Imidazo[4,5-c]pyridines (3-Deazapurines) and Their Nucleosides as Immunosuppressive and Antiinflammatory Agents

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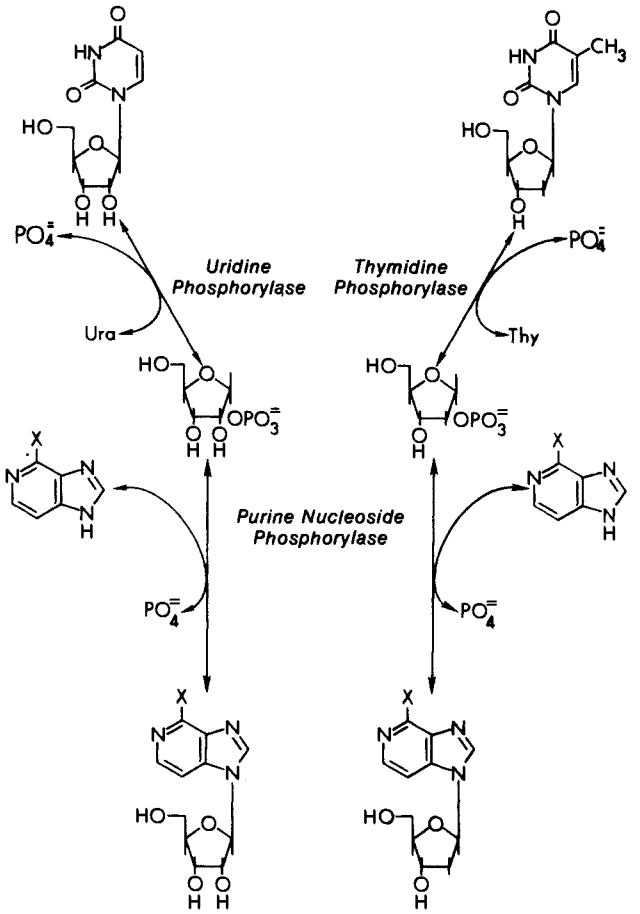
A variety of imidazo[4,5-c]pyridines (3-deazapurines) were synthesized. With use of these aglycons as pentosyl acceptors, the corresponding ribonucleosides and 2'-deoxyribonucleosides were prepared by an enzymatic method involving transfer of the pentosyl moiety from appropriate pyrimidine nucleosides. With most of the imidazo[4,5-c]pyridines, the products obtained from the enzyme-catalyzed reactions were pentosylated exclusively in the 1-position. However, some 3-pentosylation occurred with aglycons that had H or N_3 in the 4-position. In addition to the 2'-deoxy congener of the ribonucleoside of 4-amino-1*H*-imidazo[4,5-c]pyridine, the 5'-deoxy and 2',5'-dideoxy congeners were synthesized. All of the aglycons and their nucleosides were tested for toxicity to mammalian cells in culture. None were markedly cytotoxic. These compounds were also evaluated for their ability to inhibit lymphocyte-mediated cytolysis in vitro. 3-Deazaadenosine (**23**) and its 2'-deoxy congener (**38**) were the most potent inhibitors (ED_{50} = 20 μM). In addition to these two in vitro tests, in vivo inhibition of the inflammatory response in the rat carregeenan pleurisy model was determined. 3-Deazaadenosine (**23**) was the most potent compound (ED_{50} = 3 mg/kg) in this in vivo test.

The synthesis of 4-amino-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-c]pyridine (**23**) (3-deazaadenosine) was first described in 1966.[1] As synthetic methods were gradually improved,[2] larger amounts of this nucleoside became more widely available for biological testing. Antiviral[3] and antimalarial[4] activities were reported. Much attention has been focused on the finding that this adenosine analogue (**23**) acts as an alternate substrate inhibitor of *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1).[5] The direct consequence of the interaction of **23** with this enzyme is the accumulation of *S*-adenosyl-L-homocysteine and its imidazo[4,5-c]pyridine analogue. These 5'-substituted nucleoside derivatives inhibit a variety of *S*-adenosylmethionine-dependent methyltransferases.[6] It is conceivable that such biochemical effects ultimately influence a variety of specialized cellular functions which seem to involve methylation reactions.[7] However, recent evidence[8] indicates that the inhibition of lymphocyte-mediated cytolysis in vitro by **23** does not necessarily involve *S*-adenosylhomocysteine hydrolase.

