Purine Acyclic Nucleosides. 6-Dimethylamino-9-[(2-phenylalanylamido-1-substituted-ethoxy)methyl]purines as Candidate Antivirals

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Abstract □ Several acyclic puromycin analogues containing hydrocarbon substituents on the 1′-position of the aminoethoxymethyl moiety were synthesized and tested for antiviral activity. The *N*-carbobenzoxy intermediate **7c** was active in vitro against Mengo and Semliki Forest viruses.

Since the initial reports[1,2] of the potent antiviral activity of acyclovir (Zovirax), 9-[(2-hydroxyethoxy)methyl]guanine, a number of papers have been published that describe the preparation and antiviral activity of other acyclic nucleosides. Several pyrimidine acyclic nucleosides[3,4] and some purine ring modified analogs of acyclovir[5] were found to have little or no in vitro antiviral activity. Some extended chain analogues[6] and several nitrogen isosteres[7] of acyclovir were reported to be weakly active against herpes simplex virus type 1. However, substitution of a hydroxymethyl group on the 1′-carbon of the hydroxyethoxymethyl side chain of acyclovir led to the potent antiherpetic agent 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine (BW 759U).[8–12] (Also variously termed BIOLF-62[9], DHPG[10] and 2′NDG[11]). A number of analogues of BW 759U containing structural modifications in the base[13–16] and side chain[16] were reported to give derivatives with less or no in vitro antiviral activity.

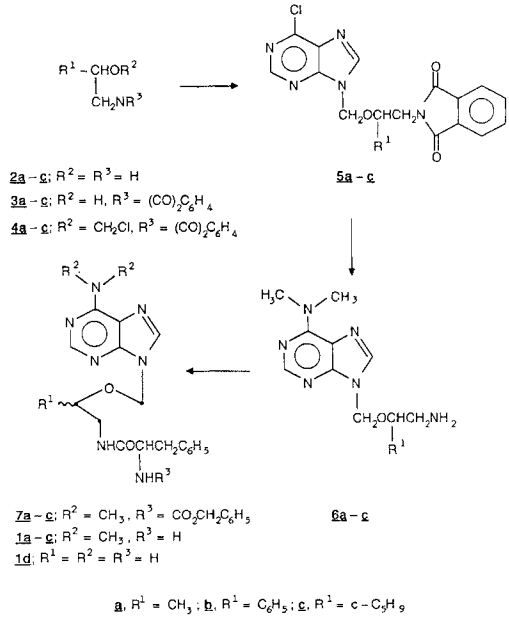
The acyclic puromycin analogue, **1d** was recently reported to be devoid of significant antiviral activity.[17] In an effort to develop analogues of **1d** with antiviral activity, hydrocarbon substituents were introduced on the 1′-position of the aminoethoxymethyl moiety of **1d**. This paper reports the preparation and antiviral evaluation of the 1′-substituted acyclic puromycin analogues **1a–1c**.

Results and Discussion

The acyclic puromycin analogues **1a–c** were synthesized as outlined in Scheme I. Phthaloylation of the 1-substituted-2-aminoethanols **2** gave the phthalimido alcohols **3** which were chloromethylated[18,19] to give the chloromethyl ethers **4**. Alkylation of 6-chloropurine with **4** gave a mixture of the 9- and 7-isomers which were separated by column chromatography to give **5** in 24–49% yield. When the 6-chloropurines **5** were treated with 40% aqueous dimethylamine, facile displacement of the 6-chloro group occurred. The product appeared to be a mixture consisting of material with an intact phthalimide ring, mixed phthalamide and deblocked amine **6**. Therefore, this material was treated with 40% aqueous methylamine[20] to afford **6** in high yield. These compounds exhibited UV spectral characteristics of 9-substituted-6-dimethylaminopurines.[21,22] The amines **6** were coupled with DL-*N*-carbobenzoxyphenylalanine using the mixed anhydride method[23] to provide **7** in high yield as a mixture of diastereoisomers which were not separated. Hydrogenolysis of **7** gave the acyclic nucleoside analogues **1a–1c**.

The antiviral activity of the nine 6-dimethylaminopurines in Table I were assayed by the plaque inhibition test.[24–26] The technique was adapted to plaque reduction assays for three DNA viruses, adeno type 5[27], herpes simplex type 1[26] and vaccinia (Lister strain, grown in HeLa cells over 72 hours); and nine RNA viruses, corona 229E,[28] influenza NWS,[29] respiratory syncytial (using BSC-1 cells),[30] measles,[31] rhinovirus type 1B[32], Bunyamwera, Mengo, Semliki Forest and Sindbis. The latter four viruses were adapted to plaque reduction assays in Vero H cells in 24 hours (Bunyamwera 72 hours) by a described technique.[26]

When tested at 50 μg per disc no significant activity was found with the acyclic puromycin analogues **1a–1c**. Thus, introduction of hydrophobic substituents on the 1′-position of **1d** did not afford active congeners. However, significant activity was found with the carbobenzoxy intermediate **7c** against Mengo and Semliki Forest viruses. When this activity was quantitated using the plaque reduction assay,[26] IC_{50} values of 5.1 μM for Mengo virus and 4.7 μM for Semliki Forest virus were obtained.



