preparation of the manuscript. This work was supported with Federal Funds from the Department of Health and Human Services under Contracts N01-AI-42554, N01-AI-62518, N01-AI-72641, and N01-AI-82518 and in part by research Grant No. CH-312 from the American Cancer Society. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or oganizations imply endorsement by the U.S. Government.

Registry No. 1, 3680-69-1; 2, 115093-90-8; 3, 22276-95-5; 4, 123148-78-7; 5, 1618-36-6; 6, 74564-16-2; 7, 123148-79-8; 8, 123148-80-1; 9, 123148-81-2; 10, 123148-82-3; 11, 123148-83-4; 12, 123148-84-5; 13, 123148-85-6; 14, 123148-86-7; 15, 123148-87-8; 16, 123148-88-9; 17, 118043-78-0; 18, 123148-89-0; 19, 123148-90-3; 20, 123168-67-2; 21, 123148-91-4; 22, 123148-92-5; 23, 123148-93-6; 24, 123148-94-7; 25, 123148-95-8; 26, 123148-96-9; 27, 123148-97-0; 28, 123148-98-1; 31, 123148-99-2.

Synthesis and Antiviral Activity of Metabolites of Rimantadine

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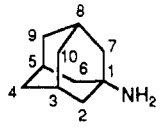
Received October 6, 1989

The hydroxy metabolites of rimantadine (3–5) were synthesized and compared to amantadine (1) and rimantadine (2) for their ability to inhibit the replication of influenza viruses in vitro. All three metabolites were inhibitory to wild-type influenza A viruses (H3N2 and H1N1). In particular, 2-hydroxyrimantadine (3) showed similar activity to amantadine, but the 3- and 4-hydroxy metabolites (4 and 5, respectively), both of which are found in rimantadine-treated patients, showed only modest inhibitory activity. A rimantadine-resistant isolate of influenza A virus exhibited cross-resistance to amantadine and to each of the metabolites 3–5. None of the compounds were effective against influenza B virus.

Amantadine (1) and rimantadine (2) are adamantane derivatives that show similar efficacy when administered orally to patients for the prophylaxis and treatment of influenza, although there are fewer side effects associated with the use of rimantadine.[1,2] However, peak plasma levels of rimantadine are 2–3-fold less than those achieved with amantadine when given at the same dose.[3] It has been proposed that the equivalence in activity in treated patients may be the consequence of selective concentration of rimantadine in the tissues of the respiratory system.[3] Alternatively, or in addition, the superior intrinsic antiviral activity of rimantadine compared to amantadine may be important.[4]



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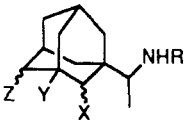

2: R = X = Y = Z = H
3: R = Y = Z = H, X = OH (α, β)
4: R = X = Z = H, Y = OH
5: R = X = Y = H, Z = OH (α, β)
6: R = Ac, X = Y = Z = H
7: R = Ac, X = Z = H, Y = Br

In treated patients, amantadine is, for the most part, excreted without metabolism.[5] In contrast, rimantadine is extensively metabolized by hydroxylation before excretion in the urine.[3,6] We were interested in synthesizing each of the hydroxy metabolites (3–5) of rimantadine and to determine whether they exhibited any antiviral activity. The presence in vivo of a metabolite of rimantadine with significant inhibitory activity against influenza A virus could contribute to the observed therapeutic efficacy of the parent compound.

Chemistry

The synthesis of 2-, 3-, and 4-hydroxyrimantadines 3, 4, and 5, respectively, followed a general route. Reduction of the 1-carboxyadamantanones 8[7] and 10 with sodium borohydride gave the hydroxy acids 11 and 12 as diastereomeric mixtures. It should be noted that although 1-carboxy-4-adamantanone (10) had been prepared by the Koch carboxylation of adamantanone[8] and by the oxidation of 1-carboxyadamantane,[9] we found it more expedient to prepare 10 by the ruthenium tetroxide oxidative degradation of 1-phenyl-4-adamantanone (19).[10] Treatment of the hydroxy acids 9,[11] 11, and 12 with a large excess of methyllithium afforded the methyl ketones 13, 14, and 15, from which the oximes 16, 17, and 18 were prepared with hydroxylamine. Reduction of the oximes 16 and 18 with lithium aluminum hydride gave the amines 3 and 5. To