The interest of this laboratory in imidazo[4,5-c]pyridines and their nucleosides intensified after it was found that

Scheme I. Enzymatic Synthesis of Ribonucleosides and 2-Deoxyribonucleosides of Imidazo[4,5-c]pyridines



23 was active in an in vivo model for acute inflammation.[9] In order to facilitate the synthesis of a variety of closely related nucleosides, a method involving nucleoside phosphorylases as catalysts was applied to the pentosylation of a variety of imidazo[4,5-c]pyridines.[10] This paper describes the syntheses of these aglycons and their nucleosides. The biological evaluation presented involves one in vivo and two in vitro tests. The latter were for cyto-

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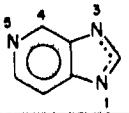
Table I. Biological Activities of Imidazo[4,5-*c*]pyridines and Their Nucleosides



compd	substit[a] 1	4	other	cell growth: % inhibn at 100 μM D-98 cells	L cells	lymphocyte-mediated cytolysis: % inhibn at 100 μM	carrageenan pleural exudate: ED_{50},[b] mg/kg
1	H	H		0	12	<5	>9
2	H	H	5-*N*-oxide	3	1	5	>9
3	H	Cl		6	29	<5	>9
4	H	SH		26	38	<5	8
5	H	SCH_3		4	11	<5	9
6	H	$SCH_2C_6H_5$		26	54	20	>9
7	H	NH_2		60	24	<5	8
8	H	$NHCH_3$		18	6	<5	inactive at 6
9	H	$NHCH_2CH_3$		3	11	<5	inactive at 6
10	H	$NHCH_2CH_2OH$		3	16	<5	>9
11	H	$NH(CH_2)_5CH_3$		53	82	100[c]	>9
12	H	NHC_6H_5		12	34	26	inactive at 9
13	H	$NHCH_2C_6H_5$		75	68	57	9
14	H	N_3		20	20	<5	inactive at 9
15	H	$N(CH_3)_2$		8	21	25	9
16	Rib	H		13	8	<5	inactive at 9
17	Rib	H	(17% 3-Rib)[d]	1	3	<5	inactive at 9
18	Rib	H	5-*N*-oxide (29% 3-Rib)[d]	5	3	<5	inactive at 9
19	Rib	Cl		76	27	<5	inactive at 9
20	Rib	SH		6	3	<5	9
21	Rib	SCH_3		24	40	<5	inactive at 9
22	Rib	$SCH_2C_6H_5$		30	43	30	9
23	Rib	NH_2		56	49	80	3
24	Rib	$NHCH_3$		8	21	11	inactive at 6
25	Rib	$NHCH_2CH_3$		4	20	8	inactive at 6
26	Rib	$NHCH_2CH_2OH$		0	3	6	inactive at 6
27	Rib	$NH(CH_2)_5CH_3$		8	41	14	inactive at 6
28	Rib	NHC_6H_5		4	18	10	>9
29	Rib	$NHCH_2C_6H_5$		32	38	5	>9
30	Rib	N_3		4	8	<5	>9
31	Rib	$N(CH_3)_2$		0	14	10	>9
32	2′-dRib	H	(75% 3-2′-dRib)[d]	6	0	<5	>9
33	2′-dRib	H	5-*N*-oxide (50% 3-2′-dRib)[d]	0	13	<5	inactive at 9
34	2′-dRib	Cl		5	25	<5	inactive at 9
35	2′-dRib	SH		10	8	<5	inactive at 9
36	2′-dRib	SCH_3		36	30	<5	>9
37	2′-dRib	$SCH_2C_6H_5$		18	19	11	inactive at 9
38	2′-dRib	NH_2		18	0	89	10
39	2′-dRib	$NHCH_2C_6H_5$		15	37	8	>9
40	2′-dRib	N_3	(38% 3-2′-dRib)[d]	4	12	13	inactive at 9
41		N_3	3-2′-dRib	14	2	<5	>9
42	2′-dRib	$N(CH_3)_2$		0	6	>5	>9
43	5′-dRib	NH_2		18	4	32	inactive at 9
44	2′,5′-ddRib	NH_2		3	12	25	inactive at 9

