Synthesis and Antiviral Evaluation of Carbocyclic Analogues of 2'-Azido- and 2'-Amino-2'-deoxycytidine

Tai-Shun Lin,* Xiao-Hui Zhang,[1] Zi-Hou Wang,[2] and W. H. Prusoff

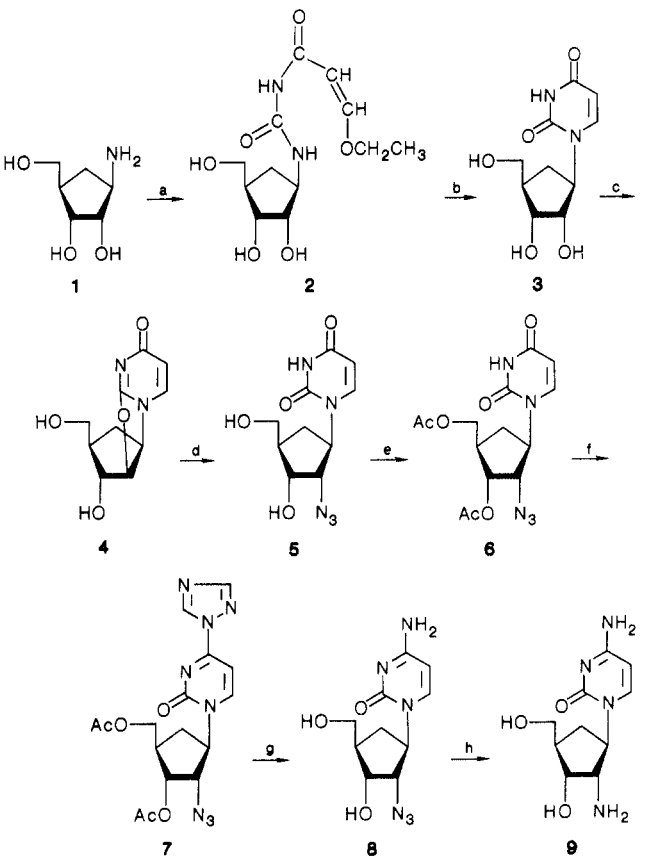
Department of Pharmacology and Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510. *Received July 13, 1987*

Carbocyclic analogues of 2'-azido- and 2'-amino-2'-deoxycytidine, compounds 8 and 9, were synthesized by an eight-step synthesis from (±)-(1α,2α,3β,5β)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (1), which was prepared from cyclopentadiene via an eight-step route. These compounds were tested in vitro against herpes simplex virus type 1 (HSV-1). The 2'-amino analogue was found to show moderate antiviral activity, with an ED_{50} of 50 μM. However, the 2'-azido analogue was not active at a concentration up to 400 μM.

2'-Azido-2'-deoxycytidine inhibits mammalian cell growth reversibly by interference with DNA replication.[3] The 5'-diphosphate derivative of 2'-azido-2'-deoxycytidine specifically inactivates the enzyme ribonucleotide reductase.[4] The diphosphate analogues of 2'-azido-2'-deoxycytidine and 2'-amino-2'-deoxycytidine were also reported to be substrates for polynucleotide phosphorylase from *Micrococcus luteus*.[5] Interest in aminonucleosides was further stimulated by the findings that 5'-amino analogues of thymidine and 5-iodo-2'-deoxyuridine have significant antiviral activity,[6,7] and 3'-amino analogues of thymidine and 2'-deoxycytidine have potent anticancer activity.[7-9] On the basis of these findings, we have synthesized the carbocyclic analogues of 2'-azido- and 2'-amino-2'-deoxycytidine for evaluation of their biological activity.

