Replacement of the 3′-CH Group by Nitrogen in the Carbocyclic Analogue of Thymidine

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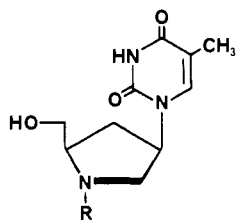
We have prepared (4*R*)-4-thyminyl-D-prolinol, an analogue of 3′-deoxythymidine in which the sugar has been replaced by D-prolinol. This strongly basic secondary amine has been converted to the corresponding hydroxylamine, an analogue of either thymidine or 2′-deoxyxylofuranosylthymine. We have also synthesized a number of simple derivatives of the amine for testing in vitro activity against herpes simplex 1 (HSV-1), human immunodeficiency virus 1 (HIV-1), and a panel of human tumor cell lines. Among these compounds, the hydroxylamine **12** proved active against the human tumor cell lines of breast, colon, and lung origin, with IC_{50} values of 0.08, 14.02, and 6.91 μM, respectively.

Many nucleoside analogues have been prepared and tested as antivirial or anticancer agents, and a number of them have found important clinical application. We decided, therefore, to synthesize a novel thymidine analogue, the hydroxylamine **12**, and to screen it as an anticancer agent. In view of the promise of 2′,3′-dideoxynucleosides and 3′-azido-3′-deoxythymidine in the treatment of viral diseases including AIDS,[1] we also screened the 3′-deoxythymidine analogue **10** and a number of its simple derivatives against human immunodeficiency virus 1 (HIV-1) and herpes simplex 1 (HSV-1). The nucleoside analogue **12** is active against a number of tumor-derived cell lines.



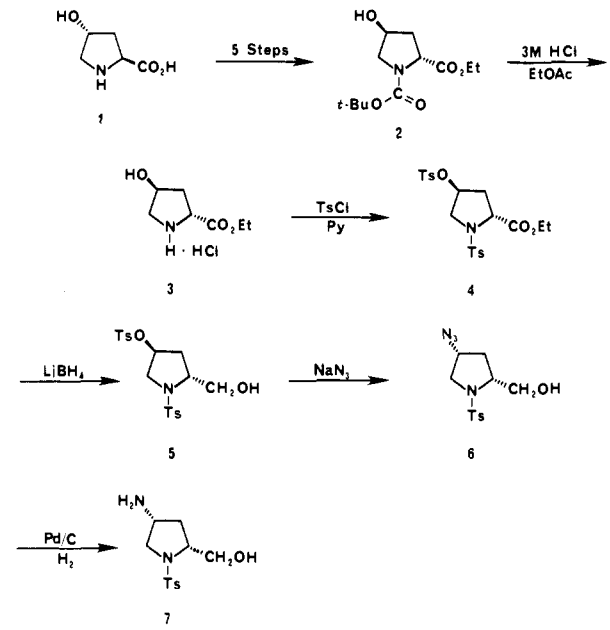
10. R = H
11. R = CH_2CH_2CN
12. R = OH
13. R = NO
14. R = CN
15. R = CO_2CH_3
16. R = CHO

Chemistry

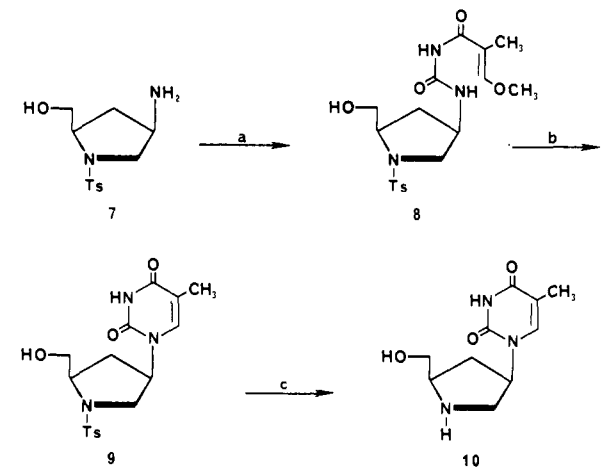
Purine and pyrimidine derivatives of L-prolinol have been described.[2] However, the L compounds are not close structural analogues of the D-nucleotides. Our synthesis of the deoxythymidine analogue **10** is a modification of the published procedure which we designed to obtain the D-enantiomer of **10** from an inexpensive starting material *trans*-4-hydroxy-L-proline.

