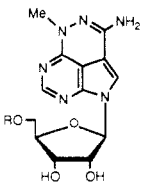
Synthesis and Cytotoxicity Studies of 8-Amino-6-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (7-Aza-TCN) and the Corresponding 2'-Deoxy- and Arabinonucleoside Analogues

Andrew M. Kawasaki,[†,‖] Linda L. Wotring,[‡] and Leroy B. Townsend*[,†,§]

Departments of Medicinal Chemistry and Pharmaceutical Chemistry, College of Pharmacy, and Department of Chemistry, College of Literature, Sciences and Arts, The University of Michigan, Ann Arbor, Michigan 48109-1065.
Received March 12, 1990

The tricyclic nucleoside 8-amino-6-*N*-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (**5**) was synthesized from 8-amino-6-*N*-methyl-4-(methylthio)-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (**2**). The 2'-deoxy analogue of **5**, 8-amino-6-*N*-methyl-2-(2-deoxy-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**11**), and the arabino analogue of (**5**), 8-amino-6-*N*-methyl-2-β-D-arabinofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (**14**) were synthesized from **5**. Nucleosides **2**, **3**, **4**, **5**, 11, and **14** were evaluated for potential anticancer activity by measuring their ability to inhibit the growth of L1210 and H. Ep. 2 tumor cells in vitro.

The nucleoside 6-amino-4-*N*-methyl-8-β-D-ribofuranosyl-1,3,4,5,8-pentaazaacenaphthylene[1] (TCN, **1a**) was found to be a potent antitumor agent, and the prodrug of TCN (TCN-P, **1b**) is currently undergoing clinical trials under the auspices of the National Cancer Institute.[2]



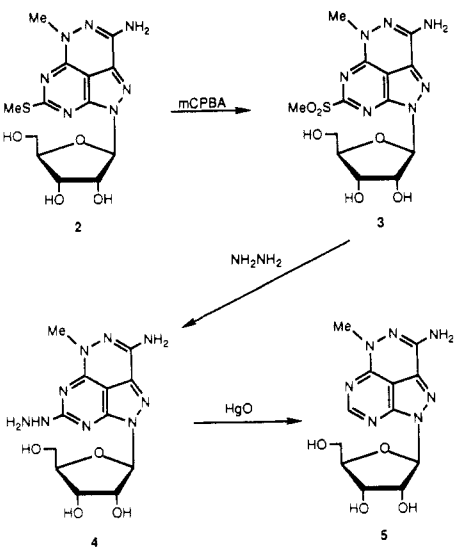
1a, R = H
1b, R = PO_3H_2

TCN is metabolized to its monophosphate derivative and is generally thought to act as an adenosine analogue.[2b,3] In TCN-treated cells, the mechanism of cytotoxicity appears to involve the inhibition of DNA synthesis and/or protein synthesis.[3a,4] It has also been reported that one metabolite of TCN, with decreased cytotoxic activity,[5] is a ring-opened compound resulting from a presumed oxidation at the C-7 position of TCN.[6] This prompted us to initiate a synthesis of 7-aza-TCN [8-amino-6-*N*-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene, **5**], which would be expected to resist this deactivating reaction. The 2'-deoxy analogue of **5** [8-amino-6-*N*-methyl-2-(2-deoxy-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene, **11**], and the arabino analogue of **5** [8-amino-6-*N*-methyl-2-β-D-arabinofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene, **14**] were also prepared for a structure–activity relationship study.

Chemical Results and Discussion

In our attempt to obtain **5** (7-aza-TCN), 8-amino-6-*N*-methyl-4-(methylthio)-2-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene was treated with Raney nickel. However, the desired product [8-amino-6-*N*-methyl-2-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene] was not obtained. Instead, two products were isolated and characterized[7] as 3-amidino-4-*N*-(methylamino)-6-(methylthio)-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine and the dethiated derivative 3-amidino-4-*N*-(methylamino)-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine. Both of these products resulted from an apparent reductive cleavage of the pyridazine ring. After further investigation, it was concluded that reductive conditions must be avoided in the dethiation of compounds containing this heterocyclic ring system and prompted a change in strategy.

Scheme I



A survey of the literature provided a nonreductive method which involved an oxidative replacement of a hydrazino group with hydrogen. This appeared to be a viable synthetic route for the synthesis of **5** if the methylthio

[†] Department of Medicinal Chemistry.
[‡] Department of Pharmaceutical Chemistry.
[§] Department of Chemistry.
[‖] Present address: ISIS Pharmaceuticals, Carlsbad, CA 92008.

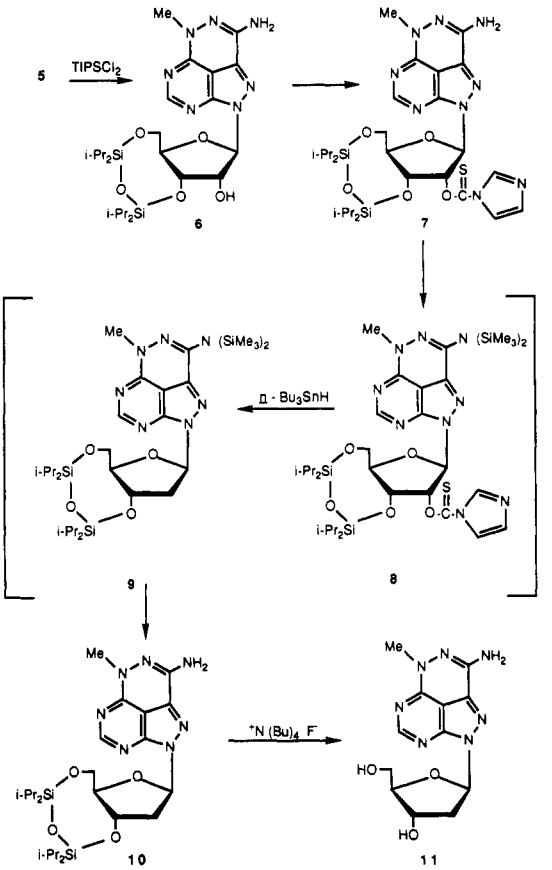
(1) Schram, K. H.; Townsend, L. B. *Tetrahedron Lett.* **1971**, 4757.
(2) (a) Feun, L. G.; Savaraj, N.; Bodey, G. P.; Lu, K.; Yap, B. S.; Ajani, J. A.; Burgess, M. A.; Benjamin, R. S.; McKelvey, E.; Krakoff, I. *Cancer Res.* **1984**, *44*, 3608. (b) Wotring, L. L.; Crabtree, G. W.; Edwards, N. L.; Parks, R. E., Jr.; Townsend, L. B. *Cancer Treat. Rep.* **1986**, *70*, 491. (c) Powis, G.; Basseches, P. J.; Kroschel, D. M.; Richardson, R. L.; O'Connell, M. J.; Kvols, L. K. *Cancer Treat. Rep.* **1986**, *70*, 359. (d) O'Connell, M. J.; Rubin, J.; Hahn, R. G.; Kvols, L. K.; Moertel, C. G. *Cancer Treat. Rep.* **1987**, *71*, 333.
(3) (a) Bennett, L. L., Jr.; Smithers, D.; Hill, D. L.; Rose, L. M.; Alexander, J. A. *Biochem. Pharmacol.* **1978**, *27*, 233. (b) Plagemann, P. G. W. *J. Natl. Cancer Inst.* **1976**, *57*, 1283.
(4) (a) Wotring, L. L.; Passiatore, J. E.; Roti Roti, J. L.; Hudson, J. L.; Townsend, L. B. *Cancer Res.* **1985**, *45*, 6355. (b) Moore, E. C.; Hurlbert, R. B.; Massia, S. P. *Biochem. Pharmacol.* **1989**, *38*, 4037.
(5) Basseches, P. J.; Powis, G. *Cancer Res.* **1984**, *44*, 4672.
(6) Schweinsberg, P. D.; Smith, R. G.; Loo, T. L. *Biochem. Pharmacol.* **1981**, *30*, 2521.
(7) Kawasaki, A. M.; Townsend, L. B. *Tetrahedron Lett.* **1989**, *30*, 3287.