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(1) Dolin, R.; Reichman, R. C.; Madore, H. P.; Maynard, R.; Linton, P. N.; Webber-Jones, J. *N. Engl. J. Med.* **1982**, *307*, 580.
(2) Van Voris, L. P.; Betts, R. F.; Hayden, F. G.; Christmas, W. A.; Douglas, R. G. *J. Am. Med. Assoc.* **1981**, *245*, 1128.
(3) Hayden, F. G.; Minocha, A.; Spyker, D. A.; Hoffman, H. E. *Antimicrob. Agents Chemother.* **1985**, *28*, 216.
(4) Belshe, R. B.; Burk, B.; Newman, F.; Cerruti, R. L.; Sim, I. S. *J. Infect. Dis.* **1989**, *159*, 430.
(5) Koppel, C.; Tenczer, J. *Biomed. Mass Spectrom.* **1985**, *12*, 499.
(6) Rubio, F. R.; Fukuda, E. K.; Garland, W. A. *Drug Metab. Dispos.* **1988**, *16*, 773.
(7) Peters, J. A.; Remijnse, J. D.; van der Wiele, A.; van Bekkum, H. *Tetrahedron Lett.* **1971**, 3065.
(8) Lantvoev, V. I. *J. Org. Chem.* (*Russian*) **1975**, *11*, 1546 (*Zh. Org. Khim.* **1975**, *11*, 1567).
(9) Miura, T.; Shibata, K.; Sawaya, T.; Kimura, M. *Chem. Pharm. Bull.* **1982**, *30*, 67.
(10) Geluk, H. W. *Synthesis* **1972**, 374.
(11) (a) Stetter, H.; Meyer, J. *Ber.* **1962**, *95*, 667. (b) Bagal, M. L.; Lantovoev, V. I. *Zh. Org. Chem.* **1973**, *9*, 291 (*Chem. Abstr.* **1973**, *79*, 46295j).

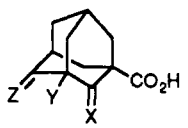
0022-2623/90/1833-1992$02.50/0

Table I. Antiviral Activity against Influenza A and B Viruses in Cell Culture

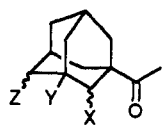
compd	A/ Mississippi	A/ Taiwan	A/NY/ 83/5	A/NY/ 83/R6	B/Ann Arbor
1	113 ± 41[a]	116 ± 53	80 ± 10	$>9 \times 10^4$	$>10^5$
2	21 ± 10	9.8 ± 7.0	12 ± 5	$>4 \times 10^4$	$>10^5$
3	150 ± 56	160 ± 27	101 ± 7	$>10^5$	$>10^5$
4	6700 ± 1900	5400 ± 2100	4000 ± 360	$>10^5$	$>10^5$
5	3400 ± 1200	1500 ± 640	804 ± 76	$>10^4$	$>10^5$

[a] 50% inhibitory dose, ng/mL. Each value is the mean ± SD of at least three determinations.

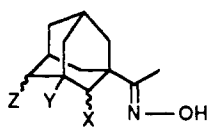
our surprise, oxime **17** was resistant to reduction with lithium aluminum hydride; however, **4** was readily prepared by reductive amination of ketone **14** with hydrogen and ammonia in the presence of Raney nickel at 100 °C.



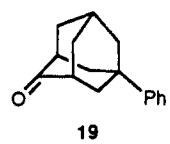
8: X = O, Y = H, Z = H_2
9: X = Z = H_2, Y = OH
10: X = H_2, Y = H, Z = O
11: X = H, OH(α, β), Y = H, Z = H_2
12: X = H_2, Y = H, Z = H, OH (α, β)



13: X = OH(α, β), Y = Z = H
14: X = Z = H, Y = OH
15: X = Y = H, Z = OH (α, β)



16: X = OH(α, β), Y = Z = H
17: X = Z = H, Y = OH
18: X = Y = H, Z = OH (α, β)



19

In an alternative and more expeditious synthesis, 3-hydroxyrimantadine (**4**) was prepared directly from rimantadine (**2**). Acetylation of rimantadine gave the acetamide **6** in 95% yield, which on treatment with bromine gave the bromo derivative **7** in 91% yield. Hydrolysis of **7** with hydrochloric acid afforded **4** in 45% yield, isolated as the hydrochloride salt.

As mentioned previously, our synthesis of **3** and of **5** produced diastereomeric mixtures. The diastereomeric mixture of **5** was ca. 3:2, and various attempts to separate this by preparative-scale high-pressure liquid chromatography were unsuccessful. No attempt was made to separate the mixture of **3**, nor was an attempt made to find a stereospecific reduction of the keto group at C-2 and C-4 in **8** and **10**, respectively. In addition, it should be noted that all the compounds used in this study are racemic.