Chemistry. The carbocyclic analogue of uridine (**3**, *C*-uridine) was synthesized from (±)-(1α,2α,3β,5β)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (**1**) by the methodology of Shealy et al.[10] The key intermediate, compound **1**, was prepared from cyclopentadiene via an eight-step route by the procedures of Shealy et al.[11] and Kam et al.[12] with minor modifications. The carbocyclic analogue of 2,2'-anhydro-1-β-D-arabinofuranosyluracil (**4**) was obtained by reaction of *C*-uridine (**3**) with diphenyl carbonate and $NaHCO_3$ in DMF at 150 °C. Nucleophilic displacement of LiN_3 at the 2'-position of the 2,2'-anhydro derivative **4** in HMPA at 150 °C[13] produced the 2'-azido analogue of *C*-uridine **5**. Compound **5** was acetylated with acetic anhydride in pyridine to afford the diacetate **6**, which was then treated with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine at room temperature[14,15] to give the 4-triazolylpyrimidinone derivatives **7**. Subsequent treatment of **7** with NH_4OH–dioxane (1:3, v/v) and then saturated methanolic ammonia overnight at room temperature yielded the 2'-azido derivative **8**. Compound **8** was then reduced in methanol under 50 psi of hydrogen, in the presence of 10% palladium on charcoal at room temperature to afford the 2'-amino carbocyclic nucleoside **9**. The synthesis is shown in Scheme I.

Scheme I[a]



[a] (a) $C_2H_5OCH{=}CHCONCO$, C_6H_6, DMF; (b) 2 N H_2SO_4; (c) $(C_6H_5O)_2C{=}O$, $NaHCO_3$, DMF; (d) LiN_3, $C_6H_5CO_2H$, HMPA; (e) Ac_2O, pyridine; (f) triazole, *p*-$ClC_6H_4OPOCl_2$, pyridine; (g) 1. NH_4OH–dioxane, 2. NH_3–CH_3OH; and (h) H_2, 10% Pd–C, CH_3OH.

Antiviral Activity. The azido and amino carbocyclic nucleosides, compounds **8** and **9**, were tested against the herpes simplex virus type 1 (HSV-1) in vitro. The 2'-amino derivative has moderate antiviral activity, with an ED_{50} of 50 μM. However, the 2'-azido analogue was not active up to 400 μM.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. The thin-layer chromatography

(1) Visiting Scientist from the Department of Pharmaceutical Chemistry, Beijing Medical College, Beijing, China.
(2) Visiting Scientist from the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China.
(3) Skoog, L.; Bjursell, G.; Thelander, L.; Hagerstron, T.; Hobbs, J.; Eckstein, F. *Eur. J. Biochem.* **1977**, *72*, 371.
(4) Thelander, L.; Larsson, B.; Hobbs, J.; Eckstein, F. *J. Biol. Chem.* **1976**, *251*, 1398.
(5) Hobbs, J.; Sternbach, H.; Sprinzl, M.; Eckstein, F. *Biochemistry* **1973**, *12*, 5138.
(6) Lin, T.-S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 109.
(7) Lin, T.-S.; Neenan, J. P.; Cheng, Y. C.; Prusoff, W. H.; Ward, D. C. *J. Med. Chem.* **1976**, *19*, 495.
(8) Lin, T.-S.; Fischer, P. H.; Prusoff, W. H. *Biochem. Pharmacol.* **1981**, *31*, 25.
(9) Lin, T.-S.; Mancini, W. R. *J. Med. Chem.* **1983**, *26*, 544.
(10) Shealy, Y. F.; O'Dell, C. A. *J. Heterocycl. Chem.* **1976**, *13*, 1015.
(11) Shealy, Y. F.; Clayton, J. D. *J. Am. Chem. Soc.* **1969**, *91*, 3075.
(12) Kam, B. L.; Oppenheimer, N. J. *J. Org. Chem.* **1981**, *46*, 3268.
(13) Verheyden, J. P. H.; Wagner, D.; Moffatt, J. G. *J. Org. Chem.* **1971**, *36*, 250.
(14) Sung, W. L. *J. Chem. Soc., Chem. Commun.* **1981**, 1089.
(15) Lin, T.-S.; Gao, Y. S.; Mancini, W. R. *J. Med. Chem.* **1983**, *26*, 1691.