0022-2623/90/1833-3170$02.50/0

group of 8-amino-6-*N*-methyl-4-(methylthio)-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (**2**) could be transformed to a hydrazino function. It was reported[8] that with the pteridine, quinoline, phthalazine, and pyridazine ring systems, hydrazino functions which are α to a ring nitrogen could be oxidized to hydrogen with mercuric oxide in good yields. Also, it was recently reported[9] that 7-hydrazino[1,2,4]triazolo[2,3-*b*][1,2,4]triazine was treated with mercuric oxide to give a good yield of the 7-hydrogen derivative. Thus, we elected to attempt the mercuric oxide reaction with 8-amino-4-hydrazino-6-*N*-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (**4**). We initially assumed that **4** could be obtained directly by a reaction of hydrazine with **2**; however, displacement of the 4-methylthio group of **2** by hydrazine required forcing conditions, and low yields were obtained. It has been reported[10-12] that a displacement of a methylsulfonyl function occurs in a more facile manner than a methylthio substituent on most heterocyclic ring systems. Thus, 8-amino-4-(methylsulfonyl)-6-*N*-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (**3**) was prepared by the oxidation of **2** with *m*-chloroperoxybenzoic acid,[13] and a facile displacement of this methylsulfonyl group of **3** by hydrazine gave **4** in a high yield. The oxidation of **4** with mercuric oxide then afforded 8-amino-6-*N*-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (**5**), the 7-aza analogue of TCN, in an overall yield of 40% from **2** (Scheme I).

For a synthesis of the 2′-deoxynucleoside analogue of **5**, 8-amino-6-*N*-methyl-2-(2-deoxy-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**11**), a literature procedure which used a Barton reduction[14] was employed. The tricyclic nucleoside **5** was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[15] (TIPSCl) in pyridine at room temperature to give the 3′,5′-tetraisopropyldisiloxandiyl (TIPS) protected derivative 8-amino-6-*N*-methyl-2-(3,5-*O*-(tetraisopropyldisiloxandiyl)-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**6**) in a 50% yield (Scheme II). Under a slight excess of acid, it has been reported[16,17] that 3′,5′-tetraisopropyldisiloxandiyl-protected ribofuranosyl nucleosides can rearrange to the 2′,3′-protected isomer. Thus, we felt that we should confirm the initial structure assignment for **6**. The ^{1}H NMR spectrum of **6** showed a multiplet at δ 1.0 which was assigned to the TIPS group. Only one hydroxyl proton was observed at δ 5.55. This signal was assigned to the C-2′-OH function since on exchange with deuterium oxide, the multiplet at δ 4.72 assigned to the C-2′-H was simplified while the multiplet assigned to the C-5′-H at δ 3.88 did not change. Also a ^{1}H NMR homonuclear decoupling experiment further established that the assigned C-2′-H resonance was coupled to the observed hydroxyl resonance since irradiation of the C-2′-H signal resulted in a simplification of the hydroxyl resonance. The UV spectra of **6**, taken at pH 1, 7, and 11, were observed to be very similar to those of **5** which indicated that the electronic nature of the heterocyclic moiety had not been perturbed. The product **6**, which contained a trace impurity after column chromatography, was used without further purification for the reaction of **6** with 1,1′-thiocarbonyldiimidazole[18] (TCDI) in dimethylformamide (DMF). A 42% yield of the 2′-thiocarbonylimidazole derivative 8-amino-6-*N*-methyl-2-(3,5-*O*-(tetraisopropyldisiloxandiyl-2-*O*-(1-thiocarbonylimidazoyl)-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**7**) was obtained. The ^{1}H NMR spectrum of **7** showed the presence of a doublet at δ 8.39, a multiplet at δ 7.68, and a multiplet at δ 7.09. These three resonances were assigned to the imidazole protons of **7**. No hydroxyl proton signals were observed in the δ 4–6 region.

Scheme II



Before carrying out a reduction[19,20] of the thiocarbonylimidazole derivative, the aromatic amino function was silylated with bis(trimethylsilyl)acetamide (BSA) to give the N-silylated derivative **8**. This silylation was performed since we were concerned with the possible involvement of the aromatic amino group with the free radical reaction. Without further purification of **8**, treatment of the crude silylated product with tri-*n*-butyltin hydride in the presence of 2,2′-azobis(2-methylpropionitrile) (AIBN) presumedly gave the N-silylated 2′-deoxynucleoside **9**. Compound **9** was not isolated but was instead N-desilylated by heating in EtOH at reflux to give

(8) Albert, A.; Catterall, G. *J. Chem. Soc., C* **1967**, 1533.
(9) Sanemitsu, Y.; Nakayama, Y.; Shiroshita, M. *J. Heterocycl. Chem.* **1983**, *20*, 1671.
(10) Mizuno, Y.; Endo, T.; Miyaoka, T.; Ikeda, K. *J. Org. Chem.* **1974**, *39*, 1250.
(11) Ikehara, M.; Muneyama, K. *Chem. Pharm. Bull.* **1966**, *14*, 46.
(12) Ikehara, M.; Yamazaki, A.; Fujieda, T. *Chem. Pharm. Bull.* **1962**, *10*, 1075.
(13) Montgomery, J. A.; Shortnacy, A. T.; Secrist, J. A., III. *J. Med. Chem.* **1983**, *26*, 1483.
(14) Pankiewicz, K.; Matsuda, A.; Watanabe, K. A. *J. Org. Chem.* **1982**, *45*, 485.
(15) Markiewicz, W. T.; Wiewiorowski, M. *Nucleic Acids Res., Spec. Publ.* **1978**, s195. Markiewicz, W. T. *J. Chem. Res. Synop.* **1979**, 24.
(16) Pankiewicz, K. W.; Watanabe, K. A.; Takayanagi, T.; Itoh, T.; Ogura, H. *J. Heterocycl. Chem.* **1985**, *22*, 1703.
(17) Verdegaal, C. H.; Jansse, P. D.; de Rooiji, J. F.; Van Boom, J. H. *Tetrahedron Lett.* **1980**, *21*, 1571.
(18) Reid, W.; Beck, B. M. *Annalen* **1961**, *96*, 646.
(19) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans.* **1975**, *1*, 1575.
(20) Barton, D. H. R.; Subramanian, R. *J. Chem. Soc., Perkin Trans.* **1977**, *1*, 1718.

