium salt in 32% yield. The low yield was due to the possibility of side reactions, e.g. reaction of both sulphonyl chloride groups, reaction at the 3'-hydroxyl and decomposition of the sulphonyl chloride to a sulphene.[11] The compound ran as a monoanion on paper electrophoresis at pH 7.2 and the UV and NMR spectra were consistent with the assigned structure. Treatment with 0.1M sodium hydroxide at room temperature for 24 h did not hydrolyse the compound; similar treatment with 0.1M hydrochloric acid gave a trace of thymine.

Reaction of propane - 1,3 - disulphonyl chloride with the appropriate 5' - amino - 5' - deoxynucleoside gave 5' - (3 - sulphopropanesulphonylamino) - 5' - deoxyuridine (**2**) (22% yield), 5' - (3 - sulphopropanesulphonylamino) - 5' - deoxyadenosine (**3**) and (*E*) - 5 - (2 - bromovinyl) - 2' - 5' - dideoxy-5' - (3 - sulphopropanesulphonylamino)uridine (**4**). Only compound **2** was obtained in a crystalline, analytically pure state. However the NMR spectra, particularly the correspondence of the signals for the methylene protons of the side chain in the four compounds and the UV spectra (including extinction coefficients) showed that the impurities in the other compounds were very small and most probably inorganic salts.

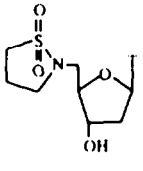
Although this method of preparing the 5' - (3 - sulphopropanesulphonylamino)nucleosides is effective, the yields are low, mainly due to the use of a difunctional reagent. As an alternative synthetic route 5' - amino - 5' - deoxythymidine was treated with 3-chloropropanesulphonyl chloride in pyridine to give 5' - (3 - chloropropanesulphonylamino) - 5' - deoxythymidine (**5**) in 45% yield. The relatively low

yield to that obtained with aromatic sulphonyl chlorides (60–70%) can be attributed to the instability of alkyl sulphonyl chlorides in the presence of basic solvents.[11] Treatment of **5** with sodium sulphite in boiling methanol gave two products; 5′ - (3 - sulphopropanesulphonylamino) - 5′ - deoxythymidine (**1**), identical to that obtained by the previous route was isolated in 50% yield and another product which crystallised from the reaction mixture. The NMR spectrum of this compound did not show a signal for the sulphonamide resonance at about δ7.4. This suggested that the compound was 5′ - deoxy - 5′ - *N*-(1,1 - dioxo - 1,2 - thiazolidin - 2 - yl)thymidine (**6**). This structure is consistent with the NMR spectrum and elemental analysis. The compound is presumably formed by the intramolecular nucleophilic attack of the sulphonamide anion on the terminal carbon atom of the 5′-side chain of **5**.



5 R = thymin-1-yl-

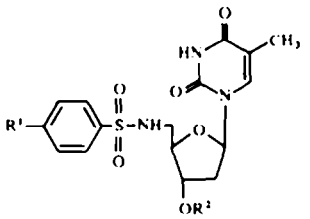
7 R = (*E*)-5-(2-bromovinyl) uracil-1-yl-



6 T = thymin-1-yl-

Although this alternative route to compound **1** gave no better yield than the first route, it did have the slight practical advantage of avoiding the unpleasant preparation of propane 1,3-disulphonyl chloride from propane 1,3-dithiol. However use of the procedure in which sulphite was used was not applicable to the production of the (*E*) - 5 - (2 - bromovinyl)uracil derivative, **4**; (*E*) - 5 - (2 - bromovinyl) - 5′ - (3 - chloropropanesulphonylamino) - 2′,5′ - dideoxyuridine (**7**) was successfully obtained, but when this was treated with sodium sulphite a mixture of anionic products was obtained. These appear to have been formed by the attack of sulphite on the bromovinyl side chain. This reaction has not been investigated further.