0022-2623/88/1831-0484$01.50/0

was performed on EM silica gel 60 F_{254} sheets (0.2 mm). IR spectra were recorded on a Perkin-Elmer 21 spectrophotometer. The UV spectra were obtained on a Beckman 25 spectrophotometer, and the NMR spectra were taken on a Bruker WM 500 spectrometer at 500 MHz using Me_4Si as internal reference. Mass spectra (at 70 eV) were provided by Yale University Chemical Instrumentation Center. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, CT.

(±)-*N*-[[(1α,2β,3β,4α)-2,3-Dihydroxy-4-(hydroxymethyl)cyclopentyl]carbamyl]-3-ethoxy-2-propenamide (2): mp 141–143 °C (lit.[10] mp 135–137 °C); NMR (Me_2SO-d_6) δ 1.20 (t, 3 H, CH_3), 1.40–2.70 (m, 3 H, 4′- and 5′-H), 3.30 (m, 2 H, 6′-H), 3.56 (m, 2 H, 2′- and 3′-H), 3.88 (m, 3 H, 1′-H and OCH_2), 4.28–4.99 (m, 3 H, 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.46 (d, 1 H, 5-H, vinyl), 7.49 (d, 1 H, 6-H, vinyl), 8.46 (d, 1 H, 1-NH), 9.90 (s, 1 H, 3-NH).

(±)-(1α,2β,3β,4α)-1-[2,3-Dihydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione (3): mp 177–179 °C (lit.[10] mp 176–179 °C); NMR (Me_2SO-d_6) δ 1.22–1.27 (m, 1 H, 4′-H), 1.91–2.01 (m, 2 H, 5′-H), 3.33–3.40 (m, 2 H, 6′-H), 3.70 (m, 1 H, 3′-H), 3.96 (m, 1 H, 2′-H), 4.55–4.66 (m, 3 H, 1′-H, 3′-, and 6′-OH, D_2O exchangeable), 4.58 (q, 1 H, 1′-H after addition of D_2O), 4.83 (d, 1 H, 2′-OH, D_2O exchangeable), 5.58 (d, 1 H, 5-H), 7.66 (d, 1 H, 6-H), 11.2 (br s, 1 H, 3-NH, D_2O exchangeable).

Carbocyclic Analogue of 2,2′-Anhydro-1-β-D-arabinofuranosyluracil (4). The carbocyclic analogue of uridine (3; 0.24 g, 1 mmol) was dissolved in 3 mL of DMF. Diphenyl carbonate (0.32 g, 1.5 mmol) and $NaHCO_3$ (8 mg) were added to this solution. The mixture was heated at 150 °C until the evolution of CO_2 had ceased (~30 min) and the solution had turned to a dark red. The reaction mixture was cooled to room temperature and poured slowly into 25 mL of ether with stirring. The deposit was collected by filtration, redissolved in methanol, decolorized with charcoal, and filtered. From the filtrate, 0.15 g (67%) of white crystals was obtained: mp 235–236 °C; NMR (Me_2SO-d_6) δ 1.75 (m, 1 H, 4′-H), 1.98 (m, 1 H, 5′-H_a), 2.33 (m, 1 H, 5′-H_b), 3.30–3.32 (m, 1 H, 6′-H_a), 3.38–3.41 (m, 1 H, 6′-H_b), 3.97 (m, 1 H, 3′-H), 4.60 (t, 1 H, 6′-OH, D_2O exchangeable), 4.83 (m, 1 H, 2′-H), 4.98 (m, 1 H, 1′-H), 5.50 (d, 1 H, 3′-OH, D_2O exchangeable), 5.82 (d, 1 H, 5-H), 7.75 (d, 1 H, 6-H); UV (0.1 N HCl) λ_{max} 258 nm (ε 9840), λ_{min} 238 nm; UV (0.1 N NaOH) λ_{max} 265 nm (ε 7940), λ_{min} 241 nm; MS (EI), *m/e* 224 (M), 205 (M − 1 − H_2O), 193 (M − CH_2OH), 188 (M − $2H_2O$), 177 (M − CH_3O_2), 166 (M − CH_2OH − C_2H_4). Anal. ($C_{10}H_{12}N_2O_4$) C, H, N.