[a] Rib = β-D-ribofuranosyl; 2′-dRib = 2-deoxy-β-D-ribofuranosyl; 5′-dRib = 5-deoxy-β-D-ribofuranosyl; 2′,5′-ddRib = 2′,5′-dideoxy-β-D-ribofuranosyl. [b] Inactive = less than 15% inhibition at the indicated dose; > = more than 15% but less than 45% inhibition at the indicated dose. [c] At 50 μM, 45% inhibition; at 26 μM 0% inhibition. [d] NMR spectra indicate that these samples were mixtures of the 1- and 3-substituted isomers. Shifts used to identify isomers were as follows. For **17**, 1-isomer: H_4 at δ 8.98 (d, $J_{4,7}$ = 0.8 Hz), H_7 at δ 7.84 (dd, $J_{4,7}$ = 0.8 Hz, $J_{6,7}$ = 5.6 Hz); 3-isomer: H_4 at δ 9.16 (d, $J_{4,7}$ = 1.0 Hz), H_7 at δ 7.73 (dd, $J_{4,7}$ = 1.0 Hz, $J_{6,7}$ = 5.7 Hz). For **18**, 1-isomer: H_4 at δ 8.73 (d), H_7 at δ 7.88 (dd, $J_{4,7}$ = 0.4 Hz, $J_{6,7}$ = 7.0 Hz); 3-isomer: H_4 at δ 9.02 (d), H_7 at δ 7.69 (dd, $J_{6,7}$ = 7.0 Hz), $J_{4,7}$ was too small to measure. For **32**, 1-isomer: H_4 at δ 8.97 (d, $J_{4,7}$ = 1.0 Hz), H_7 at δ 7.83 (dd, $J_{6,7}$ = 5.6 Hz); 3-isomer: H_4 at δ 9.12 (d, $J_{4,7}$ = 1.0 Hz), H_7 at δ 7.68 (dd, $J_{6,7}$ = 5.6 Hz). For **33**, 1-isomer: H_4 at δ 8.68 (d, $J_{4,6}$ = 1.2 Hz), H_7 at δ 7.65 (dd, $J_{4,7}$ = 0.4 Hz, $J_{6,7}$ = 7.0 Hz); 3-isomer: H_4 at δ 8.95 (d, $J_{4,7}$ = 0.5 Hz), H_7 at δ 7.83 (dd, $J_{4,7}$ = 0.5 Hz, $J_{6,7}$ = 7.0 Hz). For **40**, 1-isomer: H_2 at δ 8.77, H_7 at δ 7.98 (d, $J_{6,7}$ = 7.4 Hz), 1′H at δ 6.53; 3-isomer: H_2 at δ 8.89, H_7 at δ 7.78 (d, $J_{6,7}$ = 7.3 Hz), 1′H at δ 6.85.

toxicity to mammalian cells and inhibition of lymphocyte-mediated cytolysis. The former was the rat carrageenan pleurisy model for antiinflammatory activity.

Results and Discussion

Chemistry. Some of the imidazo[4,5-*c*]pyridines (**1**,[11] **2**,[12] **3**,[12,13] **4**,[13c,14] and **5**[14b]) listed in Table I were synthesized by procedures similar to those described in the literature. Compound **6** was synthesized by the alkylation of 4-

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Table II. Synthetic Data and Physical Constants for Some Nucleosides of Imidazo[4,5-*c*]pyridines

compd	formula	yield,[a] %	mp,[b] °C	$[\alpha]^{20}_D$, deg (*c*, DMF)	anal.
16	$C_{11}H_{13}N_3O_4$	23	200–204	−49.2 (0.5)	C, H, N
17	$C_{11}H_{13}N_3O_4 \cdot 0.75H_2O$	64	195–197	−57.7 (1.0)	C, H, N
18	$C_{11}H_3N_3O_5$	43	235–240 dec	−74.2 (0.1)	C, H, N
19	$C_{11}H_{12}ClN_3O_4$	57	198–200	−41.7 (1.0)	C, H, N, Cl
20	$C_{11}H_{13}N_3O_4S$	45	170–180 dec	−71.1 (1.0)	C, H, N, S
21	$C_{12}H_{15}N_3O_4S$	73	194–196	−55.8 (1.0)	C, H, N, S
22	$C_{18}H_{19}N_3O_4S$	72	160–165	−52.6 (1.0)	C, H, N, S
23	$C_{11}H_{14}N_4O_4$	79	226	−63.4 (1.0)	C, H, N
24	$C_{12}H_{16}N_4O_4$	64	170	−61.9 (1.0)	C, H, N
25	$C_{13}H_{18}N_4O_4$	55	178	−59.6 (1.0)	C, H, N
26	$C_{13}H_{18}N_4O_5$	34	163–165	−54.6 (0.5)	C, H, N
27	$C_{17}H_{26}N_4O_4$	74	163	−48.2 (1.0)	C, H, N
28	$C_{17}H_{18}N_4O_4$	37	113	−41.8 (0.5)	C, H, N
29	$C_{18}H_{20}N_4O_4 \cdot H_2O$	72	172 dec	−41.8 (0.5)	C, H, N
30	$C_{11}H_{12}N_6O_4$	36	217–219	−14.2 (1.0)	C, H, N
31	$C_{13}H_{18}N_4O_4 \cdot H_2O$	18	154–155	−52.0 (1.0)	C, H, N
32	$C_{11}H_{13}N_3O_3$	26	159–161	−34.2 (1.0)	C, H, N
33	$C_{11}H_{13}N_3O_4 \cdot 0.75H_2O$	12	170	−42.2 (1.0)	C, H, N
34	$C_{11}H_{12}ClN_3O_3$	85	162–165	−24.6 (1.0)	C, H, N, Cl
35	$C_{11}H_{13}N_3O_3S \cdot H_2O$	36	170	−23.0 (0.5)	C, H, N, S
36	$C_{12}H_{15}N_3O_3S \cdot H_2O$	71	179–183	−23.5 (1.0)	C, H, N, S
37	$C_{18}H_{19}N_3O_3S$	19	155–157	−23.5 (1.0)	C, H, N, S
38	$C_{11}H_{14}N_4O_3 \cdot 0.3H_2O$	70	215 dec	−25.5 (1.0)	C, H, N
39	$C_{18}H_{20}N_4O_3 \cdot 1.5H_2O$	32	95	−10.0 (0.1)	C, H, N
40	$C_{11}H_{12}N_6O_3 \cdot 0.25H_2O$	19[c]	>250 dec	−12.8 (0.5)	C, H, N
41	$C_{11}H_{12}N_6O_3 \cdot 0.50H_2O$	11[c]	187	+16.4 (0.5)	C, H, N
42	$C_{13}H_{18}N_4O_3 \cdot H_2O$	40	125–130	−23.0 (1.0)	C, H, N
43	$C_{11}H_{14}N_4O_3 \cdot H_2O$	22	140	−58.9 (0.5)	C, H, N
44	$C_{11}H_{14}N_4O_2$	17	180–181	−37.6 (0.5)	C, H, N