Biological Evaluation

The three wild-type strains of influenza A virus were susceptible to inhibition by **1** and **2** (Table I). Compound **2** was more active than **1** against both the prototype strains A/Mississippi/1/85 (H3N2) and A/Taiwan/1/86 (H1N1), and A/NY/83/5 (H3N2) isolated from a patient prior to treatment with **2**.[12] Each of the metabolites tested exhibited some antiviral activity against wild-type influenza A virus, but none was as active as **2**. Interestingly, the activity of **3** could not be distinguished from that of **1**; **4** was the least active of the metabolites. None of the compounds tested were toxic to MDCK cells (by cytopathic effect determination) at concentrations at least 10-fold greater than the respective effective antiviral concentrations (data not shown). The susceptibility of influenza A virus to each of the compounds was confirmed by ELISA determinations. The results obtained using influenza virus A/Mississippi are shown in Figure 1. At 10 μg/mL each of the compounds tested exhibited anti-influenza virus activity. Compound **4** was inactive at 3 μg/mL while **5** showed diminished activity. The activities of **1** and **3** declined at 300 and 100 ng/mL and only **2** retained full antiviral activity at 30 ng/mL compared to its activity at 10 μg/mL. Influenza virus A/NY/83/R6 (H3N2) was isolated from a patient 5 days after the initiation of treatment with **2**[12] and has been shown to have acquired resistance to **2** as a result of a mutation in the gene coding for the M2 protein.[13] Our results show that this mutation also confers resistance to **1**, **3**, **4**, and **5** (Table I). Similar results were obtained with a second pair of wild-type and rimantadine-resistant viruses in the ELISA assays (data not shown). Influenza B virus is not susceptible to inhibition by either **1** or **2**. Our results show that influenza virus B/Ann Arbor/1/86 was also not susceptible to inhibition by the metabolites **3**, **4**, and **5** (Table I).

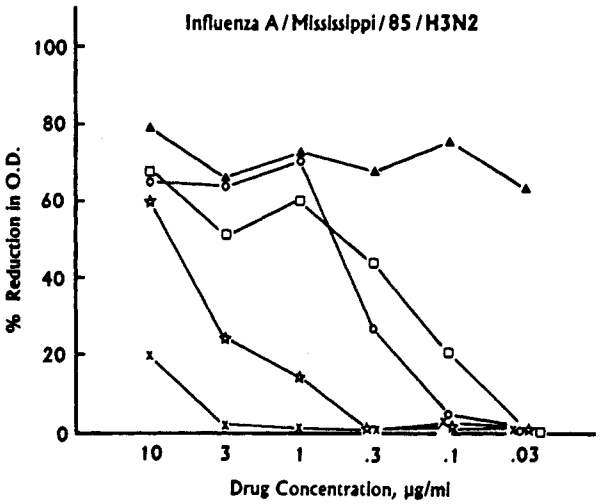


Figure 1. Cultures of MDCK cells were infected with influenza virus A/Mississippi/85/H3N2 and treated with compound at the concentrations indicated: (□) **1**, (▲), **2**, (○) **3**, (X) **4**, (☆) **5**. Cultures were incubated for 18–24 h and fixed, and the extent of virus replication was determined by ELISA.

A preliminary report suggested all three hydroxy metabolites of rimantadine were present in the urine of treated patients.[3] However, more detailed, quantitative studies have failed to identify **3** as a metabolite in man.[6] Compounds **4** and **5** each occurred as 18% and 24%, respectively, of the recovered dose (0–72 h) in the urine whereas 32% of **2** was recovered unchanged. The antiviral activity of **4** and **5** against wild-type influenza A virus was approximately 50- and 10-fold, respectively, less than that of **2**. Taken together these data suggest that it is unlikely that **4** and **5** contribute overall to the efficacy of rimantadine in the treatment of influenza A. Nonetheless, their significance in vivo may be better judged from measurements of their respective concentrations in the plasma of rimantadine-treated patients.

Experimental Section

Melting points were determined in open capillary tubes on a Büchi Tottoli or a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were determined on a Pye-Unicam SP 1000 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL 100/15 or Bruker WM 30 or Varian

(12) Hall, C. B.; Dolin, R.; Gala, C. L.; Markovitz, D. M.; Zhang, Y. Q.; Madore, P. H.; Disney, F. A.; Talpey, W. B.; Green, J. L.; Francis, A. B.; Pichichero, M. E. *Pediatrics* **1987**, *80*, 275.