Scheme I illustrates the synthesis of the key optically active intermediate *N*-tosyl-*cis*-4-amino-D-prolinol (**7**), from *trans*-4-hydroxy-L-proline (**1**), whose absolute stereochemistry is already established. The proper choice of protecting groups for the secondary amino function of **1** was vital because reactions that proceed satisfactorily on monofunctional compounds often failed or gave unstable products in this series. In our original approach to the synthesis of **9**, we planned to block the basic nitrogen of **1** with the tosyl group. However, the conversion of the *N*,*O*-ditosyl-*cis*-4-hydroxy-D-proline ethyl ester to the *N*-tosyl, O-acetyl-*trans*-4-hydroxy-D-proline ethyl ester by displacement of the tosylate group with tetraethylammonium acetate was unsuccessful. Instead, the *trans*-4-hydroxy-D-proline ethyl ester **2** was prepared from **1** by the five-step route of Stille et al.[3] with a *tert*-butoxycarbonyl protecting group on the amine. The Boc group was removed with 3 M HCl in ethyl acetate[4] to give the hydrochloride salt of the *trans*-4-hydroxy-D-proline ethyl ester (**3**) in nearly quantitative yield. We converted the amino alcohol **3** into the azide **6** using a slight modification of published procedures.[3,5] Catalytic hydrogenation of the azido group in **6** yielded *N*-tosyl-*cis*-4-

Scheme I



Scheme II[a]



[a] (a) $CH_3OCH{=}C(CH_3)CONCO$, C_6H_6, DMF, Et_2O; (b) 15 N NH_4OH; (c) HBr/HOAC, C_6H_5OH.

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amino-D-prolinol (7) in quantitative yield.

The 3'-modified analogue of thymidine **10** was synthesized from 3-methoxy-2-methylacryloyl isocyanate[6] and *N*-tosyl-*cis*-4-amino-D-prolinol (**7**) by the methodology of Shealy et al.[7] (Scheme II). Reaction of the unprotected hydroxy pyrrolidine **7** with the acyl isocyanate in anhydrous benzene–DMF–ether mixtures at low temperature gave high yield of the acryloylurea **8**. Cyclization of the acryloylurea **8** in 15 N aqueous ammonia furnished a good yield of **9**. Reductive cleavage of the *N*-tosyl residue with hydrobromic acid/acetic acid and phenol[5] gave *cis*-4-(thymin-1-yl)-D-prolinol (**10**). This amine was hygroscopic and discolored in air after a few days. Attempts to recrystallize this material failed. The product was purified as the stable hydrochloride salt. The amine **10**, in which the 3'-position is modified, is a strongly basic analogue of 3'-deoxythymidine.

The secondary amino function of **10** is readily converted to a variety of derivatives by general procedures. Cyanoethylation of **10** with acrylonitrile[8] gave the tertiary amino alcohol **11**. Oxidation with 99% *m*-chloroperoxybenzoic acid[9] in methanol gave the somewhat unstable *N*-oxide. When this compound was warmed in methanol, acrylonitrile was lost, giving the hydroxylamine **12**. This hydroxylamine cocrystallized with *m*-chlorobenzoic acid and could not be separated from it. Attempts to purify the hydroxylamine by silica gel or ion-exchange column chromatography failed because it was oxidized during the elution. The hydrochloride of **12** is hygroscopic and stable in the solid form.

The *N*-nitroso compound **13** was readily obtained by addition of sodium nitrite[10] to an acidic aqueous solution of the amine **10**. The reaction of cyanogen bromide[11] with the amine **10** affords the carbonitrile **14** as the sole product. Reaction of the amine **10** in acetonitrile with methyl chloroformate[12] and anhydrous potassium carbonate for 16 h at reflux, and subsequent isolation and treatment of the crude product with 4% methanolic sodium hydroxide at room temperature for 2 h, afforded upon workup the urethane **15** (79%). The amino alcohol **10** was readily formylated in the presence of acetic anhydride[13] to furnish the diformylprolinol, followed by selective hydrolysis with alkali[14] to give the carbaldehyde **16**.