[a] Yield was calculated on the basis of the amount of the imidazo[4,5-*c*]pyridine used. [b] dec = decomposition point. Literature values for **16**, 204–205 °C[2b], 198–199 °C dec;[15] **19**, 200–201 °C[2a], 189–190 °C;[15] **20**, 155–160 °C;[16] **21**, 195 °C;[16] **23**, 225–226 °C,[1,2a] 229–231 °C;[2b,c] **31**, 179–180 °C;[6a] **34**, 173–175 °C;[17b] **38**, 209–211 °C.[17b] [c] Yields from the same reaction mixture; total yield of both isomers was 30%.

mercapto-1*H*-imidazo[4,5-*c*]pyridine (**4**) with benzyl bromide. Compounds **8–15** were obtained by the displacement of the 4-chloro group of **3** with the appropriate amine or lithium azide. 4-Amino-1*H*-imidazo[4,5-*c*]-pyridine (**7**)[12,13b,13d,14a] was synthesized by a novel route involving the reduction of the 4-azido compound **14** under strongly acidic conditions that favored the azido over the tetrazolo[15] tautomer.

Earlier syntheses of some of the nucleosides listed in Table I utilized conventional synthetic procedures (**16**,[2b,16] **19**,[2a,16] **20**,[17] **21**,[17] **23**,[1,2] **31**,[6a] **34**,[18] **38**[10e,18b]). In this study, the nucleosides listed in Table I were synthesized by an enzymatic method previously used with purines[10a] and pyrazolo[3,4-*d*]pyrimidines.[19] Each imidazo[4,5-*c*]pyridine was pentosylated by reaction with α-D-ribose 1-phosphate or with a deoxy analogue. The enzyme catalyst was purine nucleoside phosphorylase (EC 2.4.2.1). The pentose 1-phosphate esters were generated in situ by the enzyme-catalyzed phosphorolysis of uridine, thymidine, or an appropriate congener. The catalyst for the phosphorolysis was either uridine phosphorylase (EC 2.4.2.3) or thymidine phosphorylase (EC 2.4.2.4). This enzyme-dependent synthetic route is outline in Scheme I.

The nucleoside products of the enzyme-catalyzed reactions were, in most instances, pentosylated exclusively at the 1-position of the imidazo[4,5-*c*]pyridine. With those imidazo[4,5-*c*]pyridines which lacked a substituent in the 4-position (**1**, **2**), both the 1- and 3-pentosyl isomers were formed. Attempts to separate these isomers were unsuccessful and isolated products (**17**, **18**, **32**, **33**) were mixtures as indicated by NMR spectra. Synthesis of the 1-isomer of the ribonucleoside **16**[2b,16] of unsubstituted imidazo-[4,5-*c*]pyridine was achieved by reductive dechlorination of **19**.

The extent of enzymatic 3-isomer formation was greater with the 2′-deoxyribonucleosides than with the ribonucleosides. With 4-azido-1*H*-imidazo[4,5-*c*]pyridine (**14**), two batches of 2′-deoxyribonucleoside were isolated; one (**40**) consisted of a mixture of the 1- and 3-isomers (62:38) and the other was the pure 3-isomer (**41**). The inability of the azido substituent to act as a directing group contrasts to the findings with other 4-substituents. This might be related to the coplanarity of the azido substituent with the imidazo[4,5-*c*]pyridine ring and/or its ability to form a tricyclic structural isomer involving the 4- and 5-positions of the parent ring (1*H*-imidazo[4,5-*c*]tetrazolo[1,5-*a*]-pyridine).[15]

The importance of the 4-substituent in determining the position of enzymatic pentosylation with the imidazo-[4,5-*c*]pyridines is not seen with purines. For example, synthesis of the 2′-deoxyribonucleoside of unsubstituted purine by this enzymatic method resulted in a product whose NMR spectra had no detectable 7-isomer present (unpublished data, G. W. Koszalka and T. A. Krenitsky).