In order to obtain compound **8**, 5′ - amino - 5′ - deoxythymidine was treated with p-nitrobenzenesulphonyl chloride to give 5′ - deoxy - 5′ - p - nitrobenzenesulphonylaminothymidine (**9**) in 74% yield.[12] This was reduced with sodium dithionite to give the corresponding amino compound (**10**) in 78% yield. An alternative synthesis of **10** was achieved by reacting 5′ - amino - 5′ - deoxythymidine with p-acetamidobenzenesulphonyl chloride to give *p*-acetamidobenzenesulphonylamino - 5′ - deoxythymidine (**11**) in 68% yield. Hydrolysis of this with aqueous sodium hydroxide gave **10** in 79% yield. The structures of these compounds were established by elemental analysis and NMR and UV spectroscopy. Treatment of **10** with sulphur trioxide-triethylamine in pyridine gave three anionic compounds. These were separated by DEAE cellulose ion exchange chromatography to give 5′ - deoxy - 5′ - p - sulphaminobenzenesulphonylaminothymidine (**8**) (11% yield), 5′ - p - aminobenzenesulphonylamino - 5′ - deoxy - 3′ - *O* - sulphothymidine (**12**) (14% yield) and another compound which was probably a disulphated product.



8 R^1 = $HOSO_2NH$-, R^2 = H
9 R^1 = O_2N-, R^2 = H
10 R^1 = H_2N-,
11 R^1 = CH_3CONH-, R^2 = H
12 R^1 = H_2N-, R^2 = $-SO_3OH$

The *N*-sulphate (**8**) and the *O*-sulphate (**12**) are readily distinguished by both chemical and spectroscopic techniques. The *O*-sulphate gave a deep yellow colour when treated with p-dimethylaminobenzaldehyde whereas the *N*-sulphate did not. In the NMR spectrum of the *N*-sulphate the signal for the aromatic protons ortho to the sulphamino group is shifted downfield by 0.57 ppm relative to the corresponding signal in the starting material (**10**), whereas the signal in the spectrum of the *O*-sulphate is not. This downfield shift is predicted from studies on *N*- and *O*-sulphated aminoalcohols.[13]

In the NMR spectrum of the 3′ - *O* - sulphate the signals for the 2′ and 3′-protons were shifted downfield by 0.19 and 0.48 ppm respectively relative to the starting material (**10**) whereas these signals in the *N*-sulphate are the same as those in 10. These compounds were characterised by NMR and UV spectra, but the sulphates still contained traces of inorganic impurities.

These results show that the sulphation of **10** was not specific for the amino group. This is in contrast to the situation with 5′ - amino - 5′ - deoxythymidine[8] and can be explained as being due to the relatively low nucleophilicity of the aromatic amino group in **10**.

Compounds **2** and **3**, which could be considered as analogues of ribonucleoside triphosphates were tested for inhibitory action against *E.coli* RNA polymerase holoenzyme and compounds **1** and **8**, analogues of deoxyribonucleoside triphosphates were tested against *E.coli* DNA polymerase I. The results (table) showed that the RNA polymerase was not inhibited but the DNA polymerase was inhibited by relatively high concentrations of compounds **1** and **8**. Studies of the kinetics of the inhibition by compound **1** showed it to be non-competitive so it appeared that the inhibition may be due to a non-specific deactivation of the enzyme.

The ability of (*E*) - 5 - (2 - bromovinyl) - 2′,5′ - dideoxy - 5′ - (3 - sulphopropanesulphonylamino) uridine (**4**) to inhibit herpes simplex virus type I DNA polymerase has been compared with the known inhibitory activity of (*E*) - 5 - (2 - bromovinyl) - 2′ - deoxyuridine triphosphate.[14] The latter inhibited the incorporation of dTTP into "activated" DNA by this enzyme with an ID_{50} value of 22 μM whereas compound 4 showed only 10% inhibition at 300 μM.