Biological Activity

The hydroxylamine derivative **12** may be considered as an analogue of either thymidine or 2'-deoxyxylofuranosylthymine with the tetrahydrofuran ring replaced by a pyrrolidine ring, that is, with the oxygen atom and the C-3 methylene group of the furanose ring replaced by a methylene group and a nitrogen atom, respectively. In a similar way, the amine **10** can be considered as an analogue of 3'-deoxythymidine. Since amines are strongly basic, compounds **10–12** will carry a positive charge at physiological pHs. These compounds may prove useful as enzyme inhibitors.

Compounds **10–16** were examined in growth inhibition assays against a panel of human tumor cell lines comprised of breast carcinoma line (MCF-7M), colon carcinoma (HT-29), and the SK-MES-1 lung carcinoma line. The hydroxylamine **12** proved active against the breast cell line with adriamycin as a positive control, IC_{50} 0.08 and 0.015 μM, respectively. Compound **12** also proved active against the colon and lung cell lines, IC_{50} 14.02 and 6.91 μM, respectively, with 5-fluorouracil as a positive control, IC_{50} 0.15 and 0.31 μM, respectively. Compounds **10–16** were inactive in antiviral assays against herpes simplex 1 (HSV-1) and human immunodeficiency virus 1 (HIV-1) in concentrations up to 100 μM.

Experimental Section

Melting points were determined in open glass capillary tubes and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and are within ±0.4% of the theoretical values unless indicated otherwise. Mass spectra were performed on a DVG-ZAB-HF high-resolution mass spectrometer by using either CI or FAB techniques. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The ^1H NMR spectra were recorded on a Varian 390 spectrometer with DMSO-d_6, $CDCl_3$, CD_3OD, or D_2O as solvents and TMS as a reference. UV spectra were obtained with a Beckman Model 25 spectrophotometer. Flash chromatography was performed as described by Still[15] on silica gel (E. Merck, 230–400 mesh); thin-layer chromatography was carried out on E. Merck 60F-254 precoated silica gel plates. Solvents and commercial reagents were distilled and dried by conventional methods before use.

***trans*-4-Hydroxy-D-proline Ethyl Ester Hydrochloride (3).** *N*-(*tert*-Butoxycarbonyl)-*trans*-4-hydroxy-D-proline ethyl ester[3] (**2**) (20 g, 77.2 mmol) was dissolved in 3 M HCl–EtOAc (50 mL). After 3 h, the solvent was removed in vacuo and the solid was filtered and dried to yield 14.5 g (96%) of **3**: mp 135–137 °C; $[\alpha]^{20}_D$ +25.1° (*c* 0.027, EtOH); ^1H NMR (DMSO-d_6) δ 1.27 (t, J = 7.5 Hz, CH_3), 2.1–2.5 (m, 3-H), 3.0–3.6 (m, 5-H), 4.23 (q, J = 7.5 Hz, CH_2), 4.4–4.7 (m, 2-H and 4-H); MS (CI) m/e 160 (M + 1 – HCl). Anal. ($C_7H_{14}NO_3Cl$) C, H, N, Cl.

***N*,*O*-Ditosyl-*trans*-4-hydroxy-D-proline Ethyl Ester (4).** The amino alcohol **3** was protected as described[5] to yield 80.4% of **4** as white crystals: mp 75–76 °C; $[\alpha]^{20}_D$ +52.7° (*c* 0.014, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.27 (t, J = 7.5 Hz, CH_3), 2.0–2.4 (m, 3-H), 2.43 (s, CH_3), 2.47 (s, CH_3), 3.5–3.8 (m, 5-H), 4.18 (q, J = 7.5 Hz, CH_2), 4.26 (t, J = 7 Hz, 2-H), 5.0 (m, 4-H), 7.31 (d, J = 7.5 Hz, Ph), 7.33 (d, J = 7.5 Hz, Ph), 7.63 (d, J = 7.5 Hz, Ph), 7.73 (d, J = 7.5 Hz, Ph); MS (CI) m/e 468 (M + 1), 394 (M – CO_2Et), 296 (M – OTs), 222 (M – CO_2Et – OTs). Anal. ($C_{21}H_{25}NO_7S_2$) C, H, N, S.