In addition to the 2′-deoxyribonucleoside **38**, the two additional deoxyribonucleosides of 4-amino-1*H*-imidazo-

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[4,5-c]pyridine were synthesized by the enzymatic method. The pentosyl donor for the synthesis of the 5'-deoxyribonucleoside **43** was 5'-deoxyuridine[20] and the phosphorolytic catalyst was uridine phosphorylase; that for the 2',5'-dideoxyribonucleoside **44** was the corresponding thymidine analogue[21] and the catalyst was thymidine phosphorylase.

Yields and some physical characterization of all the nucleosides synthesized are provided in Table II.

Biology. All of the imidazo[4,5-c]pyridines and their nucleosides were relatively nontoxic to cultured human D-98 and mouse L cells (Table I). Of the aglycons studied (**1–15**), only the 4-hexylamino (**11**) and the 4-benzylamino compounds (**13**), showed more than 50% inhibition of growth of both cell lines at 100 μM. In general, the cytotoxicities of the ribonucleosides **16–31** were not markedly different from those of the aglycons. The deoxyribonucleosides **32–44** were generally less toxic than the corresponding aglycons or ribonucleosides.

The lymphocyte-mediated cytolysis test was designed to assess the ability of compounds to inhibit the cellular interactions that result in the lysis of antigen-bearing target cells in vitro.[22] This "killer cell" phenomenon is thought to play a role in tumor and graft rejection.[23] Inhibition of lymphocyte-mediated cytolysis by the aglycons **1–15** was generally not detectable or weak (Table I). Only the 4-hexylamino and 4-benzylamino congeners **11** and **13**, respectively, the most cytotoxic aglycons, inhibited cell lysis more than 50% at 100 μM. The ribonucleosides **16–31** were generally inactive or weakly inhibitory except for the adenosine analogue **23**. The congener with a benzylthio substituent in the 4-position (**22**) ranked second in potency among the ribonucleosides. Of the deoxyribonucleosides tested, the 2'-deoxyadenosine analogue **38** was the most active. It was similar in potency to the corresponding ribonucleoside **23**. On titration, **23** and **38** both gave an ED_{50} of 20 μM. The corresponding 5'-deoxyribonucleoside **43** and 2',5'-dideoxyribonucleoside **44** were less potent.

The carrageenan pleurisy test was designed to evaluate compounds for antiinflammatory activity.[24] In this in vivo test, some of the aglycons listed in Table I had appreciable activity. Imidazo[4,5-c]pyridines substituted in the 4-position with a thio (**4**), methylthio (**5**), amino (**7**), benzylamino (**13**), or dimethylamino (**15**) group had ED_{50} values ranging from 8 to 9 mg/kg. 1-Ribosylation appreciably increased the activity of 4-amino-1*H*-imidazo[4,5-c]pyridine (**7** vs. **23**). Deoxyribosylation generally decreased antiinflammatory activity. Compound **38** was the most active deoxyribonucleoside.

Of the compounds listed in Table I, 3-deazaadenosine (**23**) had the most potent antiinflammatory activity with an ED_{50} of 3 mg/kg when administered by intraperitoneal injection. It had similar potency when administered orally. Evaluation of this nucleoside for clinical use in diseases with inflammatory involvement is being pursued.

Experimental Section

Cytotoxicity Evaluation. Cells originally derived from human sternal marrow (D-98) and mouse connective tissue (L) were cultured and inhibition of growth by compounds was tested as previously described.[19b]

Inhibition of Lymphocyte-Mediated Cytolysis. Detailed descriptions of this assay have been published.[25] Briefly, ^{51}Cr-labeled EL4 leukemia cells (target cells) were exposed to an equal number (2.5×10^5) of cytolytic lymphocytes (killer cells). The number of target cells lysed was determined by the amount of ^{51}Cr released after incubation with killer cells at 37 °C. This incubation was performed with rocking in phosphate-buffered saline and 10% heat-inactivated fetal calf serum for 70 min. Test compounds were added at the start of the incubation of the cell mixture. Approximately 20% lysis was observed in uninhibited mixtures. This test showed a reproducibility of ±10%.