These results show that although the analogues prepared above have structural resemblances to nucleosides 5′-triphosphates, they are poor analogues in

terms of their recognition of RNA and DNA polymerases. This could be due to their lack of an acidic function in the position corresponding to the α-phosphorus atom and to an inability to complex with Mg^{2+}.[6]

EXPERIMENTAL

NMR spectra were recorded at 100 MHz with $(CD_3)_2SO$ as the solvent. TLC was carried out on silica gel (MN Keiselgel G/UV_{254}), column chromatography on Keiselgel 60, 70-120 mesh ASTM type 7734, reversed phase chromatography on Keiselgel 60, 230–400 mesh ASTM type 9385 precoated with n-octadecylsilyl groups and ion exchange chromatography on Whatman DEAE cellulose 32. For paper electrophoresis, Whatman No. 2 paper was used. All reactions were carried out under anhydrous conditions unless otherwise indicated.

Synthesis of 5' - (3 - *sulphopropanesulphonylamino*) - 5' - *deoxyribonucleosides*

To dimethylformamide (DMF) cooled to 0° (20 ml) there was added simultaneously and with stirring, 5' - amino - 5' - deoxyribonucleoside (2.0 mmole) and triethylamine (2.4 mmole) dissolved in DMF (30 ml) and propane - 1,3 - disulphonyl chloride (2.4 mmole) dissolved in DMF (20 ml). When the addition was complete (*ca* 15 min) the reaction mixture was stirred for a further 10 min and then triethylamine (0.5 g) in water (50 ml) was added. After 24 h at room temperature the mixture was evaporated under reduced pressure to give an oil which was dissolved in 0.1M triethylammonium bicarbonate (TEAB) (200 ml). The solution was applied to a DEAE cellulose column (400 ml) and elution carried out with a TEAB gradient (0.01→0.15M). The fractions containing monoanionic nucleoside material were pooled, evaporated to dryness and co-evaporated several times with water. The residue was dissolved in water and passed down a Dowex 500W- × 8 Na^+ ion exchange column. The eluant was lyophilized to give the 5' - (3 - sulphopropanesulphonylamino) - 5' - deoxyribonucleoside as a white solid. The following compounds were obtained as their sodium salts: 5 - Deoxy - 5' - (3 - sulphopropanesulphonylamino)thymidine (**1**) (yield from 5' - amino - 5' - deoxythymidine,[15,16] 32%); λ_{max} 268 nm (ε 9400), λ_{max} 235 nm at pH 7.8; δ 1.80 (3H, s, $-CH_3$), 2.05 (4H, m, H-2' and $-CH_2-CH_2-CH_2$), 2.57 (2H, t, $-CH_2SO_2N$). 3-19 (4H m, H-5' and $CH_2-SO_3^-$) 3.82 (1H, m, H-4'), 4.24 (1H, m, H-3'), 5.40 (1H, bd, OH-3'), 6.17 (1H, t, H-1'), 7.35 (1H, bd, SO_2NH), 7.62 ppm (1H, s, H-6).

5' - Deoxy - 5' - (3 - sulphopropanesulphonylamino) uridine (**2**) (yield from 5' - amino - 5' - deoxyuridine,[15,16] 22%); (Found: C, 31.4; H, 4.20; N, 9.40; $C_{12}H_{18}N_3NaO_{10}S_2$ requires C, 31.9; H, 4.00; N, 9.30%); λ_{max} 261 nm (ε, 9500), λ_{min} 231 nm at pH 7.8; λ_{max} 262 nm (ε, 7400), λ_{min} 243 nm at pH 13; δ 1.96 (2H, m, $CH_2-CH_2-CH_2$), 2.56 (2H, t, CH_2SO_2N), 3.19 (4H, m, $CH_2SO_3^-$ and H-5'), 3.96 (3H, m, H-4', H-3' and H-2'), 5.3 (2H, bd, OH-2' and OH-3'), 5.61 (1H, d, H-5), 5.72 (1H, d, H-1'), 7.25 (1H, bd, SO_2NH), 7.70 (1H, d, H-6), 11.2 ppm (1H, bd, NH).