(±)-(1α,2β,3β,4α)-1-[2-Azido-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione (5). The 2,2′-anhydro derivative (4; 0.22 g, 1 mmol) and LiN_3 (0.35 g, 7.1 mmol) in 6 mL of anhydrous HMPA were stirred at 150 °C until most of the solid dissolved. Benzoic acid (0.12 g, 1 mmol) was then added, and the solution was cooled to room temperature and diluted with 8 mL of H_2O. The aqueous solution was extracted with $CHCl_3$ (3 × 20 mL). The $CHCl_3$ extract was back-extracted with H_2O (2 × 8 mL). The combined water solution was extracted three times with $CHCl_3$ and then evaporated in vacuo to dryness to give 1.16 g of light-yellow half-solid residue. The residue was treated with EtOH–acetone (3 mL:8 mL) and filtered to remove the insoluble material. The filtrate was chromatographed on a silica gel (100 g) column and eluted with acetone. The acetone solution containing the product was evaporated to dryness under reduced pressure to afford an oily residue, which was crystallized from ethanol to give 0.12 g (45%) of crystalline product: mp 176–178 °C; IR (KBr) ν_{max} 2110 cm^{-1} (azido); UV (0.1 N HCl) λ_{max} 266 nm (ε 10 830), λ_{min} 230 nm; UV (0.1 N NaOH) λ_{max} 266 nm (ε 7230), λ_{min} 243 nm; NMR (Me_2SO-d_6) δ 1.45 (m, 1 H, 4′-H), 2.03 (m, 1 H, 5′-H_a), 2.10 (m, 1 H, 5′-H_b), 3.42 (m, 2 H, 6′-H), 3.79 (m, 1 H, 3′-H), 4.01 (m, 1 H, 2′-H), 4.73 (s, 1 H, 6′-OH, D_2O exchangeable), 5.64 (d, 1 H, 5-H), 7.72 (d, 1 H, 6-H), 11.3 (s, 1 H, 3-NH, D_2O exchangeable); MS (CI) *m/e* 268 (M + 1), 240 (M + 1 − N_2), 222 (240 − H_2O), 204 (222 − H_2O), 190 (204 − CH_2), 139 (uracil-1-yl + C_2H_4), 113 (uracil-1-yl + 2 H). Anal. ($C_{10}H_{13}N_5O_4$) C, H, N.

(±)-(1α,2β,3β,4α)-1-[2-Azido-3-acetoxy-4-(acetoxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione (6). The azido derivative 5 (0.38 g, 1.42 mmol) was dissolved in 15 mL of dry pyridine and cooled in an ice–water bath with stirring. Acetic anhydride (1.34 mL, 14.2 mmol) was added slowly to the solution (~10 min). The reaction mixture was stirred at room temperature for 24 h and evaporated in vacuo (~35 °C) to yield a syrup, which was dissolved in 25 mL of $CHCl_3$, washed with H_2O (2 × 10 mL), saturated $NaHCO_3$ solution (2 × 10 mL), and H_2O (2 × 10 mL) again, and dried over anhydrous $MgSO_4$. The drying agent was removed by filtration. The filtrate was evaporated under reduced pressure to yield 0.61 g of syrup, which was used directly for the next preparation without further purification.

(±)-4-Amino-1-[(1α,2β,3β,4α)-2-azido-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2(1*H*)-pyrimidinone (8). The diacetate (6; 0.61 g, 1.73 mmol) was dissolved in 10 mL of pyridine, and 1,2,4-triazole (0.36 g, 5.2 mmol) was added. The reaction mixture was stirred in a cold-water bath, and 4-chlorophenyl phosphorodichloridate (0.43 mL, 2.6 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 72 h and then evaporated in vacuo (~30 °C) to afford a dark-brown syrup, which was dissolved in 20 mL of CH_2Cl_2 and washed with H_2O (2 × 10 mL), saturated $NaHCO_3$ (2 × 10 mL), and H_2O (10 mL) again. The solution was clarified with charcoal and dried over anhydrous $MgSO_4$. The filtrate was evaporated in vacuo to give 0.35 g (61%) of a brown-yellow solid, which was homogeneous on TLC (R_f 0.49, CH_2Cl_2–acetone, 7:3). This 4-triazolylpyrimidinone derivative 7 was used immediately for the next reaction step.