(13) Belshe, R. B.; Smith, M. H.; Hall, C. B.; Betts, R.; Hay, A. J. *J. Virol.* **1988**, *62*, 1508.

XL-200 spectrometer, chemical shifts are presented in ppm (δ) from internal tetramethylsilane as reference. Mass spectra (MS) were obtained with a Kratos MS 902 mass spectrometer.

***N*-(1-Adamantylethyl)acetamide (6).** To a stirred mixture of rimantadine hydrochloride (**2**) (5.0 g, 23.2 mmol) in Et_2O (150 mL) and KOH (2.9 g, 51 mmol) in water (100 mL) was added AcCl (4.2 g, 52.5 mmol). The resulting two-phase solution was stirred vigorously for 15 min, the layers were separated, and the organic extract was washed with saturated $NaHCO_3$ (30 mL) and water (30 mL). The dried (Na_2SO_4) solution was evaporated to dryness and the resulting foam was crystallized from acetone to give 4.90 g (95%) of **6** as white crystals: mp 135–6 °C; IR (Nujol) 3250 (NH), 1635 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.02 (3 H, d, J = 8 Hz, CH_3CH), 1.40–1.78 (12 H, m, 6 × CH_2), 2.00 (6 H, m, CH_3CO and 3 × CH), 3.72 (1 H, dq, J = 8 and 10 Hz, $CHNH$), 5.35 (1 H, d, J = 10 Hz, NH); MS m/z 222 $(M + H)^+$. Anal. ($C_{14}H_{23}NO$) C, H, N.

***N*-[1-(3-Bromo-1-adamantyl)ethyl]acetamide (7).** A solution of acetamide **6** (3.0 g, 13.6 mmol) in Br_2 (50 mL) was heated to reflux for 18 h. The cooled solution was evaporated to dryness and the residue was partitioned between 2 N NaOH (200 mL) and CH_2Cl_2 (200 mL). The organic extract was washed with a saturated sodium thiosulfate solution until the organic layer became colorless. The extract was dried (Na_2SO_4) and evaporated to afford 3.95 g of a white solid. Recrystallization from acetone gave 3.7 g (91%) of **7** as colorless crystals: mp 171–2 °C; IR (Nujol) 3245 and 3200 (NH), 1630 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.05 (3 H, d, J = 8 Hz, CH_3CH), 1.45–1.75 (6 H, m, 3 × CH_2), 2.03 (3 H, s, CH_3CO), 2.05–2.40 (8 H, m, 3 × CH_2 and 2 × CH), 3.83 (1 H, dq, J = 8 and 10 Hz, $CHNH$), 5.31 (1 H, d, J = 10 Hz, NH); MS m/z 299 (M^+). Anal. ($C_{14}H_{22}BrNO$) C, H, N.

1-(1-Aminoethyl)-3-adamantanol Hydrochloride (4). A. A stirred suspension of acetamide **7** (1.80 g, 6 mmol) in 2 N HCl (60 mL) was heated to reflux for 48 h, during which time all starting material dissolved. The solution was cooled, made alkaline with 2 N NaOH, and extracted with Et_2O (3 × 100 mL). The ethereal extracts were dried (Na_2SO_4), and ethereal HCl was added to give 620 mg (45%) of hydrochloride of **4** as a white precipitate: mp 324–5 °C; IR (Nujol) 2500–3500 cm^{-1} (NH and OH); ^1H NMR (d_6-Me_2SO) δ 1.10 (3 H, d, J = 8 Hz, CH_3CH), 1.30–1.65 (12 H, m, 6 × CH_2), 2.15 (2 H, s, 2 × CH), 2.85 (1 H, q, J = 8 Hz, CH_3CH), 4.52 (1 H, s, OH), 7.70 (3 H, s, $NH_3^+Cl^-$); MS m/z 196 $(M + H)^+$. Anal. ($C_{12}H_{22}ClNO$) C, H, N.