5' - Deoxy - 5' - (3 - sulphopropanesulphonylamino) adenosine (**3**) (yield from 5' - amino - 5' - deoxyadenosine,[16] 18%); λ_{max} 257 nm (ε, 13,700), λ_{min} 231 nm at pH 1; δ 2.0 (2H, m, $-CH_2-CH_2-CH_2-$), 2.62 (2H, t, CH_2SO_2N), 3.20 (4H, m, H-5' and $CH_2SO_3^-$), 4.15 (2H, m, H-3' and H-4'), 6.79 (1H, t, H-2'), 5.88 (1H, d, H-1'), 8.18 (1H, s, H-2), 8.33 ppm (1H, s, H-8).

(*E*) 5 - (2 - Bromovinyl) - 2',5' - dideoxy - 5' - (3 - sulphopropanesulphonylamino)uridine (**4**) (yield from 5' - amino - (*E*) - 5 - (2 - bromovinyl) - 2',5' - dideoxyuridine,[17] 15%) λ_{max} 289 nm (ε, 9,200) 251 nm (ε, 13,900), λ_{min} 267 nm at pH 7.2; δ 2.05 (4H, m, H-2' and $-CH_2-CH_2-CH_2$), 2.54 (2H, t, CH_2SO_2N), 3.2 (4H, m, H-5' and $-CH_2SO_3^-$), 3.8 (1H, m, H-4'), 4.15 (1H, m, H-3'), 5.35 (1H, m, OH-3'), 6.10 (1H, t, H-1'), 6.85 (1H, d, vinylic H), 7.25 (1H, t, SO_2NH), 7.28 (1H, d, vinylic H), 7.48 ppm (1H, s, H-6).

5' - (3 - *Chloropropanesulphonylamino*) - 5' - *deoxythymidine* (**5**)

To a solution of 5' - amino - 5' - deoxythymidine (0.80 g, 3.3 mmole) in pyridine at 5° there was added with stirring over 30 min, 3-chloropropanesulphonyl chloride (0.64 g, 3.6 mmole) in 5 portions. The reaction mixture was kept at 5° for 18 h and then water (1 ml) added. The solvent was then evaporated off under reduced pressure and last traces of pyridine removed by co-evaporation with water and then with ethanol. The resulting oil was chromatographed on a column of silica gel (100 g). Elution of the column with dichloromethane-methanol (9.1) gave a white solid (0.47 g, 41% yield) which was crystallised from ethyl acetate to give 5 m.p. 236–237° (Found: C, 41.1; H, 5.20; N, 11.3; S, 8.10; $C_{13}H_{20}ClN_3O_8S$ requires C, 40.9; H, 5.28; N, 11.0; S, 8.40%); λ_{max} 236 nm (ε, 9,900), λ_{min} 235 nm in ethanol; δ 1.8 (3H, s, $-CH_3$), 2.08 (3H, m, $-CH_2-CH_2-CH_2$ and H-2'), 3.15 (4H, m, CH_2SO_2 and H-5'), 3.68 (2H, t, $ClCH_2$), 4.15 (1H, m, H-3') 5.25 (1H, d, OH-3'), 6.12 (1H, t, H-1'), 7.35 (1H, bd, SO_2NH), 7.48 ppm (1H, s, H-6).

Action of sodium sulphite on 5' - (3 - *chloropropanesulphonylamino*) - 5' - *deoxythymidine*

A solution of compound **5** (200 mg, 0.52 mmole) and sodium sulphite (100 mg, 0.79 mmole) in methanol water (4:1) (10 ml) was boiled under reflux for 3 h. The solution was then evaporated to a small volume and kept at 5° for about 18 h. The crystals which separated were filtered off, washed with water and dried to give 5' - deoxy - 5' - N - (1,1 - dioxo - 1,2 - thiazolidin - 2 - yl)thymidine (**6**) (50 mg), m.p. 209° (Found: C, 44.9; H, 5.50; N, 12.4. $C_{13}H_{19}N_3O_6S$ requires C, 45.2; H, 5.60; N, 12.2%), λ_{max} 266 nm (ε, 9,800), λ_{min} 234 nm in ethanol, δ 1.80 (3H, s, CH_3), 2.15 (4H, m, H-2' and $CH_2-CH_2-CH_2$), 3.20 (6H, m, H-5', CH_2NSO_2 and CH_2SO_2N), 3.80 (1H, m, H-4'), 4.15 (1H, m, H-3'), 5.28 (1H, d, OH-3'), 6.23 (1H, t, H-1'), 7.43 (1H, s, H-6).