***N*,*O*-Ditosyl-*trans*-4-hydroxy-D-prolinol (5).** The ester **4** was reduced by lithium borohydride as described[3] to yield 95% of the alcohol **5** as white needles: mp 85–86 °C; ^1H NMR ($CDCl_3$) δ 1.8–2.2 (m, 3-H), 2.40 (s, CH_3), 3.4–4.0 (m, 2-H, 5-H and CH_2O), 4.8–5.0 (m, 4-H), 7.30 (d, J = 7.5 Hz, Ph), 7.63 (d, J = 7.5 Hz, Ph), 7.70 (d, J = 7.5 Hz, Ph); MS (CI) m/e 426 (M + 1), 394 (M – CH_2OH), 254 (M – OTs), 222 (M – 1 – CH_2OH – OTs). Anal. ($C_{19}H_{23}NO_6S_2$) C, H, N, S.

***N*-Tosyl-*cis*-4-azido-D-prolinol (6).** The azido **6** was synthesized from **5** with sodium azide as described;[5] yield was 98% of **6** as an oil: ^1H NMR ($CDCl_3$) δ 1.7–2.1 (m, 3-H), 2.43 (s, CH_3), 3.0–3.3 (m, 5-H), 3.3–3.5 (m, 5-H), 3.5–3.9 (m, 2-H, 4-H and CH_2O), 7.33 (d, J = 7.5 Hz, Ph), 7.73 (d, J = 7.5 Hz, Ph); MS (CI) m/e 297 (M + 1), 254 (M – N_3). Anal. ($C_{12}H_{16}N_4O_3S$) C, H, N, S.

***N*-Tosyl-*cis*-4-amino-D-prolinol (7).** The azide **6** (1.165 g, 3.9 mmol) in methanol (50 mL) was hydrogenated in the presence of 10% palladium on charcoal (0.2 g) at room temperature and 1 atm pressure. After 16 h the catalyst was filtered off and the solvent was removed under vacuum. The residue was crystallized from ethyl acetate to give **7** as brownish needles (1.025 g, 97.3%): mp 119–120 °C; ^1H NMR (DMSO-d_6) δ 1.3–2.0 (m, 3-H), 2.40 (s, CH_3O), 2.7–3.8 (m, 2-H, 4-H, 5-H, and CH_2O), 7.40 (d, J = 7.5 Hz, Ph), 7.70 (d, J = 7.5 Hz, Ph); MS (CI) m/e 271 (M + 1), 239

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(M - CH_2OH). Anal. ($C_{12}H_{18}N_2O_3S$) C, H, N, S.

***N*-Tosyl-*cis*-4-[[[(3-methoxy-2-methyl-1-oxo-2-propenyl)amino]carbonyl]amino]-D-prolinol (8).** Silver cyanate (18 g), which had been dried for 3 h in vacuo over phosphorous pentoxide in the dark at 135 °C, was added to a solution of 8.9 g (66 mmol) of 3-methoxy-2-methylacryloyl chloride[6] in 100 mL of anhydrous benzene. The resulting mixture was heated under reflux for 0.5 h and allowed to cool to room temperature. After the solid phase had settled, 50 mL of the supernatant solution was transferred to an addition funnel. The acylisocyanate solution (theoretically 30 mmol) was added over a period of 30 min to a solution at −15 °C containing 7.9 g (29 mmol) of **7**, 130 mL of anhydrous dimethylformamide, and 40 mL of anhydrous ether. The reaction mixture was stirred at −15 °C for 2 h and stored at +5 °C overnight. During all of the preceding operations, water was rigorously excluded. The reaction solutions were kept under a current of dry nitrogen until the reaction was terminated. The volatile components were evaporated in vacuo (aspirator and oil pump), and ethanol (100 mL) was added to the residual syrup and evaporated in vacuo. The residual oily solid was purified by flash chromatography, using 98:2 chloroform–methanol as eluent. Recrystallization from ethyl acetate–ether gave 10.7 g (90%) of **8**: mp 129–130 °C; ^{1}H NMR ($CDCl_3$) δ 1.77 (s, CH_3), 1.8–2.3 (m, 3-H), 2.47 (s, CH_3), 3.2–3.6 (m, 5-H), 3.83 (s, CH_3), 3.6–4.2 (m, 2-H, 4-H and CH_2O), 7.33 (s, vinyl), 7.35 (d, J = 7.5 Hz, Ph), 7.73 (d, J = 7.5 Hz, Ph). Anal. ($C_{18}H_{25}N_3O_6S\cdot{}^1/_2H_2O$) C, H, N, S.