B. A mixture of ketone **14** (1.0 g, 5.1 mmol), 40 mL of MeOH, 20 mL of liquid ammonia, and 4.0 g of Raney nickel was kept under an atmosphere of hydrogen at 1375 psi and 100 °C for 6 h. The mixture was cooled to 10 °C and filtered. The filtrate was evaporated and the residue was chromatographed on 50 g of silica (70–230 mesh). Elution with 50 mL of AcOEt, followed by 200 mL of 5% NH_4OH in MeOH (15-mL fractions), afforded 620 mg (62%) of **4** (free base): mp 112–114 °C (from CH_2Cl_2–hexane); IR ($CHCl_3$) 3365, 3265 (NH and OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00 (3 H, d, J = 6 Hz, CH_3CH), 1.4–1.8 (15 H, m, 6 × CH_2, OH and NH_2), 2.20 (2 H, br s, 2 × CH), 2.51 (1 H, q, J = 6 Hz, CH_3CH); ^{13}C NMR ($CDCl_3$) δ 17.0 (CH_3), 30.4 (CH), 35.7 (CH_2), 36.7 (CH_2), 36.8 (CH_2), 44.8 (2 × CH_2), 45.8 (C-1), 54.9 ($CHNH_2$), 68.4 (C-3); MS m/z 195 (M^+). Anal. ($C_{12}H_{21}NO$) C, H, N.

1-Carboxy-2-adamantanol (11). To a stirred solution of the acid **8**[7] (3.3 g, 17 mmol) in dry DMF (11 mL) at 0 °C was added $NaBH_4$ (360 mg, 9.47 mmol). The reaction mixture was allowed to warm to room temperature overnight, after which time a further 50 mg of $NaBH_4$ was added and stirring continued for 4 h. The reaction mixture was concentrated in vacuo, treated with 2 N HCl (50 mL), and extracted with CH_2Cl_2 (3 × 100 mL). Evaporation of the solvent gave 6.6 g of a clear oil which was redissolved in Et_2O (100 mL), washed with water (2 × 100 mL), dried (Na_2SO_4), and concentrated to afford 2.6 g of a white solid. Trituration with petroleum ether gave 2.43 g (73%) of **11**, mp 123–5 °C (lit.[14] mp 125.5–126.5 °C). The water washings were combined and reextracted with Et_2O (2 × 100 mL) to afford a further 575 mg of material after trituration with petroleum ether. The combined yield was 90%. IR (Nujol) 3600–2350 (CO_2H and OH), 1695 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.40–2.32 (13 H, m), 4.11 (1 H, d, J = 3 Hz, $CHOH$), 5.00–9.00 (2 H, bs, CO_2H and OH); MS m/z 197 $(M + H)^+$.

1-Acetyl-2-adamantanol (13). The hydroxy acid **11** (1.176 g, 6 mmol) in dry THF (60 mL) was cooled to 0 °C and treated with MeLi (40 mL, 1.2 M in Et_2O). The reaction mixture was heated to reflux for 2 h and cooled to 0 °C and chlorotrimethylsilane (21 mL) added. The reaction mixture was allowed to warm to room temperature and 1 N HCl (45 mL) added. Stirring was continued for 0.5 h, and the product was extracted with Et_2O (3 × 75 mL). The combined ethereal extracts were washed with water (60 mL), dried (Na_2SO_4), and evaporated to give 1.4 g of a colorless oil. Purification was effected by flash chromatography (5% MeOH–CH_2Cl_2) on Sorbsil C60-40/60 silica gel. Trituration with petroleum ether afforded 770 mg (66%) of **13** as a white solid: mp 85–87 °C; IR (Nujol) 3600–3300 (OH), 1690 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.45–2.20 (13 H, m, 5 × CH_2 + 3 × CH), 2.15 (3 H, s, CH_3CO), 3.03 (1 H, d, J = 3 Hz, $CHOH$), 4.10 (1 H, s, OH); MS m/z 195 $(M + H)^+$.

1-Acetyl-2-adamantanol Oxime (16). Methyl ketone **13** (1.37 g, 7.06 mmol) was added to a solution of hydroxylamine hydrochloride (1.31 g, 18.8 mmol) in dry pyridine (7 mL) and EtOH (7 mL). The resulting solution was heated to reflux for 2 h and then evaporated to give a white solid, to which water (17.5 mL) was added. The mixture was stirred and the product was collected by filtration to afford 1.39 g (94%) of a white solid: mp 205–7 °C; ^1H NMR ($CDCl_3$) δ 1.42–2.15 (14 H, m), 1.80 (3 H, s, CH_3C=NOH), 4.05 (1 H, d, J = 3 Hz, $CHOH$), 9.75 (1 H, s, NOH); MS m/z 210 $(M + H)^+$.