Scheme I

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. NMR data were recorded on a Varian XL-100-15-FT and T-60 spectrometers with Me_4Si as an internal standard. UV spectra were obtained on a Unicam SP 800 spectrophotometer.

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Each analytical sample had spectral data compatible with its assigned structure and showed one spot on TLC. TLC's were performed on Eastman Chromagram sheets of silica gel with fluorescent indicator. All compounds were analyzed for C, H and N; all values were within ±0.4% of the theoretical value.

2-Amino-1-cyclopentylethanol Hydrochloride (2c) (Compounds of Scheme I)—To a cooled, magnetically stirred slurry of 4.10 g (0.108 mol) of $LiAlH_4$ in 100 mL of ether was added a solution of 10.0 g (60.0 mmol) of 1-acetoxy-1-cyclopentylacetonitrile [prepared by the general method of A. Burger et al.[33] from cyclopentylmethanal and used as the crude oil] in 30 mL of ether. After 22 h at ambient temperature 15 mL of water was cautiously added in a dropwise manner, the white granular mixture was filtered, and the solids were washed with ether. The combined filtrate and washings were dried, diluted with 50 mL of hydrogen chloride saturated ethanol, and the organic phase was removed under reduced pressure. The residual semisolid was triturated with ether to afford a solid which on 2 recrystallizations gave colorless prisms (3.77 g, 37%), mp 133–135°C (lit.[33] 135–136°C).

***N*-(2-Hydroxypropyl)phthalimide (3a)**—A mixture of 148.1 g (1.0 mol) of phthalic anhydride and 75 mL (0.96 mol) of 1-amino-2-propanol was heated on a steam bath for 3.5 h. The mixture was cooled, and the solid was collected and washed with water to give an amorphous solid which was dissolved in chloroform, washed successively with 0.1 M HCl, water and brine, and was then dried. The solvent was removed under reduced pressure to give a white solid (141 g, 71%), mp 81–85°C. Recrystallization from water gave an analytical sample, mp 86–87°C.

***N*-(2-Hydroxy-2-phenethyl)phthalimide (3b)**—A mixture of 13.72 g (0.10 mol) of DL-α-hydroxyphenethylamine, 14.81 g (0.10 mol) of phthalic anhydride and 100 mL of glacial acetic acid was refluxed with stirring for 4 h. The mixture was poured into 600 mL of ice water, and the product was collected by filtration and dried under reduced pressure. Several recrystallizations from benzene afforded an analytical specimen as white flakes (21.7 g, 81%), mp 162–163°C (lit.[34] mp 164–165°C).

***N*-(2-Cyclopentyl-2-hydroxyethyl)phthalimide (3c)**—A mixture of 15.70 g (94.7 mmol) of **2c** hydrochloride, 14.15 g (95.6 mmol) of phthalic anhydride, 8.12 g (99.0 mmol) of sodium acetate and 160 mL of acetic acid was refluxed with stirring for 5 h. The cooled mixture was poured into 1 mL of ice water and stirred until flocculation occurred. The product was collected, dried under reduced pressure and recrystallized from petroleum ether (bp 60–68°C)-ethyl acetate (15.67 g, 64%), mp 83–86°C, analytical sample, mp 84–86°C.

1-Methyl-2-phthalimidoethoxymethyl Chloride (4a)—A stirred, ice bath cooled dispersion of 33.5 g (0.163 mol) of **2a** and 4.90 g (0.54 mol) of paraformaldehyde in 450 mL of 1,2-dichloroethane was saturated with dry hydrogen chloride. After 3 h the solution was dried with anhydrous calcium chloride and left overnight. The mixture was filtered, and the solvent was removed under reduced pressure. The residual material was dissolved in benzene, and the solvent was removed under reduced pressure. This process was repeated several times to afford a clear oil in quantitative yield which was used without further purification.

1-Phenyl-2-phthalimidoethoxymethyl chloride (4b) and 1-cyclopentyl-2-phthalimidoethoxymethyl chloride (4c) were obtained from **3b** and **3c** in quantitative yield.