The combined filtrate and washings were concentrated to a small volume and then chromatographed on a column of reversed phase silica. Elution with water and lyophilisation of the appropriate fraction of the eluate gave 5' - deoxy - 5' - (3 - sulphopropanesulphonylamino)thymidine (**1**) (95 mg, 50% yield) which had identical UV and NMR spectra and similar mobility upon paper electrophoresis to compound **1** obtained by the other route.

(E) - 5 - (2 - *Bromovinyl*) - 5' - (3 - *chloropropanesulphonylamino*) - 2',5' - *dideoxyuridine* (**7**)

To a solution of 5' - amino - (*E*) - 5 - (2 - bromovinyl) - 2',5' - dideoxyuridine (0.71 g, 2.1 mmole) in pyridine (20 ml) at 5° there was added with stirring, 3-chloropropanesulphonyl chloride (0.71 g, 4.0 mmole). The reaction mixture was kept at 5° for 18 h and then water (1 ml) was added. The solvents were removed by evaporation and the residue chromatographed on a column of silica gel (80 g). Elution with chloroform methanol (17:3) gave (**7**) (294 mg, 29% yield), m.p. 121–130° (d) (Found: C, 35.6; H, 4.10; N, 8.90. $C_{14}H_{19}BrClN_3O_6S$ requires C, 35.6; H, 4.10; N, 8.90%); λ_{max} 287 nm (ε, 10,600), 250 nm (ε, 13,300), λ_{min} 267 nm in ethanol; δ 2.1 (4H, m, H-2' and $-CH_2-CH_2-CH_2$), 3.2 (4H, m, H-5' and CH_2SO_2N), 3.7 (3H, m, H-4' and $ClCH_2$), 4.2 (1H, m, H-3'), 5.35 (1H, d, OH-3'), 6.15 (1H, t, H-1'), 6.85 (1H, d, vinylic H), 7.25 (1H, d, vinylic H), 7.35 (1H, t, SO_2NH), 7.82 ppm (1H, s, H-6).

5' - *Deoxy* - 5' - *p* - *nitrobenzenesulphonylaminothymidine* (**9**)[12]

To a rapidly stirred, cooled solution of 5' - amino - 5' - deoxythymidine (1.0 g, 4.2 mmole) in pyridine (50 ml), a solution of *p*-nitrobenzenesulphonyl chloride (0.99 g, 4.5 mmole) in pyridine (30 ml) was added dropwise over 2 h. The reaction mixture was kept at 5° for 18 h, methanol (2 ml) added and the solvent removed under reduced pressure. Trituration of the resulting oil with water (100 ml) gave a pale-brown solid. This was filtered off and crystallised

from ethanol to give pale-yellow crystals of (**9**) (1.31 g, 74% yield), m.p. 178–179° (Found: C, 45.2; H, 4.30; N, 13.0; S, 7.30. $C_{16}H_{18}N_4O_8S$ requires C, 45.1; H, 4.22; N, 13.1; S, 7.52%); λ_{max} 267 nm (ϵ, 28,700), λ_{min} 232 nm in ethanol; δ 1.81 (3H, s, $-CH_3$), 2.06 (2H, m, H-2'), 2.88 (2H, m, H-5'), 3.73 (1H, m, H-4'), 4.15 (1H, m, H-3'), 5.28 (1H, m, OH-3'), 5.88 (2H, s, NH_2), 6.10 (1H, t, H-1'), 6.60 (2H, d, aromatic protons), 7.06 (4H, m, aromatic protons, SO_2NH and H-6), 11.25 ppm (1H, s, NH).