8-amino-6-*N*-methyl-2-(2-deoxy-3,5-*O*-(tetraisopropyldisiloxandiyl)-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**10**) in a 61% yield from **7**. The ^{1}H NMR spectrum of **10** showed only one signal (δ 8.22) within the region of δ 7–8.5, and the signal was assigned to the aromatic proton of the heterocyclic moiety. This confirmed the loss of the imidazole group. Also, two multiplets were observed at δ 2.98 and 2.60 in the ^{1}H NMR spectrum of **10** and these chemical shifts were assigned to the methylene C-2′ protons. Desilylation of **10** was accomplished with tetra-*n*-butylammonium fluoride[21] in THF as a solvent to give the unprotected nucleoside 8-amino-6-*N*-methyl-2-(2-deoxy-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**11**) in a 63% yield. The ^{1}H NMR spectrum of **11** showed an absence of a signal in the δ 1–2 region, indicating the loss of the disiloxanyl group, with a concomitant appearance of two exchangeable signals at δ 5.28 (doublet) and at δ 4.96 (doublet of doublets) which were assigned to the C-3′-OH and C-5′-OH protons, respectively. The anomeric proton of **11** appeared as a pseudo triplet at a chemical shift of δ 6.40 which supports the β assignments.[28] To confirm that the heterocyclic moiety had remained intact, a UV spectrum of **11** showed a spectrum which was essentially the same as that obtained for **5**.

For the synthesis of the arabino nucleoside analogue of **5**, 8-amino-6-*N*-methyl-2-(β-D-arabinofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**14**), we elected to use a dimethyl sulfoxide–acetic anhydride oxidation[22,23] of the protected nucleoside **6** to give the 2′-keto nucleoside 8-amino-6-*N*-methyl-2-(3,5-*O*-(tetraisopropyldisiloxanyl)-β-D-*erythro*-pentafuran-2-ulosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**12**). It was presumed that the keto nucleoside **12** could be reduced stereoselectively with sodium borohydride to give the arabino nucleoside 8-amino-6-*N*-methyl-2-(3,5-*O*-(tetraisopropyldisiloxanyl)-β-D-arabinofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**13**) which could then be deprotected to afford **14**. Such stereoselective reductions of pentofuran-2′-ulosides have been previously reported to give moderate to good yields.[24-27] Certain 2′ and 3′-ketopentofuranosyl nucleosides have been reported to be unstable especially to basic conditions.[24,25,27] Thus, the stereoselective reduction has usually been conducted in situ without an isolation of the 2′-ketopentofuranosyl nucleoside.

Initially, the protected nucleoside **6** was oxidized with dimethyl sulfoxide and acetic anhydride by using a modification of the reported[24] procedure. A 2,4-dinitrophenylhydrazone test of the reaction mixture on a thin-layer chromatography (TLC) plate, showed that a carbonyl compound was present as the major product. In situ treatment of the reaction mixture, which presumably contained the 2′-ketopentofuranosyl nucleoside **12**, with ethanolic sodium borohydride at 0 °C afforded the protected arabinonucleoside **13** in a 35% yield from **6** (Scheme III). The ^{1}H NMR spectrum of **13** showed the expected downfield shift (0.27 ppm) of the anomeric proton resonance relative to the anomeric proton resonance of **6** due to the removal of the diamagnetic effect of a cis 2′-OH.[28]

Scheme III

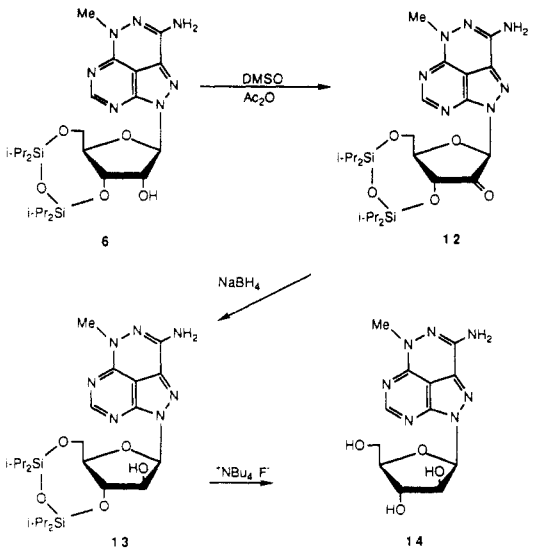


Table I. In Vitro Cytotoxicity Evaluations against L1210 and H. Ep. 2 Cells for 4-Substituted 8-Amino-6-*N*-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylenes (**2–5**), 8-Amino-6-*N*-methyl-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (**11**), and 8-Amino-6-*N*-methyl-2-β-D-arabinofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (**14**)

compd	R_1	R_2	conc, μM	L1210 growth rate,[a] %	H. Ep. 2 growth rate, %
2	SCH_3	rib[b]	100	81	nt[c]
3	SO_2CH_3	rib	100	86	nt[c]
4	$NHNH_2$	rib	10	86	88
5	H	rib	100	63[d]	67
11	H	d-rib	100	83	100
14	H	ara	50	75	62

[a] Growth rate is defined in the Experimental Section. [b] Abbreviations are as follows: rib, ribosyl, d-rib, 2-deoxyribosyl (2-deoxy-β-D-*erythro*-pentofuranosyl); ara, arabinosyl. [c] nt = not tested. [d] Average of three experiments; all other growth rates are from single experiments.

To exclude the possibility that a rearrangement of the 3′,5′-tetraisopropyldisiloxandiyl function of **6** had occurred[16,17] to give the 2′,3′-protected nucleoside, a homonuclear decoupling experiment was performed with **13**. The anomeric proton was irradiated to establish the C-2′-H resonance; then the C-2′-H resonance was irradiated. This resulted in the simplification, i.e., doublet to singlet, of the exchangeable signal which was assigned to the C-2′-OH.

Due to the low yield obtained for the isolation of the protected arabinonucleoside **13**, we elected to synthesize the unprotected arabinonucleoside **14** without the isolation of intermediates **12** or **13**. This approach has been used by other workers[24,25] to presumably minimize the decomposition of the protected intermediates. Thus, **13** was obtained as a crude product via the above mentioned procedure, then without further purification, **13** was deprotected with tetra-*n*-butylammonium fluoride to give **14**

(21) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
(22) Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1965**, *87*, 4214.
(23) Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1967**, *89*, 2416.
(24) Hansske, F.; Madej, D.; Robins, M. J. *Tetrahedron* **1984**, 125.
(25) Sakairi, N.; Hirao, I.; Zama, Y.; Ishido, Y. *Nucleosides Nucleotides* **1983**, *2*, 221.
(26) Brodbeck, U.; Moffatt, J. G. *J. Org. Chem.* **1970**, *35*, 3552.
(27) Moffatt, J. G. *Nucleoside Analogues: Chemistry, Biology, and Medical Applications*; Walker, R. T., Declerq, E., Eckstein, F., Eds.; NATO Advanced Study Institute Series; Plenum Press: New York, 1979; Vol. 26A, pp 71–164.
(28) Townsend, L. B. *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; Wiley-Interscience: New York, 1973; Vol. 2, pp 333–335.

in a 54% overall yield from 6. Judging from the improved yield, it appeared that some decomposition of the intermediates had occurred during their purification. The ^1H NMR spectrum of 14 exhibited the expected deshielding (0.28 ppm) for the anomeric proton[28] relative to that of the ribofuranosyl nucleoside 5.