1-(1-Aminoethyl)-2-adamantanol Hydrochloride (3). Oxime **16** (1.39 g, 6.65 mmol) in dry THF (22 mL) was treated with 1 M $LiAlH_4$ in Et_2O (14 mL, 14 mmol), and the mixture was heated to reflux for 4 h. The mixture was stirred at room temperature for 16 h, and water was then added together with a few drops of 2 N NaOH. The solids were collected by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (50 mL), washed with water (20 mL), dried (Na_2SO_4), and evaporated to afford 1.41 g of an oil, which was dissolved in Et_2O and treated with a saturated solution of HCl in AcOEt to give the hydrochloride salt of **3** (960 mg, 62%) as a white solid: mp 265–278 °C; IR (Nujol) 3600–2450 cm^{-1} (NH_3^+ and OH); ^1H NMR ($CDCl_3$) δ 1.15–2.20 (16 H, m), 2.60 (1 H, bs, OH), 3.09 and 3.19 (1 H, q, J = 8 Hz, $CHCH_3$), 3.78 and 3.96 (1 H, d, J = 3 Hz, $CHOH$), 6.2–8.5 (3 H, bs, NH_3^+); MS m/z 198 $(M + H)^+$. Anal. ($C_{12}H_{22}ClNO$) C, H, N.

1-Acetyl-3-adamantanol (14). Hydroxy acid **9**[11] (1.96 g, 10 mmol) was allowed to react with MeLi (50 mL, 1.2 M in Et_2O) under the conditions used for the preparation of **13** to give 1.1 g of **14** as colorless crystals (from AcOEt–hexane): mp 89–91 °C; IR ($CHCl_3$) 3395 (OH), 1680 (C=O); ^1H NMR 1.55 (OH), 1.6 (2 H, s, CH_2), 1.65–1.80 (10 H, m, 5 × CH_2), 2.1 (3 H, s, CH_3CO), 2.3 (2 H, br s, 2 × CH); ^{13}C NMR ($CDCl_3$) δ 24.39 (CH_3), 30.13 (CH), 35.0 (CH_2), 37.0 (CH_2), 44.1 (CH_2), 45.6 (CH_2), 49.7 (C-1), 67.9 (C-3), 212.5 (C=O); MS m/z 194 (M^+). Anal. ($C_{12}H_{18}O_2$) C, H.

1-Acetyl-3-adamantanol Oxime (17). Oxime **17** was prepared from ketone **14** (97 mg, 0.5 mmol), as described for the preparation of **16**, in 65% yield: mp 177–179 °C; IR (KBr) 3310 (OH), 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (2 H, s, CH_2), 1.65–1.75 (10 H, m, 5 × CH_2), 1.81 (3 H, s, CH_3), 2.25 (2 H, br s, 2 × CH), 2.45 (OH), 9.36 (OH); MS m/z 209 (M^+). Anal. ($C_{12}H_{19}NO_2$) C, H, N.

1-Carboxy-4-adamantanone (10). A vigorously stirred solution of 2.26 g (10 mmol) of 1-phenyl-4-adamantanone (**19**)[10] in 40 mL of CH_3CN and 40 mL of CCl_4 was treated with 40 g (187 mmol) of sodium periodate in 100 mL of water followed by 500 mg of ruthenium trichloride trihydrate. The mixture was boiled under reflux for 3 h, stirred at room temperature overnight, and then filtered over Celite. The filtrate was diluted with 100 mL of CH_2Cl_2 and water, and the organic phase was separated. The aqueous phase was reextracted with CH_2Cl_2 (2 × 100 mL), and the combined organic extracts were made basic with 150 mL of 1 N NaOH. The aqueous phase was separated, acidified with 1 N HCl, and extracted with CH_2Cl_2 (2 × 150 mL). The combined extracts were washed with saturated brine, dried ($MgSO_4$), and evaporated to give 1.4 g of **10** as a tan solid. This was dissolved

(14) Tabushi, I.; Aoyama, Y. *J. Org. Chem.* **1973**, *38*, 3447.

in AcOEt (75 mL) and stirred with 700 mg of neutral charcoal for 1 h. The mixture was filtered over Celite, and the filtrate was evaporated to give a colorless solid. Crystallization from 50% AcOEt–hexane (10 mL) gave 1.25 g (64%) of **10**: mp 171–172 °C; IR (KBr) 1728, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.02–2.30 (11 H, m, 5 × CH_2 + CH), 2.65 (2 H, s, 2 × CH); ^{13}C NMR ($CDCl_3$) δ 27.02 (CH), 37.36 (CH_2), 38.14 (CH_2), 39.69 (CH_2), 39.9 (C-1), 45.43 (CH), 181.43 (CO_2H), 217.3 (C=O); MS m/z 195 $(M + H)^+$. Anal. ($C_{11}H_{14}O_3$) C, H.

