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SYNTHESIS OF SOME PURINE AND PYRIMIDINE NUCLEOSIDES OF 3-AMINO-2,3,6-TRIDEOXY-L-*lyxo*-HEXOPYRANOSE (DAUNOSAMINE)

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ABSTRACT

The daunosaminyl analogue of the antibiotic puromycin and the nucleoside derivatives of daunosamine with adenine, thymine, and cytosine have been synthesised. The nucleoside derivatives of 6-dimethylaminopurine, thymine, and cytosine were prepared by melting the protected daunosamine with the protected base *in vacuo*. Daunosaminyladenine was obtained by condensing *N*-trifluoroacetyl-*O*-trifluoroacetyl-α-daunosaminyl chloride either with N^6-benzoyl-9-chloromercuryadenine in boiling xylene or with N^6-benzoyladenine in dichloromethane at room temperature in the presence of a molecular sieve. In each reaction, the β-anomeric nucleoside was obtained, as shown by p.m.r. data. The protecting groups were removed with barium hydroxide or methanolic ammonia to give the free aminonucleosides in good yield. 9-β-Daunosaminyl-6-dimethylaminopurine was coupled to *N*-benzyloxycarbonyl-*O*-methyltyrosine, giving, after hydrogenolysis, the daunosaminyl analogue of puromycin.

INTRODUCTION

Daunosamine (**1**), 3-amino-2,3,6-trideoxy-L-*lyxo*-hexopyranose, is the sugar moiety of the antitumour antibiotics daunorubicin (**2a**) and adriamycin (doxorubicin, **2b**), the latter being a promising drug for the clinical treatment of a variety of human cancers[1]. The anthracycline antibiotics form strong intercalation complexes with DNA, the structural features of the amino sugar moiety being an important requirement for the stabilization of the complex[2].

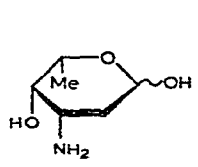
The antimicrobial and cytotoxic activity of many natural and synthetic nucleosides[3] has increased interest in the preparation of synthetic analogues as potential agents for inhibiting enzyme systems involved in nucleic acid and protein biosynthesis. Puromycin (**3**) has been extensively studied and some 5′-deoxy synthetic analogues and the corresponding free aminonucleosides have been prepared and found to possess activity comparable to that of the parent antibiotic[4–6].

The presence of daunosamine in clinically useful, antitumour compounds prompted us to prepare the daunosamine analogue (**4d**) of puromycin, as well as three new nucleosides, namely, the daunosaminyl derivatives of adenine (**6b**), thymine (**9b**),

and cytosine (**10b**). Daunosamine, the starting product for the preparation of these nucleosides, is now available both by acidic hydrolysis of daunorubicin[7], as well as by synthesis from L-rhamnose[8] and methyl α-D-mannopyranoside[9].

RESULTS AND DISCUSSION

The synthesis of 9-β-daunosaminyl-6-dimethylaminopurine (**4b**) was performed by fusion of fully acetylated daunosamine[7] (α,β-ratio 30:70) with 6-chloropurine in the presence of chloroacetic acid as catalyst. Only the acetylated β-nucleoside (**4a**) was obtained in 50% yield from the reaction mixture after treatment with methanolic dimethylamine. Hydrolysis of the protecting group with barium hydroxide gave the



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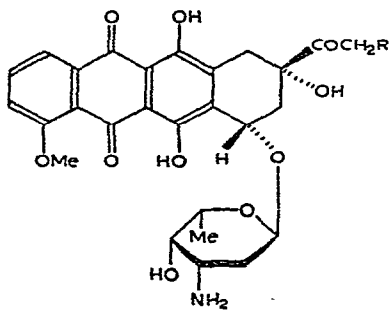
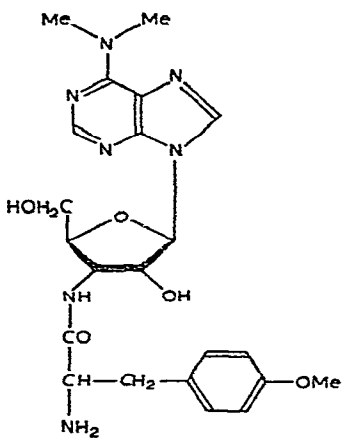