***N*-Tosyl-*cis*-4-(thymin-1-yl)-D-prolinol (9).** A solution of 8.35 g (20.3 mmol) of **8** in 200 mL of 15 N aqueous ammonia was heated under reflux for 16 h, filtered, and concentrated to a syrup in vacuo. The residue was purified by flash chromatography, using 97:3 chloroform–methanol as eluent. Recrystallization from ethyl acetate–ether gave 6.85 g (89%) of **9**: mp 122–124 °C; UV λ_{max} 228 nm (ε 18 300) and 270 (ε 13 900) at pH 7, 229 (ε 20 900) and 270 (ε 9800) at pH 12; ^{1}H NMR (DMSO-d_6) δ 1.76 (s, CH_3), 1.99 (m, 3-H), 2.17 (m, 3-H), 2.42 (s, CH_3), 3.34 (m, 5-H), 3.60 (m, 5-H), 3.71 (m, CH_2O), 4.0–4.2 (m, 2-H), 4.8–5.0 (m, 4-H), 7.47 (d, J = 7.5 Hz, Ph), 7.55 (s, pyrimidine), 7.75 (d, J = 7.5 Hz, Ph); MS (CI) m/e 380 (M + 1), 362 (M + 1 − H_2O), 253 (M − 1 − Thy), 222 (M − 1 − Thy − CH_2OH). Anal. ($C_{17}H_{21}N_3O_5S\cdot{}^1/_2H_2O$) C, H, N, S.

***cis*-4-(Thymin-1-yl)-D-prolinol Hydrochloride (10).** *N*-Tosyl-*cis*-4-(thymin-1-yl)-D-prolinol (**9**) (3.041 g, 8.02 mmol) and phenol (2.264 g, 24.1 mmol) were dissolved in a mixture of acetic acid (80 mL) and 48% hydrobromic acid (70 mL). The solution was heated at 90 °C for 16 h. The reaction mixture was then poured into water (200 mL) and extracted with ether (3 × 200 mL). The aqueous solution was applied to an AG50W-X4 (H^+ form) column (100-mL bed volume) and eluted first with water and then with 0.5 M NH_4OH. The NH_4OH fractions, which contained the product, were combined and evaporated to dryness. The final product was hygroscopic and unstable. The stable hydrochloride of **10** (1.723 g, 82.2%) was prepared by evaporation of a solution in aqueous HCl: mp 260–262 °C dec; UV λ_{max} 269 nm (ε 11 600) at pH 7, 272 (ε 8800) at pH 12; ^{1}H NMR (DMSO-d_6) δ 1.80 (s, CH_3), 1.9–2.7 (m, 3-H), 3.4–3.6 (m, 5-H), 3.7–3.9 (m, 2-H and CH_2O), 4.8–5.2 (m, 4-H), 7.63 (s, pyrimidine); MS (FAB) m/e 226 (M + 1 − HCl). Anal. ($C_{10}H_{16}N_3O_3Cl$) C, H, Cl; N: calcd, 16.06; found, 15.30.

***N*-(Cyanoethyl)-*cis*-4-(thymin-1-yl)-D-prolinol (11).** Acrylonitrile (108 mg, 2.04 mmol) was added dropwise over 15 min to a cooled (ice–salt bath) solution of amine **10** (414 mg, 1.84 mmol) in 10 mL of H_2O. Stirring was continued for 1 h at ice-bath temperature and for an additional hour with the bath removed. The reaction mixture was evaporated to dryness and purified by flash chromatography using 98:2 chloroform–methanol as eluent. Recrystallization from methanol–ether gave 378 mg (73.9%) of **11**: mp 120–122 °C; UV λ_{max} 274 nm (ε 14 000) at pH 7, 274 (ε 10 700) at pH 12; ^{1}H NMR (DMSO-d_6) δ 1.77 (s, CH_3), 2.2–2.8 (m, 3-H and CH_2CH_2CN), 2.9–3.3 (m, 5-H), 3.3–3.6 (m, 2-H and CH_2O), 4.8–5.1 (m, 4-H), 8.0 (s, pyrimidine); MS (FAB) m/e 279 (M + 1), 126 (Thy + H). Anal. ($C_{13}H_{18}N_4O_3\cdot{}^3/_4H_2O$) C, H, N.