The 4-triazolylpyrimidinone 7 (0.35 g, 0.87 mmol) was dissolved in 24 mL of dioxane–NH_4OH (3:1) and stirred at room temperature for 4 h. The solution was evaporated in vacuo and coevaporated again with methanol to give a syrup, which was dissolved in 25 mL of saturated methanolic ammonia and stirred at room temperature in a Wheaton pressure-bottle overnight. The solvent was evaporated under reduced pressure to yield a syrup, which was dissolved in a small amount of methanol and chromatographed on a silica gel column ($CHCl_3$–MeOH, 3:1) to yield 0.2 g of product. This product was chromatographed again (EtOAc–MeOH, 2:1) to afford 0.16 g (69%) of light yellow powder: mp 192–197 °C effervesced; IR (KBr) ν_{max} 2095 cm^{-1} (azido); UV (0.1 N HCl) λ_{max} 283 nm (ε 7880), λ_{min} 243 nm; UV (0.1 N NaOH) λ_{max} 274 nm (ε 5000), λ_{min} 251 mm; NMR (Me_2SO-d_6) δ 1.36–1.42 (m, 1 H, 4′-H), 1.99–2.10 (m, 2 H, 5′-H), 3.37–3.45 (m, 2 H, 6′-H), 3.76–3.94 (m, 1 H, 3′-H), 4.02 (m, 1 H, 2′-H), 4.79 (t, 1 H, 6′-OH, D_2O exchangeable), 4.94 (q, 1 H, 1′-H), 5.45 (d, 1 H, 3′-OH, D_2O exchangeable), 5.76 (d, 1 H, 5-H), 7.04–7.20 (br d, 2 H, 4-NH_2, D_2O exchangeable), 7.67 (d, 1 H, 6-H); MS (DCI, NH_3), *m/e* 282 $(M + NH_2)^+$, 267 (M + 1), 218 (M + 1 − H_2O − CH_2OH), 208 (M + 1 − N_3 − OH), 190 (208 − H_2O), 137 (cytosine + C_2H_3), 110 (cytosine base). Anal. $C_{10}H_{14}N_6O_3 \cdot 0.5CH_3OH$) C, H, N. The NMR spectrum of this compound did indicate the presence of methanol: δ 3.17 (s, CH_3), 4.15 (q, OH, D_2O exchangeable).

(±)-(1α,2β,3β,4α)-1-[2-Amino-3-hydroxy-4-(hydroxymethyl)cyclopentyl]-2(1*H*)-pyrimidinone (9). The azido derivative 8 (70 mg, 0.26 mmol) was dissolved in 45 mL of MeOH and hydrogenated under 50 psi of hydrogen pressure in the presence of 10% palladium on charcoal (20 mg) for 20 h. After filtration, the filtrate was evaporated under reduced pressure to afford a white solid residue, which was crystallized from ethanol to yield 60.4 mg (96%) of product: mp 218–220 °C dec; ninhydrin positive; UV (0.1 N HCl) λ_{max} 281 nm (ε 9130), λ_{min} 243 nm; UV (0.1 N NaOH) λ_{max} 273 nm (ε 6220), λ_{min} 251; NMR (Me_2SO-d_6) δ 1.18–1.24 (m, 1 H, 4′-H), 1.92–2.01 (m, 2 H, 5′-H), 3.11–3.14 (m, 1 H, 3′-H), 3.31–3.41 (m, 3 H, 6′-H and 3′-OH, D_2O exchangeable), 3.64 (d, 1 H, 2′-H), 4.44–4.50 (q, 1 H, 1′-H), 4.36–4.79 (br m, 3 H, 2′-NH_2 and 6′-OH, D_2O exchangeable), 5.70 (d, 1 H, 5-H), 6.85–7.05 (br d, 2 H, 4-NH_2, D_2O exchangeable), 7.57 (d, 1 H, 6-H); MS (DCI), *m/e* 241 (M + 1), 240 (M), 130 (M − cytosine), 112 (M − cytosine − H_2O).