Inhibition of the Pleural Exudate Response to Carrageenan. This assay was performed as previously described.[24a] An acute inflammatory response was produced by injection of 150 mg of the algal polysaccharide carrageenan into the pleural cavity of rats. Test compounds suspended in 0.5% (carboxymethyl)cellulose were administered by intraperitoneal injection 30 min before the intrapleural carrageenan injection. The volume of the pleural exudate was measured 3 h after the carrageenan injection. Control volumes were in the range of 0.5-0.8 mL of exudate. This test showed a reproducibility of ±15%.

Enzyme Catalysts. Uridine phosphorylase (EC 2.4.2.3), thymidine phosphorylase (EC 2.4.2.4), and purine nucleoside phosphorylase (EC 2.4.2.10) were purified from *Escherichia coli* as previously described.[10a] One unit of enzyme activity was defined as that amount which catalyzed the formation of 1 μmol of product/min under the defined assay conditions.[10a]

Physical Characterization of Compounds. All compounds listed in Table I gave elemental analyses within ±0.4% of calculated values. Analyses were performed by Integral Microanalytical Laboratories, Raleigh, NC, or Atlantic Microlabs, Atlanta, GA. Melting points were obtained on a Thomas-Hoover capillary apparatus and are uncorrected. UV spectra were recorded using a Varian Super-Scan 3 instrument. A Varian CFT-20 or FT-80A instrument provided the NMR spectra in Me_2SO-d_6. Optical rotations were obtained with a Perkin-Elmer Model 141 polarimeter. Infrared spectral data were obtained on an Analect FX-6260 FT-IR instrument using KBr disks. A difference spectrum of compounds **3** and **14** gave the bands assigned to the tetrazolo moiety in **14**.[15] Table II lists some physical constants for the nucleosides synthesized.

The imidazo[4,5-c]pyridines whose syntheses are not described below in detail had the following melting points (°C): **1**, 167–169 (lit.[11a,b] 170–171, 210–215); **2**, >260 (lit.[12] 220–250); **3**, >210 gradual decomposition (lit.[13c,d] 243–245, 223–227); **4**, >300 (lit.[14a,b] >350, >295); **5**, 212–213 (lit.[14b] 217–218) **8**, (0.9 HCl·$^1/_{20}$ H_2O), 300; **9**, 132–135; **10**, (2 HCl·$^1/_{20}$ EtOH), 232–235 (darkens at 215); **11**, (HCl) 216–218; **12**, 170–172.

Syntheses. A styrene–divinylbenzene copolymer derivatized with the cationic functional group $CH_2N^+(CH_3)_3$ (AG-1 X 2; chloride form) was purchased from Bio-Rad Laboratories. The Cl^- form was converted to the OH^- form by repeated suspension of the resin in 1 N NaOH followed by extensive washing with water. Polyacrylamide gel (P-2; 200–400 mesh) was also obtained from Bio-Rad Laboratories. A cross-linked dextran polymer (Sephadex G-10; 40–120 μm) was purchased from Pharmacia. Thin-layer chromatography was performed on cellulose (No. 13254) and silica gel (No 13181) sheets purchased from Eastman-Kodak Co. Solvents were removed in vacuo with a rotary evaporator at temperatures not above 50 °C.

4-(Benzylthio)-1*H*-imidazo[4,5-*c*]pyridine (6). A mixture of 4-mercapto-1*H*-imidazo[4,5-c]pyridine (**4**; 3.0 g, 20 mmol) and Na_2CO_3 (2.5 g, 24 mmol) in 30 mL of DMF was heated at 40 °C. Benzyl bromide (4.1 g, 24 mmol) was added and the mixture was stirred and heated until the UV spectrum no longer increased at 310 nm, indicating that the reaction was complete. The reaction mixture was poured into ice–water and the pH value of the solution was adjusted to 7 by the addition of glacial acetic acid. The solid which formed was collected and recrystallized from CH_2Cl_2/hexanes to give 1.55 g of product. Extraction of the

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aqueous filtrate with ethyl acetate, evaporation of the solvent, and recrystallization gave an additional 1.7 g of product: total yield 68%; mp 135–139 °C; UV λ_{max} nm ($\epsilon \times 10^{-3}$) at pH 1, 310 (10.6), at pH 13, 293 (11.0), 301 (sh) (10.5); NMR δ 8.29 (s, 1 H, H_2), 8.22 (d, 1 H, J = 5.6 Hz, H_6), 7.32 (m, 6 H, Ar H and H_7), 4.59 (s, 2 H, SCH_2). Anal. ($C_{13}H_{11}N_3S$) C, H, N, S.