6-Chloro-9-(1-methyl-2-phthalimidoethoxymethyl)purine (5a)—To an ice bath cooled solution of 25.3 g (0.163 mol) of 6-chloropurine and 17.6 g (0.174 mol) of triethylamine in 200 mL of dimethylformamide was added a solution of **4a** (0.163 mol) in 100 mL of dimethylformamide. After 55 h at ambient temperature the mixture was poured over 2 L of ice water with stirring. The resultant solid was collected and triturated with hot methanol to yield 13.3 g (22%), mp 181–182°C, of the 7-isomer of **5a** which contained ~10% of the 9-isomer. The aqueous filtrates were extracted with six 100-mL portions of chloroform, and the solvent was removed under reduced pressure (~1 mm Hg). The residual oil was dissolved in methanol, and the solvent was removed under reduced pressure to afford a solid which was a mixture of the 9- and 7-isomers. This material was dissolved in 200 mL of hot chloroform and introduced on a column (56 cm × 5.3 cm, 500 g) of magnesium silicates (Florisil) in chloroform. After the column had cooled it was eluted with chloroform, and 300 mL fractions were collected. Those fractions showing higher R_f values afforded three crops of **5a** (20.85 g, mp 162–166°C; 4.42 g, mp 164–168°C; and 4.84 g, mp 167–169°C; 49% total yield). Recrystallization of the latter sample from ethanol afforded the analytical sample, mp 172–173°C; NMR ($CDCl_3$): δ 8.58 (s, 1, purine H), 8.13 (s, 1, purine H), 7.70 (s, 4, Ar H), 5.68 (s, 2, NCH_2O), 4.4–3.6 (m, 5, $OCHCH_2$), and 1.31 ppm (d, 3, CH_3).

Anal.—Calc. for $C_{17}H_{14}ClN_5O_3$: C, 54.9; H, 3.80; N, 18.8. Found: C, 54.8; H, 3.84; N, 18.6.

6-Chloro-9-(1-phenyl-2-phthalimidoethoxymethyl)purine (5b), mp 177–178°C (ethanol), and 6-chloro-9-(1-cyclopentyl-2-phthalimidoethoxymethyl)purine (5c), mp 152–154°C (ethanol), were obtained from 4b and 4c in 24% and 24% yield, respectively.

9-(2-Amino-1-substituted-ethoxymethyl)-6-dimethylaminopurine Hydrochlorides (6a–c)—Compound 6a (R = CH_3)—A mixture of 10.02 g (27.0 mmol) of **5a** and 50 mL of 40% aqueous dimethylamine in a glass lined, stainless steel vessel was heated at 90°C for 18 h. The vessel was cooled and 50 mL of 40% aqueous methylamine was added. The solution was again heated at 90°C for 6.5 h. The solvent was removed under reduced pressure to afford a syrup which was dissolved in ethanol. This solution was stirred with sufficient ion-exchange resin [Rexyn 201 (OH^-)] to cause a negative test with silver nitrate solution. The mixture was filtered, and the solvent was removed under reduced pressure. The residue was repeatedly dissolved in ethanol and evaporated to remove the last traces of amine. The residual oil was dissolved in a minimum amount of ethanol and diluted with 400 mL of ether. To this stirred solution was added hydrogen chloride-saturated ethanol in 1 mL portions until no further precipitation occurred. This mixture was stirred vigorously for 0.5 h, and the solids were allowed to settle. The solvent was decanted, and the solids were again covered with ether. The solids were repeatedly triturated with fresh ether and then dissolved in a minimum of ethanol. Reprecipitation with ether gave the product (6.46 g, 83%), mp 173–184°C, which showed one spot on TLC (chloroform-ethanol:1-1). Recrystallization from ethanol-ether and finally from ethanol gave the analytical sample, mp 201–203°C (eff); UV: λ_{max} (0.1 M HCl) 267 nm (ε 18200); UV: λ_{max} (0.1 M NaOH) 275 nm (18500); ^1H NMR (D_2O): δ 8.13 (s, 1, purine H), 7.97 (s, 1, purine H), 5.67 (s, 2, NCH_2O), 4.07 (br m, 1, $OCHCH_2$), 3.23 (s, 6, $N(CH_3)_2$), 3.12 (d, 2, J = 2.5 Hz, $CHCH_2N$), and 1.23 ppm (d, 3, J = 3.0 Hz, $CHCH_3$).

Anal.—Calc. for $C_{11}H_{18}N_6O \cdot HCl$: C, 46.1; H, 6.68; N, 29.3. Found: C, 46.2; H, 6.74; N, 29.2.