Biological Results and Discussion

Compounds 2, 3, 4, 5, 11, and 14 were evaluated for cytotoxicity in the L1210 and H. Ep. 2 cell lines. The results from growth inhibition experiments are presented in Table I. 7-Aza-TCN (5) and arabinosyl-7-aza-TCN (14) showed marginal activity, but their IC_{50} values were greater than 100 and 50 μM respectively, for both cell lines. The 2'-deoxy-7-aza-TCN (11) and the various 4-substituted 7-aza-TCN's 2–4 did not show significant growth inhibitory activity. The lack of cytotoxic activity of 7-aza-TCN (5) in particular was contrary to our expectations. Therefore, we considered what biochemical properties of this compound might contribute to its drastic loss of cytotoxic activity in comparison to TCN itself (L1210 IC_{50} = 0.035 μM).[2b]

The cytotoxic activity of adenosine analogues such as arabinosyl adenine is limited by deamination to a less active product by adenosine deaminase.[29] Therefore, the possibility was investigated that deamination might limit the cytotoxic activity of 7-aza-TCN (5). The adenosine deaminase inhibitor deoxycoformycin (1 μM)[30] was added to L1210 cell cultures, and 1 hour later 100 μM 7-aza-TCN (5) was added to the deoxycoformycin-treated cultures and to control cultures. Deoxycoformycin had no influence on the effect of 7-aza-TCN (5) on cell growth. Thus, it was concluded that deamination of 7-aza-TCN (5), if it occurred, was not a limitation on its cell growth inhibitory activity.

Another possibility for the lack of cytotoxic activity of 7-aza-TCN (5) in comparison with TCN might be that it is not phosphorylated by cellular kinases, a step that is required for the cytotoxic activity of TCN.[2b] This possibility was examined by testing for the formation of nucleotides from 7-aza-TCN in intact L1210 cells. Cells (2×10^5 mL^{-1}) were incubated 12 h in the presence of 100 μM 7-aza-TCN, and their nucleotides were extracted and analyzed as described in the Experimental Section. No nucleotides of 7-aza-TCN were detected under conditions where the detection limit was approximately 0.1 nmol per 10^6 cells. For comparison L1210 cells (2×10^5 mL^{-1}) were incubated 12 h in the presence of 100 μM TCN, extracted, and analyzed as for the 7-aza-TCN-treated cells. TCN was phosphorylated to the extent of 2.5 nmol of TCN-P per 10^6 cells. (TCN-P was the only metabolite detected in these studies, and in other more extensive studies in our laboratory[31]). Thus, the extent of uptake and phosphorylation, if any, of 7-aza-TCN must be less that 4% of that of TCN. Therefore, a failure of 7-aza-TCN to be phosphorylated by cellular kinases appears to provide at least a partial explanation for its lack of cytotoxicity.

Experimental Section

Chemistry. General Methods. Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Rotary evaporations were conducted with a flash evaporator at less than 50 °C, unless otherwise specified, using a water aspirator (15 mmHg) or a vacuum pump (1 mmHg). Low-pressure chromatography was performed on Instrumentation Specialties Company Model 226 absorbance monitor with optical unit (254 nm), Model 614 chart recorder, and a fraction collector. A Michel-Miller (Ace Glass) column (4 × 30) which was packed with normal-phase silica, EM Reagent Kieselgel 60 (230–400 mesh ASTM), was used as the column unless otherwise specified. Typical flow rates for low-pressure chromatography were 5 mL/min and 20 mL/fraction were collected. Flash chromatography was performed with use of normal-phase silica, EM Reagent Kieselgel 60 (230–400 mesh ASTM), and open-bed chromatography was performed with use of normal phase silica, EM Reagent Kieselgel 60 (70–230 mesh ASTM). All eluant systems are stated as volume to volume ratios. Thin-layer chromatography (TLC) was accomplished with use of SilicAR 7GF (250-μm layer) on prescored glass plates (2.5 × 8 cm) purchased from Analtech, Inc., Newark, DE. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained with use of Bruker WM 360 (360 MHz) or Bruker WP 370 SY (270 MHz) spectrometers. ^1H NMR spectra were recorded with either deuteriochloroform as solvent and tetramethylsilane as internal standard or dimethylsulfoxide-d_6 and tetramethylsilane as internal standard. The following abbreviations were used to designate the multiplicity of individual signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, br s = broad singlet. IR spectra were recorded with use of a Perkin-Elmer 281 spectrophotometer. UV spectra were recorded on a Hewlett-Packard UV 8450 spectrophotometer. The symbol ϵ is defined as the molar extinction coefficient, and values reported as absorbances indicate relative absorbances at the specified wavelengths λ. Mass spectral data were obtained on a Finnigan Model 4025 GC/MS using electron ionization or chemical ionization. Analytical samples were dried in vacuo (vacuum pump) at 78 °C in the presence of phosphorus pentoxide for at least 12 h unless otherwise specified. Elemental analyses were obtained from M-H-W Laboratories, P. O. Box 15853, Phoenix, AZ 85018.

Practical grade 1,1'-thiocarbonyldiimidazole was purchased from Sigma Chemical Co. Absolute dimethyl sulfoxide was prepared by standing over calcium sulfate overnight followed by heating at 80 °C under nitrogen in the presence of calcium hydride for 12 h. The solvent was distilled in vacuo (vacuum pump) from calcium hydride and stored over 4-Å molecular sieves. Absolute acetic anhydride was prepared by heating at reflux temperature with excess magnesium turnings for 6 days, under nitrogen. The solvent was distilled at atmospheric pressure under nitrogen and stored over 4-Å molecular sieves.

8-Amino-4-(methylsulfonyl)-6-*N*-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (3). Nucleoside 2 (21.59 g, 58.8 mmol) was dissolved in DMF (600 mL) at reflux temperature and the solution was then allowed to cool to room temperature. Technical grade (85%) *m*-chloroperoxybenzoic acid (27.79 g, 134.4 mmol) was dissolved in DMF (52 mL), and this solution was added dropwise to a cooled (5 °C) reaction mixture over a period of 30 min. The reaction mixture was then allowed to warm to room temperature and stirred at room temperature for 5 h. The reaction mixture was cooled (5 °C) and cold (5 °C) water (1000 mL) was added. After the mixture was allowed to warm to room temperature, it was extracted with diethyl ether (3 × 600 mL). The aqueous phase was stored at 0 °C for 18 h to give pale yellow crystals which were collected by filtration and washed with water (3 × 50 mL) at 5 °C. The crystals were dried in vacuo (water aspirator) at 58 °C for 12 h to give 3 as yellow crystals (17.33 g, 75%). An analytical sample was prepared by recrystallization from water at reflux temperature to give yellow crystals of 3: mp 235–237 °C; R_f 0.35, $CHCl_3$/MeOH (85/15); ^1H NMR (360 MHz, Me_2SO-d_6) δ 7.05 (br s, 2, NH_2, D_2O exchangeable), 6.00 (d, 1, H-1', $J_{1',2'}$ = 5.0 Hz), 3.65 (s, 3, NMe), 3.36 (s, 3, SO_2Me), 3.5–6.0 (characteristic pattern of peaks for ribose moiety); UV λ_{max} nm ($\epsilon \times 10^{-3}$) (EtOH) 227 (1.27), 288 (0.49), 317 (0.55). Anal. $C_{13}H_{17}N_7O_6S$: C, H, N.