4-Azido-1*H*-imidazo[4,5-*c*]pyridine (14) (1*H*-Imidazo[4,5-*c*]tetrazolo[1,5-*a*]pyridine). A mixture of 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (**3**; 3.0 g, 20 mmol) and lithium azide (2.5 g, 50 mmol) in 20 mL of DMF was heated, with stirring, at 120 °C for 18 h. The solvent was removed in vacuo and the yellow residue was washed well with water and collected (**14**): yield 2.8 g, 85%; mp >250 °C; UV λ_{max} nm ($\epsilon \times 10^{-3}$) at pH 1, 251 (sh) (6.4), 261 (8.4), 270 (8.5), 280 (7.2), at pH 13, 260 (5.7), 267 (5.9), 291 (6.6); NMR δ 13.67 (br s, NH), 9.06 (d, 1 H, $J_{6,7}$ = 7.3 Hz, H_6), 8.55 (s, 1 H, H_2), 7.71 (d, 1 H, $J_{6,7}$ = 7.3 Hz, H_7); IR (KBr) 1004, 1052, 1087, 1115 (tetrazole), 1647, 1524 (N=N) cm^{-1}. This IR spectrum indicates that the tetrazolo tautomer is dominant in the solid. Anal. ($C_6H_4N_6$) C, H, N.

4-Amino-1*H*-imidazo[4,5-*c*]pyridine (7).[12,13b,13d,14a] Compound **14** (1.6 g, 10 mmol) was dissolved in 150 mL of 6 N HCl and 0.4 g of 10% Pd/C was added. This was hydrogenolyzed at 40 lb/in.^{2} for 36 h. The catalyst was removed and the filtrate was evaporated to dryness in vacuo. Several volumes of EtOH were added and evaporated to dryness in vacuo. This was repeated several times. The white crystalline product was the dihydrochloride salt: yield 1.8 g, 87%; mp >250 °C; UV λ_{max} nm ($\epsilon \times 10^{-3}$) at pH 1, 260 (8.1), 278 (8.4); at pH 13, 275 (7.2); NMR δ 8.48 (br s, NH), 8.47 (s, 1 H, H_2), 7.70 (d, 1 H, $J_{6,7}$ = 6.9 Hz, H_6), 7.12 (d, 1 H, $J_{6,7}$ = 6.9 Hz, H_7), 6.50 (br s, NH_2). Anal. ($C_6H_6N_4 \cdot 2HCl$) C, H, Cl, N.

4-Amino-1*H*-imidazo[4,5-*c*]pyridine dihydrochloride (3.2 g, 4.8 mmol) was dissolved in 30 mL of warm water and filtered. The filtrate (pH 8.0) was applied to a column packed with polyacrylamide gel (P-2, 5 × 13 cm) that had been conditioned by washing with 1 N NH_4OH and then with water. The eluate contained salts and a small amount of yellow material. The eluent was then changed to 1 N NH_4OH. Fractions of eluate containing UV-absorbing material were combined. The solution was concentrated in vacuo and then lyophylized to give **7** as an off-white powder: yield 1.93 g, 93%; mp 225 °C (softens 221); mass spectrum, m/e 134; NMR δ 12.44 (br s, NH), 8.07 (s, 1 H, H_2), 7.63 (d, 1 H, $J_{6,7}$ = 5.8 Hz, H_6), 6.75 (d, 1 H, $J_{6,7}$ = 5.8 Hz, H_7), 6.07 (s, 2 H, NH_2). Anal. ($C_6H_6N_4$) C, H, N.

4-(Benzylamino)-1*H*-imidazo[4,5-*c*]pyridine (13). A mixture of 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (**3**; 2.0 g, 13 mmol), benzylamine (5 mL), and a few drops of water was heated at reflux for 4 days. The reaction mixture was poured onto ice and water and the cold mixture was extracted twice with diethyl ether. The ether was removed in vacuo and the residual oil was triturated twice with hexane. The oil was suspended in water and the aqueous phase was neutralized with glacial acetic acid. The aqueous phase was taken to dryness in vacuo, resuspended in water (15 mL), and applied to a column of Dowex 50(H^+) resin (10 g). The column was washed with water until the ultraviolet absorbance of the eluate was no longer detectable. The column packing was removed and heated with several portions of concentrated ammonium hydroxide. Filtrates from these extractions were combined (400 mL), cooled, and neutralized with glacial acetic acid. The yellow solid was collected and dried in vacuo at 40 °C to give the 0.25 hydrate of 4-(benzylamino)-1*H*-imidazo[4,5-*c*]pyridine (**13**): yield 0.94 g, 31.5%; mp 60–64 °C; UV λ_{max} nm ($\epsilon \times 10^{-3}$) at pH 1, 263 (sh) (12.1), 277 (13.0), at pH 13, 278 (11.9); NMR δ 12.45 (br s, NH), 8.08 (s, 1 H, H_2), 7.68 (d, 1 H, J = 5.8 Hz, H_6), 7.33 (m, 6 H, Ar and NH), 6.74 (d, 1 H, J = 5.8 Hz, H_7), 4.71 (d, 2 H, J = 6.3 Hz, NCH_2). Anal. ($C_{13}H_{12}N_4 \cdot 0.25H_2O$) C, H, N.