Antiviral Determination. Vero cells are grown to confluency in 25-cm^2 Falcon flasks with Dulbecco's medium supplemented with 10% fetal calf serum. The cells are then infected with herpes simplex virus type 1 at a multiplicity of infection (MOI) of 10. After a 1-h absorption period at 37 °C, the viral inoculum is removed, and the flask is washed once with phosphate-buffered saline. The test compounds are dissolved in Dulbecco's medium supplemented with serum, and then the mixture is added to the flask. The infected cultures are incubated at 37 °C for 40 h and then frozen until virus titrations are performed. Virus is released by freezing and thawing the media–cell suspension one time. The

cell lysates are diluted directly, and the virus yield is assayed by plaque formation on Vero cells. The number of plaque-forming units (PFU) of virus in the drug-treated cultures relative to that found in the drug-free condition is determined.

The *cytotoxicity* of various test compounds on the uninfected host Vero cells is determined. Vero cells in Dulbecco's medium (2.5 mL) supplemented with 10% fetal calf serum are added to eight 25-cm^2 Falcon flasks at a concentration equivalent to 0.1 confluency for each compound under assay. After incubation at 37 °C in 5% CO_2–95% air for 1 day, the test compound, dissolved in 2.5 mL of the above growth medium, is added, and two flasks are harvested immediately by decanting the medium, washing once with 5 mL of buffered saline, and then incubating at 37 °C for 15 min with a 5 mL solution of trypsin (0.125%) and EDTA (0.02%). The cells dislodged from the flask by this latter procedure are generally in clumps and are dispersed by repeated forceful pipetting of the suspension. Trypan blue solution (0.2 mL) is added, and the number of cells are counted with a haemocytometer. Each day for the next 3 days, two of the remaining flasks are harvested in the manner just described for determination of cell number.

Acknowledgment. This research was supported by USPHS Grants CA-45410 and CA-28852. We also acknowledge the support of Northeast NMR Facility at Yale University for the high-resolution NMR spectra, made possible by a grant from the Chemical Division of the NSF (Grant CHE-7916210).

Registry No. 1, 62138-01-6; 2, 62102-29-8; 3, 59967-83-8; 4, 69975-22-0; 5, 111795-56-3; 6, 111795-57-4; 7, 111795-58-5; 8, 111795-59-6; 9, 111795-60-9; EtOCH=CHCONCO, 57796-78-8.

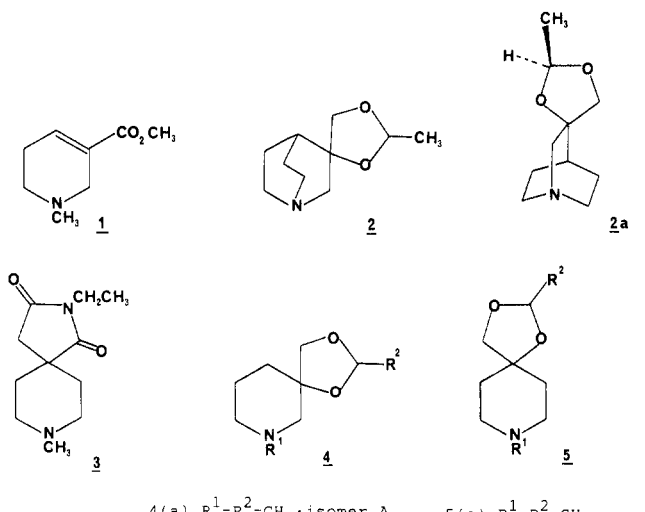
2-Methyl-1,3-dioxaazaspiro[4.5]decanes as Novel Muscarinic Cholinergic Agonists