8-Amino-4-hydrazino-6-*N*-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (4). Nucleoside 3 (17.33 g, 43.4 mmol) was dissolved in a mixture of EtOH (1700 mL) and water (850 mL) at reflux temperature and anhydrous hydrazine

(29) Adamson, R. H.; Zaharevitz, D. W.; Johns, D. G. *Pharmacology* **1977**, *15*, 84.

(30) (a) Crabtree, G. W.; Agarwal, R. P.; Parks, R. E., Jr.; Lewis, A. F.; Wotring, L. L.; Townsend, L. B. *Biochem. Pharmacol.* **1979**, *28*, 1491. (b) Wotring, L. L.; Townsend, L. B. *Cancer Res.* **1979**, *39*, 3018.

(31) Wotring, L. L.; Townsend, L. B.; Jones, L. M.; Borysko, K. Z.; Gildersleeve, D. L.; Parker, W. B. *Cancer Res.* **1990**, *50*, 4891.

(2.75 mL, 86.8 mmol) was added dropwise to the reaction mixture. The reaction mixture was heated to reflux temperature for 1.5 h to give a gelatinous mass. After the reaction mixture was cooled to room temperature, water (1500 mL) was added to give a solid which was collected by filtration and washed with room temperature water (3 × 200 mL). The solid was dried in vacuo (water aspirator) at 65 °C to give **4** as a pink solid (14.43 g, 95%). Recrystallization from DMF at reflux temperature afforded the analytical sample of **4** as a white solid: mp 300 °C dec; R_f 0.35, $CHCl_3$/MeOH (75/25); ^1H NMR (360 MHz, Me_2SO-d_6) δ 8.13 (br s, 1, NHN, D_2O exchangeable), 6.40 (br s, 2, NH_2 D_2O exchangeable), 5.84 (d, 1, H-1′, $J_{1',2'}$ = 4.3 Hz), 4.27 (br s, 2, NNH_2, D_2O exchangeable), 3.49 (s, 3, NMe), 3.5–6.0 (characteristic pattern of peaks for a ribose moiety); UV λ_{max} nm (ε × 10^{-3}) (EtOH) 227 (1.27), 288 (0.49), 317 (0.55). Anal. $C_{12}H_{17}N_9O_4$: C, H, N.

8-Amino-6-*N*-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (5). Compound **4** (18.96 g, 54.0 mmol) was dissolved in DMF (965 mL) at 110 °C to give an orange solution. Absolute EtOH (482 mL) was added to the reaction mixture which was then heated to reflux temperature (100 °C). Powdered yellow mercuric oxide (35.10 g, 162 mmol) was added to this reaction mixture in four portions over a period of several hours. After heating the reaction mixture for a total time of 4 h, the hot reaction mixture was filtered through a Celite pad (2 × 15 cm) and the solids were washed with hot (140 °C) DMF (3 × 100 mL). The solvents were evaporated in vacuo (water aspirator) at 50 °C, followed by evaporation in vacuo (vacuum pump) at 60 °C to give an orange solid. This solid was dissolved in DMF (250 mL) at 95 °C and powdered Na_2SO_4 (150 g) was added to the solution. Evaporation of the solvent in vacuo (vacuum pump) at 70 °C gave a slightly wet solid. This solid was coevaporated with *p*-xylene (100 mL) in vacuo (water aspirator) at 70 °C to give a dry solid. This sample was subjected to open-bed chromatography by using a short column (12 × 8.5 cm) of normal-phase silica. $CHCl_3$/MeOH (80/20) was used as eluant and fractions 4–14 (11 × 400 mL) were evaporated in vacuo (water aspirator) followed by evaporation in vacuo (vacuum pump) to give **5** as a yellow solid (7.48 g, 43%). The analytical sample was obtained by a recrystallization from DMF at reflux temperature with H_2O as cosolvent to give **5** as a pale yellow solid: mp 280–281 °C; R_f 0.3, $CHCl_3$/MeOH (85/15); ^1H NMR (270 MHz, Me_2SO-d_6) δ 8.29 (s, 1, ArH), 6.75 (br s, 2, NH_2, D_2O exchangeable), 5.94 (d, 1, H-1′, $J_{1',2'}$ = 5.3 Hz), 5.45 (d, 1, 2′-OH, D_2O exchangeable), 5.19 (d, 1, 3′-OH, D_2O exchangeable), 5.11 (m, 1, 5′-OH, D_2O exchangeable), 4.73 (dd, 1, H-2′), 4.22 (dd, 1, H-3′), 3.95 (m, 1, H-4′), 3.56 (s, 3, NMe), 3.55 (m, 2, H-5′); UV λ_{max} nm (ε × 10^{-3}) (pH 7), 278 (8.55), 316 (13.88), (pH 1) 300 (11.37); (pH 11) (235 (8.53), 278 (7.54), 316 (12.75); EI-mass spectrum, *m/z* 321 (M^+). Anal. $C_{12}H_{15}N_7O_4$: C, H, N.

8-Amino-6-*N*-methyl-2-(3,5-*O*-(tetraisopropyldisiloxandiyl)-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (6). Compound **5** (8.12 g, 25.3 mmol) was suspended in absolute pyridine (500 mL) and the suspension was cooled to 5 °C. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (8.77 mL, 27.8 mmol) was then added to this sealed, cold (5 °C) reaction mixture, and the reaction mixture was stirred at room temperature under a nitrogen atmosphere. After stirring for 3.0 h, the solution was evaporated in vacuo (vacuum pump) at room temperature to give a yellow solid which was dissolved in chloroform (700 mL). This solution was extracted with water (4 × 200 mL) and the chloroform phase was dried with magnesium sulfate. Evaporation of the solvent in vacuo (water aspirator) gave a viscous oil which was dissolved in 200 mL of $CHCl_3$/MeOH (96/4). This sample was subjected to open-bed chromatography on a short column (14.5 × 14.5 cm) of normal-phase silica using $CHCl_3$/MeOH (96/4) as eluant. Fractions 5–10 were combined (6 × 250 mL) and evaporated in vacuo (water aspirator) to give an oil. This oil, in four portions, was subjected to low-pressure chromatography using $CHCl_3$/MeOH (96/4) as the eluant. Fractions 35–50 of each chromatographic separation were combined (16 × 20 mL) and evaporated in vacuo (water aspirator) to give **6** as a white foam (7.13 g, 50%). TLC using the same solvent system showed that this product contained trace impurities but it was used without further purification for the synthesis of **7**. **6**: ^1H NMR (270 MHz, Me_2SO-d_6) δ 8.22 (s, 1 H, ArH), 6.54 (br s, 2, HN_2, D_2O exchangeable), 5.89 (s, 1, H-1′), 5.55 (d, 1, 2′-OH, $J_{2',2'\text{-OH}}$ = 4.6 Hz, D_2O exchangeable), 4.97 (dd, 1, H-3′), 4.72 (m, 1, H-2′), 3.95 (m, 1, H-4′), 3.88 (br s, 2, H-5′), 3.58 (s, 3, NMe), 1.0 (m, 28, *i*-Pr); UV λ_{max} nm (ε × 10^{-3}): (pH 7) 282, 315 (10.3); (pH 1) 305 (7.5); (pH 11) 282, 320 (11.3).