2a R = H
2b R = OH



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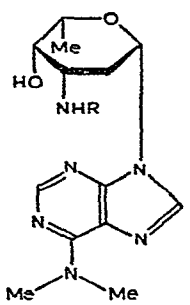
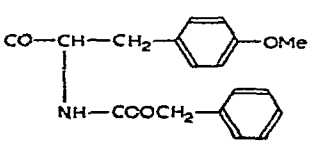
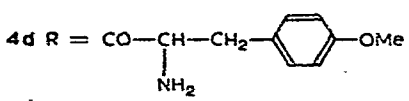


4a R = Ac
4b R = H



4c R =



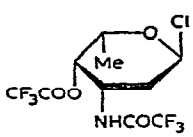
4d R =

aminonucleoside (**4b**), which was coupled[10] to *N*-benzyloxycarbonyl-*O*-methyltyrosine in the presence of dicyclohexylcarbodiimide and *N*-hydroxysuccinimide to give **4c**. Hydrogenolysis of the benzyloxycarbonyl group gave the puromycin analogue **4d**.

A useful intermediate for the synthesis of the other daunosamine-derived nucleosides was *N*-trifluoroacetyl-*O*-trifluoroacetyl-α-daunosaminyl chloride[11] (**5**) which was prepared by conversion of daunosamine into the 3-*N*-trifluoroacetyl-1,4-bis-*O*-trifluoroacetyl derivative and subsequent treatment with dry hydrogen chloride. The chloro derivative is sufficiently stable to be isolated as a white solid and can be stored for some days under anhydrous conditions[12].

Two of the currently used methods for the synthesis of adenine nucleosides, namely, the condensation of N^6-benzoyl-9-chloromercuryadenine with protected glycosyl halides and the reaction of N^6-benzoyladenine with glycosyl halides in the presence of a molecular sieve have been employed. Each procedure gave only the β anomer of the protected nucleoside of adenine. After removal of the 4-*O*-trifluoroacetyl group by treatment with methanol, the condensation product **6a** was hydrolyzed with methanolic ammonia, affording the free nucleoside **6b**.

The low yield (10%) of the product of reaction of **5** with N^6-benzoyl-9-chloromercuryadenine in boiling xylene is attributed, at least in part, to the concurrent conversion of **5** into 1,5-anhydro-2,3,6-trideoxy-3-trifluoroacetamido-4-*O*-trifluoroacetyl-L-*lyxo*-hex-1-enitol (**7**) and *N*-trifluoroacetyldaunosamine[13] (**8**) which were isolated from the reaction mixture before treatment with methanol.



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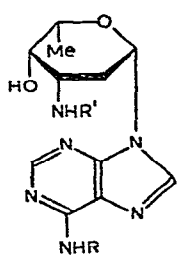
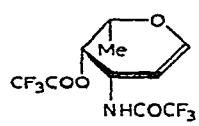


6a R = Bz, R' = CF_3CO
6b R = R' = H



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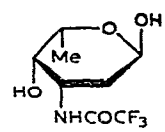

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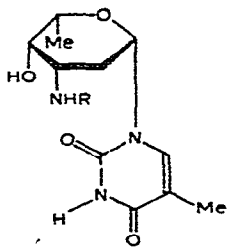
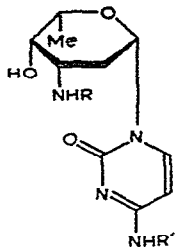
The synthesis of pyrimidine nucleosides of daunosamine has been performed by the fusion method[14], starting from the trimethylsilyl derivatives of the pyrimidine bases[15] and the chloro derivative **5**; the condensation products were recovered after removal of the 4'-*O*-trifluoroacetyl group by treatment with methanol. The *N*-protecting groups were removed by hydrolysis with methanolic ammonia, affording the free nucleosides **9b** and **10b**. The instability of **5** under the reaction conditions is in agreement with literature data about the chemical behaviour of 2,6-dideoxy-1-halo sugars[15] and explains the rather poor yields of the fusion procedures.