***N*-Hydroxy-*cis*-4-(thymin-1-yl)-D-prolinol Hydrochloride (12).** A filtered solution containing 62 mg (0.36 mmol) of 99% *m*-chloroperoxybenzoic acid in 10 mL of MeOH was added dropwise in 30 min to a cooled, stirred solution of 100 mg (0.36 mmol) of **11** in 20 mL of MeOH. After 1 h of additional stirring at 0–2 °C, the reaction mixture was evaporated to dryness and washed with anhydrous Et_2O (3 × 20 mL). The crude *N*-oxide was immediately dissolved in 20 mL of MeOH and warmed at 40 °C for 15 min. The solution mixture was then evaporated to dryness. The hydroxylamine **12** and *m*-chlorobenzoic acid did not separate. The mixture was treated with excess of 1 M HCl solution, filtered, and evaporated to dryness. Recrystallization from ethanol–acetone gave 75 mg (75%) of the hydrochloride of **12**: mp 178–180 °C dec; UV λ_{max} 270 nm (ε 12 500) at pH 7, 272 (ε 9200) at pH 12; ^{1}H NMR (CD_3OD) δ 1.88 (s, CH_3), 2.3–2.5 (m, 3-H), 2.6–2.8 (m, 3-H), 3.8–4.1 (m, 5-H and CH_2O), 4.1–4.3 (m, 2-H), 5.0–5.1 (m, 4-H), 7.55 (s, pyrimidine); MS (CI) m/e 224 (M + 1 − H_2O − HCl), 192 (M + 1 − H_2O − HCl − CH_3OH), 126 (Thy + H). Anal. ($C_{10}H_{15}N_3O_4\cdot1.1HCl\cdot H_2O$) C, H, Cl; N: calcd, 14.04; found, 13.09.

***N*-Nitroso-*cis*-4-(thymin-1-yl)-D-prolinol (13).** An ice-cold solution of **10** (270 mg, 1.2 mmol) in water (5 mL) containing concentrated hydrochloric acid (0.11 mL) was treated with a solution of sodium nitrite (96 mg, 1.39 mmol) in 5 mL of water for 30 min with stirring. The mixture was extracted with ethyl acetate and the dried extract evaporated. The residue was purified by flash chromatography, using 95:5 chloroform–methanol as eluent. Recrystallization from methanol–ether gave 223 mg (73.2%) of **13**: mp 212–213 °C; UV λ_{max} 220 nm (ε 14 300) and 270 (ε 13 200) at pH 7, 226 (ε 17 900) and 268 (ε 10 700) at pH 12; ^{1}H NMR (DMSO-d_6) δ 1.80 (s, CH_3), 2.2–2.7 (m, 3-H), 3.17 (bs, 5-H), 3.9–4.3 (m, 5-H and CH_2O), 4.4–4.7 (m, 2-H), 4.7–5.2 (m, 4-H), 7.60 (s, pyrimidine); MS (FAB) m/e 255 (M + 1), 126 (Thy + H). Anal. ($C_{10}H_{14}N_4O_4$) C, H, N.

***N*-Cyano-*cis*-4-(thymin-1-yl)-D-prolinol (14).** The cooled solution of 180 mg (1.7 mmol) of cyanogen bromide in 10 mL of methanol was added to a solution of 362 mg (1.61 mmol) of **10** and 225 mg (1.65 mmol) of sodium acetate in 20 mL of 95% methanol. The resulting solution was allowed to stand at room temperature for 90 min, and the solvent was then evaporated in vacuo. The residue was purified by flash chromatography, using 97:3 chloroform–methanol as eluent. Recrystallization from methanol–ether gave 303 mg (75.3%) of **14**: mp 244–247 °C dec; UV λ_{max} 206 nm (ε 13 900) and 271 (ε 13 800) at pH 7, 216 (ε 19 000) and 272 (ε 11 000) at pH 12; ^{1}H NMR (DMSO-d_6) δ 1.77 (s, CH_3), 1.8–2.4 (m, 3-H), 3.3–4.0 (m, 2-H, 5-H, and CH_2O), 4.8–5.3 (m, 4-H), 7.60 (s, pyrimidine); MS (CI) m/e 251 (M + 1), 127 (Thy + 2H). Anal. ($C_{11}H_{14}N_4O_3\cdot{}^1/_3H_2O$) C, H, N.