8-Amino-6-*N*-methyl-2-(3,5-*O*-(tetraisopropyldisiloxandiyl)-2-*O*-(1-thiocarbonylimidazolyl-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (7). Compound **6** (1.99 g, 3.5 mmol) and practical grade 1,1′-thiocarbonyldiimidazole (2.77 g, 14 mmol) were dissolved in absolute DMF (20 mL) under a nitrogen atmosphere. The reaction vessel was then sealed and kept under a nitrogen atmosphere. After stirring the mixture at room temperature for 16 h, the reaction mixture was partitioned into ethyl acetate (360 mL) and water (100 mL). The ethyl acetate phase was extracted with water (3 × 100 mL) and dried with magnesium sulfate. The solvent was removed in vacuo (water aspirator) at 35 °C to give a yellow oil which was dissolved in ethyl acetate (25 mL). This solution was filtered, and the filtrate was subjected to low-pressure chromatography using ethyl acetate as eluant. Compound **7** was isolated as a foam (1.0 g, 42%). An analytical sample was prepared by recrystallization from EtOH to give **7** as white crystals: mp 179–181 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 8.44 (s, 1, ArH), 8.39 (d, 1, ImH), 7.68 (m, 1, ImH), 7.09 (m, 1, ImH), 6.51 (s, 1, H-1′), 6.36 (d, 1, H-2′), 5.43 (dd, 1, H-3′), 4.67 (br s, 2, NH_2, D_2O exchangeable), 4.10 (m, 3, H-4′, H-5″, H-5′), 3.73 (s, 3, NMe), 1.05 (m, 28, *i*-Pr). Anal. $C_{28}H_{43}N_9O_5SSi_2$: C, H, N.

8-Amino-6-*N*-methyl-2-(2-deoxy-3,5-*O*-(tetraisopropyldisiloxandiyl)-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (10). Compound **7** (1.20 g, 1.8 mmol) was azeotroped with toluene (200 mL) then dissolved in absolute toluene (25 mL), and the reaction vessel was sealed. *Bis*(trimethylsilyl)acetamide (4.4 mL, 18 mmol) was added to the mixture to give a solution which was stirred at room temperature for 12 h. The mixture was carefully evaporated in vacuo (vacuum pump) at 30 °C to give an oil which was presumed to be **8**. Without further purification, **8** was dissolved in absolute toluene (12 mL) and the reaction vessel was kept under a nitrogen atmosphere. 2,2′-Azobis(2-methylpropionitrile) (195 mg, 1.2 mmol) was added to the reaction mixture, then tri-*n*-butyltin hydride (1.94 mL, 7.2 mmol) in absolute toluene (12 mL) was added dropwise to the reaction mixture at reflux. After heating the mixture for 2.5 h, the solvent was removed in vacuo (water aspirator) at 60 °C followed by evaporation in vacuo (vacuum pump) at 70 °C. This furnished an oil which was assumed to be **9**. This oil was dissolved in EtOH (100 mL) and heated at reflux temperature for 30 min. The solvent was evaporated in vacuo (water aspirator) to give an oil which was dissolved in 10 mL of hexane/ethyl acetate (25/75). This sample was subjected to low-pressure chromatography using normal phase silica and hexane/ethyl acetate (25/75) as eluant. Fractions 30–40 were evaporated to give **10** as a white foam (604 mg, 61%). An analytical sample was obtained by dissolving the product **10** (604 mg), isolated by chromatography, in EtOH (6 mL) at reflux and this was followed by filtration of the solution. Water (1 mL) was then slowly added to the filtrate at reflux until turbidity. The solution was cooled to room temperature and then to ice-bath temperature to give a fine white suspension with a white precipitate. The suspension was decanted, and the precipitate was triturated with cold (ice-bath temperature) aqueous EtOH (50/50) (2 mL). The solvent was decanted and the white precipitate was dried under a stream of nitrogen for 1 h to give an oil which was dried in vacuo (vacuum pump) at 78 °C to give **10** as a white solid: mp 152–153.5; ^1H NMR (270 MHz, Me_2SO-d_6) δ 8.22 (s, 1, ArH), 6.58 (br s, 2, NH_2, D_2O exchangeable), 6.35 (d, 1, H-1′, $J_{1',2'}$ = 7.9 Hz), 5.27 (m, 1, H-3′), 3.75 (m, 3, H-4′, 2 H-5′), 3.57 (s, 3 H, NMe), 2.98 (m, 1, H-2′), 2.60 (m, 1, H-2′), 1.00 (m, 28, *i*-Pr). Anal. $C_{24}H_{41}N_7O_4Si_2$: C, H, N.

8-Amino-6-*N*-methyl-2-(2-deoxy-β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (11). Compound **10** (548 mg, 1.0 mmol) was dissolved in THF (12 mL). Tetra-*n*-butylammonium fluoride (3.0 mL, 3.0 mmol) as a 1.0 M THF solution was added dropwise in three portions over a period of 2 h to the reaction mixture which was stirred at room temperature. After stirring at room temperature for an additional 2 h, the clear solution was evaporated in vacuo (water aspirator) to give an oil which was then suspended in $CHCl_3$ (10 mL). The solvent was evaporated in vacuo (water aspirator) to give a yellow solid which

was triturated with $CHCl_3$ (5 × 5 mL) to give a light green solid. This solid was washed with H_2O (5 mL) at room temperature and the solid was then air dried. After dissolving the solid in DMF (5 mL) at 50 °C, the sample was absorbed onto powdered Na_2SO_4 (4.0 g) by evaporation in vacuo (vacuum pump) followed by coevaporation with *p*-xylene (10 mL) in vacuo (water aspirator) at 60 °C. This sample was subjected to low-pressure chromatography using normal-phase silica and $CHCl_3$/MeOH (85/15). Fractions 30–45 were evaporated in vacuo (water aspirator) to give a white solid (192 mg, 63%). The analytical sample was prepared by dissolving the product, isolated by chromatography, in MeOH (50 mL) at reflux temperature. The solution was filtered and the solvent was evaporated in vacuo (water aspirator) to give a solid which was triturated with cold (ice temperature) MeOH (5 mL). The solid was filtered and washed with cold (ice temperature) MeOH (5 × 2 mL) and dried in vacuo (vacuum pump) to give **11** as a white solid: mp 244.5–245 °C; ^1H NMR (270 MHz, Me_2SO-d_6): δ 8.28 (s, 1, ArH), 6.71 (br s, 2, NH_2, D_2O exchangeable), 6.40 (t, 1, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.28 (d, 1, 3′-OH, D_2O exchangeable), 4.96 (dd, 1, 5′-OH), 4.47 (m, 1, H-3′), 3.85 (m, 1, H-4′), 3.57 (s, 3, NMe), 3.58 (m, 1, H-5′), 3.42 (m, 1, H-5′), 2.96 (m, 1, H-2′), 2.30 (m, 1, H-2′); UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 7) 283, 314 (12.3); (pH 1) 300 (9.5); (pH 11) 226 (12.0), 283, 314 (12.11). Anal. $C_{12}H_{15}N_7O_3$: C, H, N.