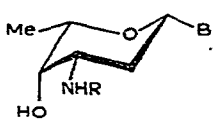
9a R = CF_3CO
9b R = H



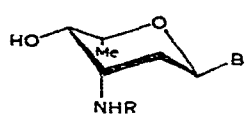
10a R = CF_3CO, R′ = Bz
10b R = R′ = H

Attempts to carry out these condensation reactions under milder conditions by stirring the components at room temperature in dichloromethane in the presence of a Friedel–Crafts catalyst[17,18] (*e.g.*, stannic chloride), or by treatment of the pyrimidine derivative with the glycosyl halide **5** in the presence of mercuric cyanide and nitromethane, failed to give the expected products.

The β-anomeric configuration was assigned to the new daunosamine nucleosides on the basis of p.m.r.-spectral data. For the adenine derivatives **4a–d** and **6a–b** at 36°, the anomeric proton gives two doublets with vicinal coupling constant values in the range 10.0–11.0 Hz ($J_{1ax,2ax}$) and 2.5–4.0 Hz ($J_{1ax,2eq}$). The presence of the diaxial coupling dictates the equatorial orientation of the heterocyclic base, as in the $^1C_4$ conformation **11**.



11 (β, $^1C_4$)



12 (α, $^4C_1$)

The $^4C_1$ conformation **12** would be destabilised by a *syn*-diaxial interaction between the substituents at positions 3 and 5. For the thymine derivatives at 36°, the signal for the anomeric proton was a broadened triplet (spacing ~6.5 Hz). The pair of doublets with axial–axial and axial–equatorial coupling constants appeared if the temperature was lowered to 28° for **9b** and to 15° for **9a**. This behaviour can be interpreted in terms of an equilibrium between $^1C_4$ and $^4C_1$ conformations. Lower temperatures favour the $^1C_4$ conformer, whereas higher temperatures shift the equilibrium towards the $^4C_1$ form*. For the cytosine derivatives, the anomeric-proton signal was also a broadened triplet at 36°, but this pattern did not change by lowering the temperature to ~10° for **10b**, whereas, for **10a**, the pair of doublets with $J_{ax,ax}$ and $J_{ax,eq}$ appeared at 15°. Therefore, the β configuration may be assigned to daunosaminylcytosine.

*However, it has been reported that the nucleosides of deoxypyranoses, namely 2-deoxy-D-*erythro*-pentopyranosyl-9*H*-purines, exist in a flexible conformation at room temperature[19].

EXPERIMENTAL

General. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. T.l.c. was performed on silica gel 60 F (Merck), and the spots were detected under u.v. light or by charring with sulphuric acid. P.m.r. spectra were determined on Varian A-60A or HA-100 instruments with Me_4Si as internal reference in the indicated solvents. Rotations were determined with a Roussel–Jouan digital polarimeter at 25°. Evaporations were performed at 40° under reduced pressure (water aspirator).

9-(N-Acetyl-β-daunosaminyl)-6-dimethylaminopurine (**4a**). — A mixture of 1.28 g (4.68 mmol) of 3-*N*-acetyl-1,4-di-*O*-acetyldaunosamine (α,β ratio, 30:70) and 0.726 g (4.70 mmol) of 6-chloropurine was rapidly heated to 120°, and chloroacetic acid (15 mg) was added. Heating was continued for 45 min at 0.55 mmHg until evolution of acetic acid had subsided. After cooling, the residue was dissolved in 100 ml of chloroform, and the solution was washed with saturated, aqueous sodium hydrogen carbonate and 30% aqueous sodium chloride, and then concentrated. The residue (1.6 g) was treated for 12 h with 25 ml of 40% aqueous dimethylamine at room temperature. The solution was then concentrated, and a solution of the white, solid residue in 50 ml of ethyl acetate was washed with 30% aqueous sodium chloride, concentrated to a small volume, and chilled. After 15 h, **4a** (0.77 g, 49%) was collected; m.p. 222–223° (from ethyl acetate). P.m.r. data ($CDCl_3$): δ 1.30 (d, *J* 6.5 Hz, Me-5′), 2.00 (s, AcO), 3.53 (s, NMe_2), 5.83 (dd, $J_{ax,ax}$ 10, $J_{ax,eq}$ 4 Hz, H-1′), 7.98 and 8.35 (2 s, H-2 and H-8).