The corresponding methyl ester, prepared with CH_2N_2 in CH_2Cl_2, had mp 53–54 °C (from hexane); IR ($CHCl_3$) 1722 (ester C=O), 1688 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.00–2.20 (11 H, m, 5 × CH_2 + CH), 2.57 (2 H, s, 2 × CH), 3.65 (3 H, s, OCH_3); ^{13}C NMR ($CDCl_3$) δ 27.14 (CH), 37.7 (CH_2), 38.2 (CH_2), 40.1 (CH_2), 40.2 (C-1), 45.6 (CH), 51.9 (CH_3), 176.0 (C=O ester), 216.3 (C=O, ketone); MS m/z 208 (100, M^+). Anal. ($C_{12}H_{16}O_3$) C, H.

1-Carboxy-4-adamantanol (12). A solution of 1-carboxy-4-adamantanone (**10**) (970 mg, 5 mmol) in 10 mL of EtOH was treated with $NaBH_4$ (900 mg). The mixture was stirred at 50 °C for 30 min and at room temperature for 3.5 h and was worked up as for the preparation of **11**. Crystallization from AcOEt–hexane gave 420 mg (43%) of **12**: mp 151–155 °C; IR (KBr) 3460 (OH), 1708 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) revealed CH-4 at δ 3.84 and 3.92 in a ratio of 2:3; ^{13}C NMR ($CDCl_3$) δ 26.8 (CH), 29.7 (CH_2), 31.9 (CH_2), 33.8 (CH_2), 34.1 (CH), 35.2 (CH_2), 37.7 (CH_2), 38.6 (CH_2), 72.9 and 73.4 (C-4), 182.8 and 182.9 (CO_2H); MS m/z 196 (M^+). Anal. ($C_{11}H_{16}O_3$) C, H.

1-Acetyl-4-adamantanol (15). Hydroxy acid **12** (576 mg, 2.9 mmol) in 12 mL anhydrous THF was allowed to react with 20 mL of 1.6 M (ethereal) MeLi under the conditions specified for the preparation of **13** to give 380 mg of crude **15**. Chromatography over 35 g of silica (70–23 mesh) with 60% AcOEt in hexane as eluant gave 220 mg of **15** as colorless crystals: mp 66–74 °C; IR ($CHCl_3$) 3610 (OH), 1692 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.4–2.2 (14 H, m, 5 × CH_2 + 3 × CH + OH), 2.10 (3 H, s, CH_3CO), 3.90 (1 H, br s, CH-4); ^{13}C NMR ($CDCl_3$) δ 24.4 (CH_3), 27.2 (CH), 30.0 (CH_2), 31.8 (CH_2), 34.0 (CH), 34.4 (CH), 35.3 (CH_2), 37.3 (CH_2), 38.3 (CH_2), 72.7 and 73.3 (C-4), 214 (C=O); MS m/z 194 (M^+), 151. Anal. ($C_{12}H_{18}O_2$) C, H.

1-Acetyl-4-adamantanol Oxime (18). Oxime **18** was prepared from ketone **15** (97 mg, 0.5 mmol) and hydroxylamine hydrochloride (100 mg) according to the procedure given for **16**. Crystallization from AcOEt gave 63 mg (60%) of **18**: mp 208–215 °C; IR 3450–3100 (OH), 1658 (weak, C=N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.4–2.1 (13 H, m, 5 × CH_2 + 3 × CH), 1.84 (3 H, s, CH_3), 3.88 (1 H, br s, CH-4), 7.61 (OH); ^{13}C NMR ($CDCl_3$–d-Me_2SO) δ 8.9 (CH_3), 27.5 (CH), 30.23 (CH_2), 33.8 (CH_2), 34.2 (CH), 34.3 (CH), 35.6 (CH_2), 38.1 (C-1), 38.5 (CH_2), 39.6 (CH_2), 73.4 (CH-4), 160.3 (C=NOH); MS m/z 209 (M^+). Anal. ($C_{12}H_{19}NO_2$) C, H, N.