8-Amino-6-*N*-methyl-2-(3,5-*O*-(tetraisopropyldisiloxandiyl)-β-D-arabinofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthylene (13). Compound **6** (563.8 mg, 1.0 mmol) was added to a flame-dried reaction flask, and the flask was sealed. Absolute dimethyl sulfoxide (4 mL) was added to **6** via a syringe and this solution was cooled to 0 °C to give a slurry. Absolute acetic anhydride (0.5 mL, 5.3 mmol) was then added by a syringe to the cold, sealed reaction mixture. The mixture was allowed to slowly warm to room temperature, and stirring for 21 h at room temperature produced a yellow suspension. A TLC of the reaction mixture using ethyl acetate as eluant showed that no starting material remained at R_f 0.6 and that there was only one major spot present at R_f 0.5 which gave a positive 2,4-dinitrophenylhydrazone test. The reaction mixture was cooled to 0 °C and sodium borohydride (231 mg, 6.1 mmol), as an ethanolic (12.5 mL) solution, was added dropwise over a 40-min period to the cold reaction mixture. The reaction mixture was stirred at 0 °C for 1.5 h. At this time, TLC using ethyl acetate as eluant showed only one major spot which gave a negative 2,4-dinitrophenylhydrazone test. A cold (0 °C) aqueous solution of saturated sodium chloride (15 mL) was added to the cold (0 °C) reaction mixture. The reaction mixture was then extracted with ethyl acetate (3 × 15 mL), and the organic phases were combined and dried with magnesium sulfate. The solids were collected by filtration, and the filtrate was evaporated in vacuo (water aspirator) at 60 °C followed by evaporation in vacuo (vacuum pump) at 60 °C to give an oil (2.57 g). This oil was almost completely dissolved in ethyl acetate (10 mL) and the fine suspension was filtered. The filtrate was subjected to low-pressure chromatography using hexane/ethyl acetate (25/75) (1640 mL) followed by ethyl acetate (750 mL) as eluants. Fractions 86–97 (12 × 30 mL) were combined and evaporated in vacuo (water aspirator) followed by evaporation in vacuo (vacuum pump) at 60 °C to give a pale yellow foam (190 mg, 34%). An analytical sample was prepared by dissolving the product in ethyl acetate at room temperature, and this solution was filtered and the filtrate evaporated in vacuo (water aspirator) at 50 °C to give a foam. This foam was pulverized and dried in vacuo (vacuum pump) at the reflux temperature of acetone in the presence of phosphorous pentoxide to give **13** as a yellow solid: mp > 60 °C; ^1H NMR (270 MHz, Me_2SO-d_6) δ 8.19 (s, 1, ArH), 6.44 (br s, 2, NH_2, D_2O exchangeable), 6.16 (d, 1, H-1′, $J_{1',2'}$ = 7.1 Hz), 5.60 (d, 1, 2′-OH, D_2O exchangeable), 5.18 (m, 1, H-3′), 4.54 (dd, 1, H-2′), 4.31 (m, 1, H-4′), 3.83 (m, 2, H-5′), 3.57 (s, 3, NCH_3), 1.18 (m, 28, Si-*i*-Pr); R_f 0.25, hexane/ethyl acetate (25/75). Anal. $C_{24}H_{41}N_7O_5Si_2$: C, H, N.

8-Amino-6-*N*-methyl-2-β-D-arabinofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (14). Compound **6** (1.128 g, 2.0 mmol) was added to a flame-dried reaction flask, and the flask was sealed. Absolute DMSO (8 mL) was added to **6** via a syringe and this solution was cooled to 0 °C to give a slurry. Absolute Ac_2O (1.0 mL, 10.6 mmol) was then added by syringe to the cold, sealed reaction mixture. The mixture was allowed to slowly warm to room temperature and the reaction mixture stirred for 24 h at room temperature to produce a yellow suspension. A TLC of the reaction mixture, using hexane/EtOAc (25/75) as solvent, showed that no starting material remained at R_f 0.35. There was only one major spot present (R_f 0.5) as determined by TLC. The spot at R_f 0.5 gave a positive 2,4-dinitrophenylhydrazone test. The reaction mixture was cooled to 0 °C and sodium borohydride (462 mg, 12.2 mmol), as an ethanolic (25 mL) solution, was added dropwise over a 20-min period to the cold reaction mixture. The reaction mixture was stirred at 0 °C for 2 h. A TLC of the reaction mixture, using hexane/EtOAc (25/75) as eluant, showed only one major spot at R_f 0.3 which gave a negative 2,4-dinitrophenylhydrazine test. There was a trace spot at R_f 0.5 which corresponded to the 2′-keto starting material and a trace spot at R_f 0.15. A cold (0 °C) aqueous solution, saturated with NaCl (30 mL), was added to the cold (0 °C) reaction mixture, and the reaction mixture was extracted with EtOAc (30 mL). The aqueous phase was extracted twice more with EtOAc (2 × 20 mL), and the organic phases were combined and evaporated in vacuo (water aspirator) to give a foam. The crude product was dissolved in THF (50 mL) and 1 M tetra-*n*-butylammonium fluoride (4 mL, 4 mmol) as a THF solution was slowly added to the reaction mixture. After the reaction mixture was stirred for 16 h at room temperature, additional 1 M tetra-*n*-butylammonium fluoride (4 mL, 4 mmol) was added as a THF solution, and the reaction mixture was stirred at room temperature for 6 h. The solution was evaporated in vacuo (water aspirator) to give an oil. Water (125 mL) was then added to this oil to give a suspension. This suspension was extracted with EtOAc (3 × 50 mL), the aqueous phase was evaporated in vacuo (water aspirator) and then evaporated in vacuo (vacuum pump) to give a yellow solid (12.27 g). This solid was dissolved in H_2O at 60 °C and pulverized Na_2SO_4 (6.5 g) was added to the solution. The solution was evaporated in vacuo (vacuum pump) at 70 °C to give a pale-yellow solid. This solid was subjected to low-pressure chromatography using $CHCl_3$/MeOH (85/15). Fractions 33–53 (21 × 20 mL) were evaporated in vacuo (water aspirator) and then evaporated in vacuo (vacuum pump) at 60 °C to give a yellow solid (872 mg). This solid was dissolved in H_2O (40 mL) at 95 °C, the solution was filtered, and the filtrate was evaporated in vacuo (vacuum pump) at 60 °C to give a gelatinous mass. This solid was triturated with MeOH (10 mL) at reflux temperature, and the suspension was cooled to 0 °C. The solid was collected by filtration and washed with MeOH at 0 °C (4 × 3 mL) to give a pale yellow solid. This solid was dried in vacuo (water aspirator) at 40 °C for 12 h to give an analytical sample of **14** as a pale yellow solid (294 mg, 45%): mp 261–263 °C dec; R_f 0.3, $CHCl_3$/MeOH (85/15); ^1H NMR (270 MHz, Me_2SO-d_6) δ 8.26 (s, 1, ArH), 6.66 (br s, 2, NH_2, D_2O exchangeable), 6.22 (d, 1, H-1′, $J_{1',2'}$ = 6.2 Hz), 5.41 (d, 1, OH, D_2O exchangeable), 5.39 (s, 1, OH, D_2O exchangeable), 5.02 (m, 1, 5′-OH, D_2O exchangeable), 4.36 (m, 2, H-2′, H-3′), 3.77 (m, 3, 4′-H, 5′-H), 3.58 (s, 3, NCH_3); UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 7) 313 (11.22); (pH 1) 302 (8.34); (pH 11) 226 (10.16), 313 (10.90). Anal. $C_{12}H_{15}N_7O_4$: C, H, N.