Anal. Calc. for $C_{15}H_{22}N_6O_3$: C, 53.88; H, 6.63. Found: C, 53.55; H, 6.48.

9-β-Daunosaminyl-6-dimethylaminopurine (**4b**). — A mixture of 2.28 g (6.8 mmol) of **4a** and 115 ml of 0.1M barium hydroxide was kept for 20 h at 100°. The cooled solution was neutralized with solid carbon dioxide and concentrated. The white, solid residue was treated with hot ethanol, and the solution was filtered and concentrated to dryness. The residue was dissolved in 50% aqueous methanol and placed on a column containing 75 g of Amberlite IRC-50(HO^-) resin. The column was washed with 50% aqueous methanol until the eluate was free of u.v.-absorbing material, and then with 2M ammonium hydroxide in 50% aqueous methanol which gave **4b** (1.75 g, 88%), m.p. 232–233° (from propan-2-ol), $[\alpha]_D$ −4.5° (*c* 0.9, methanol). P.m.r. data (Me_2SO-d_6): δ 1.16 (d, *J* 6.5 Hz, Me-5′), 3.46 (s, NMe_2), 3.76 (q, *J* 6.5 Hz, H-5′), 5.74 (dd, $J_{ax,ax}$ 11, $J_{ax,eq}$ 2.5 Hz, H-1′), 8.21 and 8.30 (2 s, H-2 and H-8).

Anal. Calc. for $C_{13}H_{20}N_6O_2$: C, 53.41; H, 6.89. Found: C, 53.10; H, 6.88.

9-{3-[(N-Benzyloxycarbonyl-D-methoxyphenyl-L-alanyl)amino]-2,3,6-trideoxy-β-L-lyxo-hexopyranosyl}-6-dimethylaminopurine (**4c**). — A mixture of 0.35 g (1.197 mmol) of **4b**, 0.416 g (1.261 mmol) of *N*-benzyloxycarbonyl-*O*-methyltyrosine, and 0.145 g (1.261 mmol) of *N*-hydroxysuccinimide in 16 ml of anhydrous *N*,*N*-dimethylformamide was stirred at room temperature for 15 min, and dicyclohexylcarbodiimide (0.26 g, 1.261 mmol) was then added. After stirring for 16 h, the mixture was filtered,

and concentrated under high vacuum. A solution of the residue in 50 ml of ethyl acetate and 25 ml of 1-butanol was washed with 25 ml of aqueous 10% sodium chloride, 5% aqueous sodium hydrogen carbonate (3 × 25 ml), and 25 ml of saturated, aqueous sodium chloride, then dried, and concentrated to give **4c** as an amorphous solid (0.62 g, 85%), m.p. 105–108°, $[\alpha]_D$ −10° (*c* 1, methanol). P.m.r. data ($CDCl_3$): δ 1.26 (d, *J* 6.5 Hz, Me-5'), 3.53 (s, NMe_2), 3.76 (s, OMe), 5.05 (s, $COOCH_2$), 5.78 (dd, $J_{ax,ax}$ 10.5, $J_{ax,eq}$ 3.5 Hz, H-1'), 7.93 and 8.35 (2 s, H-2 and H-8).

6-Dimethylamino-9-[2,3,6-trideoxy-3-(p-*methoxyphenyl-*L-*alanylamino)-*β-L-*lyxo-hexopyranosyl]purine* (**4d**). — A solution of **4c** (1.5 g, 2.485 mmol) in 150 ml of glacial acetic acid was shaken in the presence of 0.75 g of Pd/C (10%) under hydrogen at 1 atmos. After 30 min, evolution of carbon dioxide ceased, and the solution was filtered through Celite and concentrated. The white residue was eluted from silica gel with chloroform–methanol (4:1) to give **4d** (0.73 g, 63%), m.p. 126–128° (from propan-2-ol–ethyl ether), $[\alpha]_D$ −8° (*c* 0.84, methanol). P.m.r. data (Me_2SO-d_6): δ 1.18 (d, *J* 6.5 Hz, Me-5'), 3.46 (s, NMe_2), 3.70 (s, OMe), 5.86 (dd, $J_{ax,ax}$ 10, $J_{ax,eq}$ ~3 Hz, H-1'), 8.22 and 8.30 (2 s, H-2 and H-8).