John Saunders,*‡ Graham A. Showell,‡ Roger J. Snow,‡ Raymond Baker,‡ Elizabeth A. Harley,† and Stephen B. Freedman†

Chemistry and Biochemistry Departments, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, U.K. *Received April 10, 1987*

Many nonquaternary ammonium muscarinic agonists have been developed over the last few years, but most of the existing compounds (e.g., arecoline, RS-86, AF-30) behave as weak partial agonists at cholinergic receptors in tissues of limited receptor reserve. The current paper describes the synthesis and biochemical assessment of analogues of AF-30 designed to have sufficient conformational freedom to allow greater receptor flexibility and hence activation. The new compounds and important standards were tested in a new biochemical assay designed to measure both receptor affinity and intrinsic activity of each compound and for their ability to stimulate phosphatidylinositol turnover in rat cerebral cortex. Two azaspirodecanes (**5a** and **5b**) were shown to have far greater predicted efficacy than AF-30.

The tertiary amines arecoline (**1**), AF-30 (**2**), and RS-86 (**3**) have been described as muscarinic agonists, but they behave as weak partial agonists incapable of eliciting a full response in some tissues.[1] For example, unlike the full agonists carbachol and acetylcholine, these compounds are capable of only marginally stimulating the breakdown of phosphatidylinositol (PI) in rat cerebral cortex, in vitro.[2]



4(a) $R^1=R^2=CH_3$; isomer A
(b) $R^1=R^2=CH_3$; isomer B
(c) $R^1=H; R^2=CH_3$; isomer A
(d) $R^1=H; R^2=CH_3$; isomer B

5(a) $R^1=R^2=CH_3$
(b) $R^1=H; R^2=CH_3$
(c) $R^1=CH_3; R^2=C_2H_5$

In attempting to explain this, it is necessary to consider those phenomena that describe the interaction of a ligand with its receptor.[3] The stimulation produced by an agonist in a given tissue is governed not only by the affinity of the compound for a specific receptor but also by its intrinsic activity (efficacy). This latter property depends on the number of receptors in that tissue and hence the efficiency of the coupling mechanism between receptor activation and the appropriate secondary messenger system. RS-86 has similar affinity for muscarinic receptors located in the heart and cortical tissues.[4] It produces a potent dose-dependent decrease in adenylate cyclase activity in cardiac membranes with a maximum response equivalent to that invoked by carbachol and other classical agonists having a quaternary ammonium group. In contrast, the compound produces a stimulation of phospholipid metabolism in slices of cerebral cortex that is less than 10% that of the maximum achieved by carbachol.[2] Thus, in tissues such as the cerebral cortex where there is relatively inefficient coupling between receptor activation and secondary messenger metabolism ("low receptor reserve"), the low efficacy of RS-86 is insufficient to produce a full agonist response.

Previous studies[5] in these laboratories have focussed on the completely rigid muscarinic agonist **2** and have shown that the most active stereoisomer (**2a**) has the 3*R*,2′*S* configuration indicated. By use of a recently developed[6]

‡ Chemistry Department.
† Biochemistry Department.

(1) Palacios, J. M.; Bolliger, G.; Closse, A.; Enz, A.; Gmelin, G.; Manalowski, J. *Eur. J. Pharmacol.* **1986**, *125*, 45.
(2) Freedman, S. B. *Br. J. Pharmacol.* **1986**, *87*, 29P.
(3) Kenakin, T. P. *Pharmacol. Rev.* **1984**, *36*, 165.
(4) Freedman, S. B.; Harley, E.; Iversen, L. L., manuscript in preparation.
(5) Saunders, J.; Showell, G. A.; Baker, R.; Freedman, S. B.; Hill, D.; McKnight, A.; Newberry, N.; Salamone, J. D.; Hirshfield, J.; Springer, J. P. *J. Med. Chem.* **1987**, *30*, 969.

0022-2623/88/1831-0486$01.50/0