The filtrate from the trituration of the analytical sample with MeOH (10 mL) was evaporated in vacuo (water aspirator) to give a residue. This residue was coevaporated with toluene (2 × 50 mL) in vacuo (water aspirator) at 60 °C to give a residue which was triturated with EtOH (10 mL) at room temperature. The resulting solid was filtered and dried at room temperature for 6 h to give additional **14** as a beige solid (60 mg, 9%).

Biological Studies. Chemicals. Deoxycoformycin was kindly provided by the Division of Cancer Treatment, Drug Research and Development, National Cancer Institute.

Cell Culture. The in vitro cytotoxicity against L1210 cells was evaluated as described previously.[30b] L1210 cells were grown in static suspension culture with Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the presence of the indicated concentrations of the test compound, by counting the cells once each day. Growth rate was defined as the slope of the plot of the log of the cell number against time for a treated culture, as a percentage of the slope for the control culture. Experimentally, this parameter was determined by calulating the ratio of the population doubling time of control cells (average, 12 h) to the population doubling time of treated cells. The IC_{50} was defined as the concentration re-

quired to reduce the growth rate to 50% of the control.

The in vitro cytotoxicity against H. Ep. 2 human epidermoid carcinoma cells (ATCC CCL23) was evaluated in monolayer cultures. H. Ep. 2 cells were maintained in exponential growth in Basal Medium of Eagle with Hanks' salts (BME) (GIBCO Laboratories, Grand Island, NY) and 0.5 g of $NaHCO_3$ per L, supplemented with 15% heat inactivated (56 °C, 30 min) bovine calf serum (Hyclone Laboratories, Logan, UT). Cells were harvested from the surface of culture flasks, for subculturing or for counting, by using 0.005% trypsin (2 × crystalline, 3080 NF units/mg, GIBCO Laboratories, Grand Island, NY) and 0.1% EDTA, disodium salt, in BME without serum. For growth rate determinations, 2×10^4 cells were placed in replicate 25 cm^2 flasks, in control medium. After 1 day of incubation the medium was changed to compound-containing medium. Then growth was monitored by harvesting and counting the cells in two flasks from each treatment group, on days 1, 2, and 4 after adding the compounds. The data was plotted and analyzed as described previously[30b] and above for L1210 cells. The average control population doubling time was 19 h.

Nucleotide Extractions. L1210 cells were incubated as described in the Results and Discussion and the neutralized perchloric acid extracts were prepared as described previously.[2b] The nucleotides were separated by anion exchange chromatography (HPLC) as described previously[32] except that the pH of both elution buffers was 4.75 for studies on 7-aza-TCN and 4.65 for TCN. The UV absorbance of the eluate was monitored at 254 nm for detection of natural nucleotides, and at 312 nm (λ_{max} at pH 4.75) for detection of 7-aza-TCN nucleotides or 292 nm (λ_{max} at pH 4.65) for detection of TCN nucleotides.

Acknowledgment. This investigation was supported in part by research grants CH-299 and CH-312 from the American Cancer Society, in part by funds from the Department of Health and Human Services (Grant No. AI-25739), and in part by a grant to L.L.W. from the Office of the Vice President for Research, the University of Michigan. The authors (L.B.T.) would also like to thank Warner-Lambert/Parke-Davis for a grant for graduate student research support and Ms. Rae Miller for her expert preparation of the manuscript. The authors thank K. Z. Borysko, R. D. Newcomb, and T. J. Franks for their expert technical assistance.

Registry No. **2**, 125284-49-3; **3**, 129572-01-6; **4**, 129572-02-7; **5**, 109947-70-8; **6**, 129572-03-8; **7**, 129572-04-9; **10**, 129572-05-0; **11**, 129572-06-1; **13**, 129572-07-2; **14**, 129572-08-3.

(32) Wotring, L. L.; Townsend, L. B. *Cancer Res.* **1989**, *49*, 289.

Synthesis and Biochemical Evaluation of Tritium-Labeled 1-Methyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxamide, a Useful Radioligand for $5HT_3$ Receptors

David W. Robertson,* William Bloomquist, Marlene L. Cohen, Leroy R. Reid, Kathryn Schenck, and David T. Wong

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285.
Received April 17, 1990

The advent of potent, highly selective $5HT_3$ receptor antagonists has stimulated considerable interest in $5HT_3$ receptor mediated physiology and pharmacology. To permit detailed biochemical studies regarding interaction of the indazole class of serotonin (5HT) antagonists with $5HT_3$ receptors in multiple tissues, we synthesized 1-methyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxamide (LY278584, compound **9**) in high specific activity, tritium-labeled form. This radioligand was selected as a synthetic target because of its potency as a $5HT_3$-receptor antagonist, its selectivity for this receptor viz a viz other 5HT-receptor subtypes, and the ability to readily incorporate three tritia via the indazole *N*-CH_3 substituent. Alkylation of *N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxamide (**8**) with sodium hydride and tritium-labeled iodomethane, followed by HPLC purification, resulted in [^{3}H]-**9** with a radiochemical purity of 99% and a specific activity of 80.5 Ci/mmol. This radioligand bound with high affinity to a single class of saturable recognition sites in membranes isolated from cerebral cortex of rat brain. The K_d was 0.69 nM and the B_{max} was 16.9 fmol/mg of protein. The specific binding was excellent, and accounted for 83–93% of total binding at concentrations of 2 nM or less. The potencies of known $5HT_3$-receptor antagonists as inhibitors of [^{3}H]-**9** binding correlated well with their pharmacological receptor affinities as antagonists of 5HT-induced decreases in heart rate and contraction of guinea pig ileum, suggesting the central recognition site for this radioligand may be extremely similar to or identical with peripheral $5HT_3$ receptors.

Introduction

Although pharmacological data have long suggested that subtypes of serotonin (5-hydroxytryptamine, 5HT) receptors exist, the seminal work of Peroutka and Snyder provided biochemical evidence for the existence of $5HT_1$- and $5HT_2$-receptor subtypes.[1] During the past decade an explosion of information has emerged regarding the multiple molecular forms of 5HT receptors, and at least six subtypes are now accepted.[2]

The $5HT_3$-receptor subtype has attracted considerable attention recently, and our understanding of these receptors has increased dramatically over the past few years because of the discovery and widespread availability of potent and selective antagonists of these receptors.[3] The structures of some of the better characterized $5HT_3$-receptor antagonists are displayed in Chart I. These agents produce a variety of peripheral and central pharmacological effects in laboratory animals, and are being studied in man for the treatment of gastrointestinal motility disorders, migraine pain, chemotherapy-induced emesis, schizophrenia, anxiety, substance abuse, and other central nervous system (CNS) disorders.[3] Interest in the CNS

(1) Peroutka, S. J.; Snyder, S. H. *Mol. Pharmacol.* **1979**, *16*, 687.
(2) Robertson, D. W.; Fuller, R. W. *Annu. Rep. Med. Chem.* **1988**, *23*, 49.
(3) Fozard, J. R. In *The peripheral actions of 5-Hydroxytryptamine*; Fozard, J. R., Ed.; Oxford Medical Publications: Oxford, 1989; p 354.

0022-2623/90/1833-3176$02.50/0