Anal. Calc. for $C_{23}H_{31}N_7O_4$: C, 58.83; H, 6.65. Found: C, 58.98; H, 6.57.

6-Benzoyl-9-(N-trifluoroacetyl-β-daunosaminyl)adenine (**6a**). — *Method A.* To an azeotropically dried suspension of 3.32 g (7 mmol) of N^6-benzoyl-9-chloromercuryadenine in 100 ml of xylene, 2.45 g (6.85 mmol) of *N*-trifluoroacetyl-*O*-trifluoroacetyl-α-daunosaminyl chloride (**5**) dissolved in 20 ml of xylene and 20 ml of ethyl ether were added at 45° during 15 min with stirring. The mixture was boiled under reflux for 90 min, then cooled, and filtered. The filtered material was extracted with 100 ml of chloroform, and the filtrate and washings were combined and concentrated. A solution of the residue in 100 ml of chloroform was filtered, washed with 30% aqueous potassium iodide (3 × 40 ml) and then water, dried (Na_2SO_4), and concentrated. The solid residue was extracted with hot hexane. Insoluble material was extracted for 30 min with boiling methanol, the extract was concentrated, and the residue was eluted from silica gel with acetone–benzene (5:1) to afford **6a** (0.33 g, 10%), m.p. 142–146°. Mass spectrum: *m/e* 464 (M^+). P.m.r. data ($CDCl_3$): δ 1.33 (d, *J* 6.5 Hz, Me-5'), 5.94 (dd, $J_{ax,ax}$ 10, $J_{ax,eq}$ 4 Hz, H-1'), 7.3–8.1 (m, C_6H_5), 8.30 and 8.76 (2 s, H-2 and H-8).

Concentration of the hexane solution and fractional crystallization of the residue from di-isopropyl ether–hexane gave 0.25 g of 1,5-anhydro-2,3,6-trideoxy-3-trifluoroacetamido-4-*O*-trifluoroacetyl-L-*lyxo*-hex-1-enitol (**7**), m.p. 73–74° [mass spectrum: *m/e* 322 (M + 1)] and 0.18 g of *N*-trifluoroacetyldaunosamine (**8**), m.p. 145–147° [mass spectrum: *m/e* 226 (M − 17)].

Method B. A solution of 1.22 g (3.42 mmol) of freshly prepared **5** in 50 ml of dry dichloromethane was added to a mixture of 1.33 g (5.55 mmol) of N^6-benzoyladenine and 4.3 g of molecular sieve (4 Å). The reaction mixture was stirred for 5 days at room temperature, then filtered, and concentrated. The syrupy residue was treated for 1 h with 50 ml of boiling methanol. The solution was concentrated and the residue was eluted from silica gel with acetone–benzene (5:1). The fractions containing the pure

nucleoside were combined and concentrated to give **6a** (0.49 g, 31%), m.p. 143–145°, which was identical with the product obtained by Method A.

9-β-Daunosaminyladenine (**6b**). — A solution of 0.380 g of **6a** in 25 ml of methanol was saturated at 0° with ammonia. After storage for 1 week in a closed vessel at room temperature, the solution was concentrated under vacuum until crystallization began. The precipitate, collected after cooling overnight, was crystallized from propan-2-ol to afford **6b** (0.2 g, 92.5%), m.p. 243–246°, $[\alpha]_D$ −8° (*c* 1.24, methanol). P.m.r. data (Me_2SO-d_6): δ 1.10 (d, *J* 6.5 Hz, Me-5′), 3.72 (dq, *J* 6.5, *J*′ ~1 Hz, H-5′), 5.66 (dd, $J_{ax,ax}$ 10.5, $J_{ax,eq}$ 3 Hz, H-1′), 8.15 and 8.28 (2 s, H-2 and H-8).