5' - p - Acetamidobenzeneslphonylamino - 5' - deoxythymidine (**11**)

A solution of p-acetamidobenzenesulphonyl chloride (1.05 g, 4.48 mmole) in pyridine (40 ml) was added dropwise with stirring to a solution of 5' - amino - 5' - deoxythymidine (1.0 g, 4.2 mmole) in pyridine (100 ml) at 5°. Stirring was continued for a further hour and then the reaction mixture was kept at 5° for 18 h. Methanol (2 ml) was then added and the solvent removed under reduced pressure. The residue was co-evaporated with ethanol and then chromatographed on a column of silica gel (150 g). Elution with chloroform ethanol (4:1) gave the required compound (1.25 g, 68% yield) which was crystallised from aqueous ethanol to give **11** m.p. 145° (Found: C, 47.4; H, 5.30; N, 12.1. $C_{18}H_{22}N_4O_7S$ requires C, 47.4; H, 5.3; N, 12.3%); λ_{max} 262.5 nm (ϵ, 28,000), λ_{min} 229 nm in ethanol; δ 1.79 (3H, s, H-5'), 2.08 (5H, m, CH_3CO and H-2'), 2.96 (2H, m, H-5'), 3.73 (1H, m, H-4'), 4.14 (1H, m, H-3'), 5.23 (1H, d, OH-3'), 6.12 (1H, t, H-2'), 7.48 (1H, s, H-6), 7.74 (5H, m, aromatic H and SO_2NH), 10.27 (1H, s, CONH), 11.23 ppm (1H, s, NH).

5' - *p* - *Aminobenzenesulphonylamino* - 5' - *deoxythymidine* (**10**)

(a) A solution of compound **9** (200 mg, 0.47 mmole) and sodium dithionite (500 mg, 4.6 mmole) in aqueous M sodium hydroxide (20 ml) was boiled for 1 h. The solution was cooled to room temperature, neutralised (dilute HCl) and the solution evaporated to a small volume. Upon standing and cooling, crystals were formed. These were filtered off to give **10** (172 mg, 78% yield) m.p. 208–210° (Found: C, 48.7; H, 5.40; N, 13.9; S, 8.0. $C_{16}H_{20}N_4O_6S$ requires C, 48.5; H, 5.10; N, 14.1; S, 8.1%); λ_{max} 267 nm (ϵ 28,700), λ_{min} 232 nm in ethanol; δ 1.81 (3H, s, CH_3), 2.06 (2H, m, H-2'), 2.88 (2H, m, H-5'), 3.73 (1H, m, H-4'), 4.15 (1H, m, H-3'), 5.28 (1H, m, OH-3'), 5.88 (2H, s, $-NH_2$), 6.10 (1H, t, H-1'), 6.60 (2H, d, aromatic H), 7.06 (4H, m, aromatic H, SO_2NH and H-6), 11.25 ppm (1H, s, NH).

(b) A solution of compound **11** (220 mg, 0.50 mmole) in aqueous M sodium hydroxide (20 ml) was boiled for 2.5 h. The cooled solution was neutralized (dilute HCl) and the resulting precipitate filtered off and crystalised from aqueous ethanol to give a product which was identical with that prepared by method (a) (yield 79%).

Reaction of sulphur trioxide-triethylamine with compound **10**

A solution of compound **10** (230 mg, 0.58 mmole) and sulphur trioxide-triethylamine (90 mg, 0.65 mmol) in pyridine (30 ml) was heated at 60° for 4 h, (paper electrophoresis at pH 7.2 of a sample of the mixture revealed the presence of uncharged, mono-anionic and dianionic species). The pyridine was removed by evaporation under reduced pressure and co-evaporation with water. The residue was dissolved in aqueous 0.01M triethylammonium bicarbonate and applied to a DEAE cellulose column (2 × 30 cm) and elution carried out with a triethylammonium bicarbonate gradient (0.01→0.15M in 4 l.).