9-(2-Amino-1-phenylethoxymethyl)-6-dimethylaminopurine hydrochloride (6b), mp 170–171°C (ethanol), and 9-(2-amino-1-cyclopentylethoxymethyl)-6-dimethylaminopurine hydrochloride (6c), mp 153–154°C (dec.) (ethanol-ether), were obtained from 5b and 5c in 80% and 86% yield, respectively.

9-(2-*N*-Carbobenzoxyphenylalanylamido-1-methylethoxymethyl)-6-dimethylaminopurine (7a)—To a stirred, ice bath cooled solution of 7.60 g (25.4 mmol) of DL-*N*-carbobenzoxyphenylalanine and 3.8 mL (27.2 mmol) of triethylamine in 120 mL of tetrahydrofuran was added 2.72 g (25.2 mmol) of ethyl chloroformate and 10 mL of tetrahydrofuran. A precipitate formed within a few minutes and after 10 min a solution of 3.58 g (12.5 mmol) of **6** hydrochloride and 5.5 ml (39.4 mmol) of triethylamine in 150 mL of chloroform was added in one portion. After 1.5 h at ambient temperature the reaction was diluted with 50 mL of ethanol, and the solvent was removed under reduced pressure. The residual solid was dissolved in 250 mL of chloroform and washed with four 50 mL portions of water, five 50 mL portions of 5% $NaHCO_3$, 50 mL of water and dried. The solvent was removed under reduced pressure. The residual white solid was crystallized from ethyl acetate-petroleum ether (bp 60–68°C) to yield 5.49 g (82%) of **7a**, mp 150–155°C which showed one spot on TLC (benzene:ethanol; 10:1). The analytical sample was obtained from ethanol, mp 149–152°C; UV: λ_{max} (0.1 M HCl + 10% C_2H_5OH) 269 nm (ε 16100); UV: λ_{max} (0.1 M NaOH + 10% C_2H_5OH) 277 nm (ε 16100).

Anal.—Calc. for $C_{28}H_{33}N_7O_4$: C, 63.3; H, 6.26; N, 18.4. Found: C, 63.2; H, 6.26; N, 18.2.

9-(2-*N*-Carbobenzoxyphenylalanylamido-1-phenylethoxymethyl)-6-dimethylaminopurine (7b), mp 185–186°C (ethanol), and 9-(2-*N*-carbobenzoxyphenylalanylamido-1-cyclopentylethoxymethyl)-6-dimethylaminopurine (7c), mp 146–147°C (ethyl acetate-petroleum ether, bp 60–68°C), were obtained from 6b and 6c in 67% and 80% yield, respectively.

9-(2-Phenylalanylamido-1-phenylethoxymethyl)-6-dimethylaminopurine (1b)—A mixture of 2.78 g (4.70 mmol) of **7b** dissolved in 200 mL of acetic acid and 0.43 g of 10% Pd-C was shaken in the presence of H_2 at 50–80 psi for 6.5 h. The mixture was filtered, and the solvent was removed under reduced pressure. The residue was

dissolved in ethanol, and the solvent was removed under reduced pressure. The residual syrup was crystallized from ethanol-petroleum ether (bp 60–68°C) to yield 1.69 g (77%), mp 97–102°C, which showed one spot on TLC (benzene:ethanol; 1:1). Recrystallization from ethanol-ether afforded analytically pure material (0.712 g, 32%), mp 142–144°C which retained 0.25 mol of H_2O after drying for 24 h at 100°C in the presence of P_2O_5; UV: γ_{max} (0.1 M HCl + 10% C_2H_5OH) 269 nm (ϵ 16000), UV: γ_{max} (0.1 M NaOH + 10% C_2H_5OH) 277 nm (ϵ 16700); NMR (Me_2SO-d_6): δ 8.22 (s, 1, purine H), 8.14 (s, 1, purine H), 7.99 (br t, 1, CH_2NH), 7.4–7.0 (m, 10, ArH), 5.48 (q, 2, J = 11 Hz, NCH_2O), 4.65 (m, 1, $OCHCH_2$), 3.36 (s, 6, $N(CH_3)_2$), 3.30 (m, 2, $OCHCH_2N$), 3.02 ppm (br s, 2, NH_2).

Anal.—Calc. for $C_{25}H_{29}N_7O_2 \cdot 0.25\ H_2O$: C, 64.7; H, 6.41; N, 21.1. Found: C, 64.5; H, 6.35; N, 21.0.

9-(2-Phenylalanylamido-1-methylethoxymethyl)-6-dimethylaminopurine (1a), mp 77–80°C (ether), and **9-(2-phenylalanylamido-1-cyclopentylethoxymethyl)-6-dimethylaminopurine (1c),** mp 149–151°C (ethanol ether), were obtained from **7a** and **7c** in 90% and 40% yield, respectively.

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