***N*-[(Methyloxy)carbonyl]-*cis*-4-(thymin-1-yl)-D-prolinol (15).** The reaction mixture of **10** (255 mg, 1.13 mmol), methyl chloroformate (430 mg, 4.55 mmol), and anhydrous potassium carbonate (940 mg, 6.81 mmol) in acetonitrile (15 mL) was refluxed for 16 h. It was filtered and washed with methanol, and the filtrate was evaporated to dryness. The crude product was treated with 4% methanolic sodium hydroxide at room temperature for 2 h and then evaporated to dryness. The residue was purified by flash chromatography, using 97:3 chloroform–methanol as eluent. Recrystallization with methanol–ether gave 254 mg (79.4%) of **15**: mp 209–210 °C; UV λ_{max} 208 nm (ε 11 400) and 271 (ε 12 400) at pH 7, 214 (ε 14 900) and 272 (ε 9600) at pH 12; ^{1}H NMR (DMSO-d_6) δ 1.80 (s, CH_3), 2.0–2.4 (m, 3-H), 3.1–3.4 (m, 5-H), 3.5–3.7 (m, 2-H), 3.63 (s, OCH_3), 3.8–4.0 (m, CH_2O), 4.7–5.1 (m, 4-H), 7.60 (s, pyrimidine); MS (CI) m/e 284 (M + 1), 266 (M + 1 − H_2O), 252 (M − CH_2OH), 126 (Thy + H). Anal. ($C_{12}H_{17}N_3O_5$) C, H, N.

***N*-Formyl-*cis*-4-(thymin-1-yl)-D-prolinol (16).** Acetic anhydride (5 mL) was added dropwise to a mixture of **10** (260 mg, 1.16 mmol) in 15 mL of 96% formic acid at 5 °C. After the addition was complete, the mixture was stirred at room temperature for 1 h. Ice–water (10 mL) was added, and the mixture was concentrated at reduced pressure. The residue was purified by flash chromatography, using 97:3 chloroform–methanol as eluent. Recrystallization from methanol–ether gave 200 mg (70.2%) of diformylprolinol: mp 172–173 °C; UV λ_{max} 208 nm (ε 15 300) and 271 (ε 10 400) at pH 7, 215 (ε 16 400) and 271 (ε 8200) at pH 12; ^{1}H NMR (DMSO-d_6) δ 1.80 (s, CH_3), 1.85–2.15 (m, 3-H), 2.2–2.6 (m, 3-H), 2.9–3.5 (m, 5-H), 3.8–4.2 (m, 2-H), 4.2–4.5 (m, CH_2O), 4.6–5.0 (m, 4-H), 7.60 (s, pyrimidine), 8.23 (bs, aldehyde), 8.27 (d, J = 1.5 Hz, aldehyde); MS (CI) m/e 282 (M + 1), 254 (M + 1 − CO), 236 (M − CO_2H), 127 (Thy + 2H). Anal. (C_{12}-

$H_{15}N_3O_5$) C, H, N.

The diformylprolinol (40 mg, 0.142 mmol) was treated with 3 M NH_4OH (10 mL) at room temperature for 0.5 h. The solvent was removed in vacuo, and recrystallization from methanol–ether gave 30.5 mg (84.9%) of **16**: mp 225–226 °C; UV λ_{max} 208 nm (ϵ 14000) and 271 (ϵ 9200) at pH 7,214 (ϵ 15200) and 271 (ϵ 7200) at pH 12; ^{1}H NMR (DMSO-d_6) δ 1.77 (s, CH_3), 2.0–2.6 (m, 3-H), 3.1–3.7 (m, 5-H and 2-H), 3.93 (bs, CH_2O), 4.9–5.2 (m, 4-H), 7.62 (s, pyrimidine), 8.18 (s, aldehyde); MS (FAB) m/e 254 (M + 1), 126 (Thy + H). Anal. ($C_{11}H_{15}N_3O_4 \cdot {}^1/_2H_2O$) C, H, N.