Anal. Calc. for $C_{11}H_{16}N_6O_2$: C, 49.99; H, 6.10. Found: C, 49.65; H, 6.18.

1-β-Daunosaminylthymine (**9b**). — A mixture of 4.16 g (11.6 mmol) of **5** and 4.74 g (17.5 mmol) of bis(trimethylsilyl)thymine was heated for 45 min at 130°/20 mmHg. The melt was cooled, 100 ml of 80% aqueous methanol were added, and the suspension was boiled under reflux for 30 min, then filtered, and concentrated. The solid residue was extracted with hot dichloromethane, and the extract was dried (Na_2SO_4) and concentrated to give 2.25 g (55%) of 1-(*N*-trifluoroacetyl-β-daunosaminyl)thymine (**9a**), m.p. 247–249° (from ethanol), $[\alpha]_D$ −120° (*c* 1, methanol). P.m.r. data (Me_2SO-d_6): δ 1.18 (d, *J* 6.5 Hz, Me-5′), 1.80 (s, Me-5), 5.72 (dd, $J_{ax,ax}$ 10, $J_{ax,eq}$ 2 Hz, H-1′), and 7.53 (s, H-6).

A solution of 1.13 g of **9a** in 3 ml of methanol previously saturated with ammonia at 0° was kept at room temperature for 1 week in a stoppered vessel, then concentrated to a small volume, and diluted with ether. The resulting precipitate was collected, and crystallized from methanol–ethanol, yielding **9b** (0.66 g, 80%), m.p. 266–267°, $[\alpha]_D$ −183° (*c* 1, methanol). P.m.r. data (Me_2SO-d_6): δ 1.13 (d, *J* 6.5 Hz, Me-5′), 1.4–1.9 (m, H-2′), 1.76 (d, *J* <1 Hz, Me-5), 3.64 (dq, *J* 6.5, *J*′ ~1 Hz, H-5′), 5.50 (dd, $J_{ax,ax}$ 9.7, $J_{ax,eq}$ 4 Hz, H-1′), 7.60 (q, *J* <1 Hz, H-6).

Anal. Calc. for $C_{11}H_{17}N_3O_4$: C, 51.75; H, 6.71; N, 16.46. Found: C, 51.58; H, 6.77; N, 16.36.

1-β-Daunosaminylcytosine (**10b**). — A mixture of 0.5 g (1.4 mmol) of **5** and 0.75 g (2.1 mmol) of N^4-benzoyl-bis(trimethylsilyl)cytosine was heated at 150° for 1 h in a closed, previously evacuated glass-flask. After cooling, 30 ml of 80% methanol were added, and the resulting suspension was boiled under reflux for 30 min, then filtered, and concentrated. The white residue was crystallized from methanol to give 0.13 g (21%) of N^4-benzoyl-1-(*N*-trifluoroacetyl-β-daunosaminyl)cytosine (**10a**), m.p. 259–261°, $[\alpha]_D$ −64° (*c* 1, methanol). P.m.r. data (Me_2SO-d_6): δ 1.22 (d, *J* 6.5 Hz, Me-5′), 5.85 (dd, $J_{ax,ax}$ 8.5, $J_{ax,eq}$ 3.5 Hz, H-1′), 7.3–7.7 and 7.9–8.3 (2 m, H-5,6 and aromatic protons).

Protecting groups were removed by dissolving 0.2 g of **10a** in 60 ml of methanol saturated with ammonia at 0°. The solution was kept in a pressure flask at room temperature for 1 week and then concentrated, and the residue was eluted from silica gel with 80% methanol to provide **10b** (0.105 g, 96%), m.p. 152–155° (from propan-

2-ol), $[\alpha]_D$ −88° (*c* 1, methanol). P.m.r. data (Me_2SO-d_6): δ 1.08 (d, *J* 6.5 Hz, Me-5'), 1.3–1.8 (m, H-2'), 5.56 (broad t, H-1'), 5.72 and 7.60 (2 d, *J* 7.5 Hz, H-5,6).

Anal. Calc. for $C_{10}H_{16}N_4O_3$: C, 49.99; H, 6.71; N, 23.32. Found: C, 49.79; H, 6.78; N, 23.18.

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