1-(1-Aminoethyl)-4-adamantanol Hydrochloride (5). A solution of oxime **18** (31.3 mg, 0.15 mmol) in anhydrous THF (4 mL) was treated with $LiAlH_4$ (65 mg). The mixture was boiled under reflux for 16 h, cooled to room temperature, and treated cautiously with 5 mL of water followed by 1 mL of 1 N NaOH and 15 mL of water. The mixture was extracted with CH_2Cl_2 (2 × 25 mL), and the extract was washed with saturated brine (25 mL), dried ($MgSO_4$), and evaporated to give 24 mg of **5** as a gum: NMR ($CDCl_3$) δ 0.92 (3 H, d, J = 7 Hz, CH_3), 1.3–2.2 (15 H, m), 2.45 (1 H, q, J = 6 Hz, $CHNH_2$), 3.85 (1 H, br s, CH-4); MS m/z 195 (M^+). A solution of **5** in Et_2O was treated with HCl gas in 1 mL of Et_2O to give a precipitate of the corresponding hydrochloride, mp 245–255 °C. Anal. ($C_{12}H_{22}ClNO$) C, H, N.

Antiviral Assay. The compounds were assayed for antiviral activity in Madin Darby Canine Kidney (MDCK) cells by using a viral cytopathogenicity inhibition assay as described previously.[4] To confluent monolayers of cells in 96-well microtiter plates was added compound at the desired concentration in serial 2-fold dilutions across the plate. Virus suspension containing 100 $TCID_{50}$ was added to the monolayers, and plates were incubated at 35 °C for 48 h. Cells were fixed, stained, and evaluated microscopically for cytopathic effect (cpe). The results are expressed as the dose of compound required to inhibit virus cpe by 50% when infected, control cultures just reached 100% cpe. The susceptibility of influenza A virus isolates to inhibition by test compounds was confirmed by ELISA in a test modified from that described previously.[4,13] The validation of the test using the appropriate controls has been described previously.[4,13] MDCK cells were grown in microtiter plates and infected with virus in the presence or absence of test compound. Plates were incubated at 37 °C for 18–24 h. Cells were fixed with 0.05% glutaraldehyde in PBS at room temperature for 15 min, washed, and incubated for 1 h at 37 °C with 50 μL of a 10^{-3} dilution of ferret antiserum to influenza A H3N2 (Mississippi/85-like) virus in PBS containing 0.5% bovine serum albumin (BSA). The plates were washed and incubated for 1 h at 37 °C with 50 μL of a 10^{-3} dilution of protein A-horseradish peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA) in PBS and incubated at room temperature for 2–5 min with 50 μL o-phenylenediamine (Abbott Laboratories, North Chicago, IL) and buffer containing 0.02% H_2O_2; the reaction was stopped by the addition of 100 μL of 1 M H_2SO_4, and ODs at 450 nm were determined.

Acknowledgment. We very much appreciate the technical assistance provided by C. Enny and J. Leone in the preparation of some of the intermediates.

Synthesis of 1-Methyl-5-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)uracil and 1-Methyl-5-(3-azido-2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)uracil. The C-Nucleoside Isostere of 3′-Azido-3′-deoxythymidine and Its 2′-"Up"-Fluoro Analogue[1]

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1-Methyl-5-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)uracil (C-AZT), a C-nucleoside isostere of the potent anti-AIDS nucleoside 3′-azido-3′-deoxythymidine (AZT), was synthesized. 1-Methyl-2′-deoxy-5′-*O*-tritylpseudouridine (**2a**) was oxidized with CrO_3/pyridine/Ac_2O complex to 1-methyl-5-(5-*O*-trityl-β-D-*glycero*-pentofuranos-3-ulosyl)uracil (**12a**), which was selectively reduced to 1-methyl-5-(5-*O*-trityl-β-D-*threo*-pentofuranosyl)uracil (**13a**). Mesylation of **13a** to **14a** followed by nucleophilic displacement of the mesyloxy group with azide afforded 3′-azido-2′,3′-dideoxy-5′-*O*-trityl-1-methylpseudoridine (**15a**), which was detritylated to C-AZT. In a similar manner, 1-methyl-5-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (C-FMAU, a potent antiherpetic nucleoside) was converted into the 3′-azido analogue (3′-azido-C-FMAU). Both C-AZT and 3′-azido-C-FMAU, however, did not exhibit any significant inhibitory activity against HIV in H9 cells.

The primary pathogen that causes the acquired immunodeficiency syndrome (AIDS) and AIDS related complex (ARC) has been identified as a retrovirus, human T-lymphotropic virus type III (HTLV-III), also called lympha-

0022-2623/90/1833-1995$02.50/0