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Synthesis and Antirhinovirus Activity of 6-(Dimethylamino)-2-(trifluoromethyl)-9-(substituted benzyl)-9*H*-purines

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A series of 6-(dimethylamino)-2-(trifluoromethyl)-9-(substituted benzyl)purines was synthesized and tested for antirhinovirus activity. Most of the compounds were synthesized by alkylation of 6-chloro-2-(trifluoromethyl)-9*H*-purine with the appropriate benzyl halide followed by displacement of the chloro group with dimethylamine. Alternatively, 6-(dimethylamino)-2-(trifluoromethyl)purine was alkylated with the appropriate benzyl halide. Although several different aryl substituents provided compounds with IC_{50}'s = 0.03 μM against rhinovirus serotype 1B, no congener was significantly more active than the parent **2**. Twenty-three compounds were tested against 18 other serotypes, but none exhibited a uniform profile of activity.

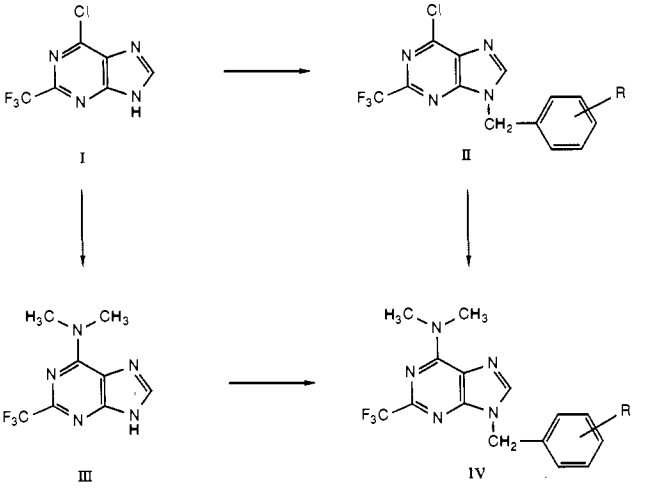
Recently, we reported the structure–activity relationships of some 6-(dimethylamino)-9-benzyl-9*H*-purines with activity against rhinovirus in vitro.[1-3] The most active compounds had a lipophilic, electron-withdrawing 2-substituent.[3] The 2-CF_3 analogue **1** was the most potent compound with an IC_{50} = 0.03 μM against serotype 1B. Although **1** had potent in vitro activity against rhinovirus 1B, most other serotypes were less sensitive.[3] To develop an agent with a broader spectrum of activity, we investigated the effect of various aryl substituents on the antirhinovirus activity of **1**. The synthesis and antirhinovirus activity of aryl-substituted analogues of **1** are reported.

Chemistry

Most of the compounds in Table I were prepared in two steps from 6-chloro-2-(trifluoromethyl)-9*H*-purine (I) and the appropriate benzyl halide (Scheme I). The (trifluoromethyl)purine I was prepared from 5-aminoimidazole-4-carboxamide by a modification of the literature procedure.[4,5] Condensation of 5-aminoimidazole-4-carboxamide with trifluoroacetamide gave 1,9-dihydro-2-(trifluoromethyl)-6*H*-purin-6-one (**67**),[4] which was converted to I by the Vilsmeier–Haack method.

Alkylation of I with the appropriate benzyl halide gave a mixture of the 9-benzylpurine II and the 7-isomer.[6] The 9-isomers were easily separated by flash chromatography and were usually used without further purification. The chloro group in II was displaced with ethanolic dimethylamine to give the target purines **1**, **3–10**, **12**, **15**, **17**, **26**, **30**, **36–40**, **42–45**, **49–54**, and **57–60** (methods A and B). Alternatively, I was treated first with ethanolic dimethylamine to give the 6-(dimethylamino)-2-(trifluoromethyl)purine III, which was alkylated with the appropriate benzyl halide to give purines **1**, **2**, **11**, **14**, **16**, **25**, **27**, **28**, **32**, and **33** (method C).

Scheme I



The 9-(aminobenzyl)purines **21**, **35**, **41**, **46**, and **55** were prepared from the nitro precursors by catalytic hydrogenation (method D). Several 9-[(dimethylamino)benzyl] analogues (**18**, **34**, **47**, and **61**) were prepared from the amines by Borch reductive alkylation (method E).[7] Re-

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