The first component eluted was starting material, compound **10**, the second component was identified as 5' - deoxy - 5' - p - sulphaminobenzenesulphonylaminothymidine (**8**) (32 mg, 11% yield) (mobility on paper electrophoresis at pH 7.2, 0.51 relative to thymidine 5'-triphosphate); λ_{max} 258 nm (ϵ, 19,700), λ_{min} 228 nm in water at pH 7.8; δ 1.81 (3H, s, CH_3), 2.06 (2H, m, H-2'), 2.90 (2H, m, H-5'), 3.74 (1H, m, H-4'), 4.15 (1H, m, H-3'), 6.10 (1H, t, H-1'), 7.17 (2H, d, aromatic H *meta* to sulphamino group) 7.57 ppm (3H, m, H-6 and aromatic H *ortho* to sulphamino group). The third component was identified as 5' - p - aminobenzenesulphonylamino - 5' - deoxy - 3' - O - sulphothymidine (**12**) (50 mg, 14% yield) (mobility on paper electrophoresis at pH 7.2, 0.50 relative to thymidine 5'-phosphate); λ_{max} 261 nm (ϵ, 23,400), λ_{min} 225 nm in water at pH 7.8, δ 1.81 (3H, s, CH_3), 2.25 (1H, m, H-2'), 2.92 (2H, m, H-5'), 4.00 (1H, m, H-4'), 4.63 (1H, m, H-3'), 5.88 (2H, s, NH_2), 6.03 (1H, t, H-1'), 6.60 (2H, d, aromatic H o to the amino group), 7.42 (2H, d, aromatic H m to the amino group), 7.61 ppm (1H, s, H-6).

A fourth component was eluted from the column. This had a mobility of 0.95 relative to thymidine 5'-triphosphate upon paper electrophoresis at pH 7.2 and so was presumably a disulphated product.

Enzyme inhibition studies

The inhibitory activities of the synthetic analogues were measured by determining the incorporation of radioactive labelled nucleoside triphosphates into a poly[d(A – T)] template at various inhibitor concentrations.

In the experiments with *E.coli* RNA polymerase holoenzyme the reaction mixture contained $MgCl_2$ (8 mM), tris-HCl, pH 8.0 (40 mM), KCl (200 mM), dithiothreitol (5 mM), [^{14}C] UTP (0.5 mM), [^{14}C] ATP (0.5 mM), poly[d(A – T)] (1 OD/ml), enzyme (21 μg/ml) and analogue in the range 1–27.5 mM. In the experiments with *E.coli* DNA polymerase I the reaction mixture contained $MgCl_2$ (10 mM), tris-HCl, pH 7.5 (100mM), dithiothreitol (2 mM), dTTP (0.4 mM), [^{14}C]dATP (0.4 mM), poly[d(A – T)] (1 OD/ml), enzyme (9.1 μg/ml) and analogue in the range 2.5–30 mM. The incubation was carried out at 37° for 4 min. The polymeric material separated chromatographically and

Table 1. Inhibition of enzymes by nucleoside 5'-triphosphate analogues

Analogue	Concn of analogue (mM)	% inhibition of enzyme: *E.coli* RNA polymerase	% inhibition of enzyme: *E.coli* DNA polymerase I
5'-Deoxy-5'-(3-sulphopropanesulphonylamino)uridine (**2**)	5	0	–
	27.5	15	–
5'-Deoxy-5'-(3-sulphopropanesulphonylamino)adenosine (**3**)	1	0	—
	27.5	0	—
5'-Deoxy-5'-(3-sulphopropanesulphonylaminothymidine (**1**)	5	– –	0
	10	– –	26
	20	–	82
	30	—	97
5'-Deoxy-5'-*p*-sulphaminobenzenesulphonylaminothymidine (**8**)	3	—	32
	12	–	60

the radioactivity determined by scintillation counting. The results (Table 1) are expressed as a percentage decrease of radioactive label in the polymer as compared to standard assays carried out in the absence of the analogue. The kinetics of the inhibition of *E.coli* DNA polymerase I by compound 1 (10 mM) was investigated. Both K_m and V_{max} were reduced thus showing that the inhibition was non competitive.

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