4-(Dimethylamino)-1*H*-imidazo[4,5-*c*]pyridine Hydrochloride (15). A solution of the 4-chloro derivative **3** (5.0 g, 33 mmol) in anhydrous dimethylamine (100 mL) was heated in a stainless steel bomb at 140 °C for 24 h. The solvent was removed in vacuo and the residue was dissolved in water. The solution was acidified by the addition of 1 N HCl and evaporated to dryness. Recrystallization from MeOH gave **15**: yield 1.92 g, 30%; mp >250 °C; UV λ_{max} nm ($\epsilon \times 10^{-3}$) at pH 1, 270 (11.3), 290 (10.8), at pH 13, 285 (10.0); NMR δ 12.5 (br s, NH), 8.43 (s, 1 H, H_2), 7.68 (d, 1 H, $J_{6,7}$ = 6.9 Hz, H_6), 7.14 (d, 1 H, $J_{6,7}$ = 6.9 Hz, H_7), 3.32 (s, 6 H, $N(CH_3)_2$). Anal. ($C_8H_{10}N_4 \cdot HCl$) C, H, Cl, N.

1-β-D-Ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (16).[2b,16] A solution of 4-chloro-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]-pyridine (**19**; 1.0 g, 3.5 mmol) in ethanol/water (1:1, v/v; 200 mL) was hydrogenated at 40 lb/in.^{2} in the presence of 10% Pd/C catalyst (0.3 g). After the reduction was completed (monitored by TLC), the catalyst was removed by filtration, and the filtrate was evaporated to dryness in vacuo. The residue was chromatographed on a silica gel column using a mixture of CH_2Cl_2 and MeOH as the eluent. Fractions of the eluate containing a single component by TLC silica gel (EtOAc/MeOH, 9:1; R_f 0.25) were pooled and dried: yield 0.2 g (23%); mp 200–204 °C [lit.[2b] mp 204–205 °C; lit.[16] mp 198–199 °C dec]; $[\alpha]^{20}_D$ −49.2° (*c* 0.5, DMF); UV λ_{max} nm ($\epsilon \times 10^{-3}$) at pH 1, 255 (3.8), 265 (4.0), at pH 13, 250 (4.5), 263 (sh) (3.7), 270 (sh) (2.7) [lit.[2b] λ_{max} at pH 1, 257 (4.1), 263 (4.3); at pH 11, 243 (7.0), 260 (sh) (4.9), 267 nm (4.0); lit.[16] λ_{max} at pH 1.72, 257–258 (4.1), 263–264 (4.7); at pH 12.35, 248 (4.7), 259 (sh) (4.1), 268 (sh) (2.8)]; NMR δ 8.99 (d, 1 H, $J_{4,7}$ = 0.8 Hz, H_4), 8.61 (s, 1 H, H_2), 8.37 (d, 1 H, $J_{6,7}$ = 5.6 Hz, H_6), 7.83 (dd, 1 H, $J_{4,7}$ = 0.8 Hz and $J_{6,7}$ = 5.6 Hz, H_7), 5.94 (d, 1 H, J = 6.2 Hz, $H_{1'}$). Anal. ($C_{11}H_{13}N_3O_4$) C, H, N.

4-Amino-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (23). 4-Amino-1*H*-imidazo[4,5-*c*]pyridine (**7**; 0.32 g, 2.4 mmol), uridine (1.3 g, 5.3 mmol), and K_2HPO_4 (0.4 mmol) were dissolved in 51 mL of deionized water. The pH of this solution was 8.2. Uridine phosphorylase (21 units) and purine nucleoside phosphorylase (1600 units) were added. After 3 days at 37 °C, the reaction mixture was evaporated to dryness and the residue was suspended in 10 mL of water. After filtration of the suspension, the solids were washed with 3 mL of water. To the combined filtrate and wash was added 4 mL of methanol. This solution was applied to a column packed with an anion exchange resin (AG-1; 2.5 × 10 cm) that had been preequilibrated with a mixture of water and methanol (70/30, v/v). After the sample was applied, the resin was washed with 200 mL of this mixture and then with 150 mL of a water–methanol mixture containing a greater proportion of methanol (10/90, v/v). Product which had a R_f value of 0.40 on TLC (cellulose/H_2O) was eluted with a mixture of 0.07 N formic acid and methanol (10/90, v/v). After removal of the solvent from the appropriate fractions, 0.5 g of the anhydrous product was obtained. The yield calculated on the basis of the amount of 4-amino-1*H*-imidazo[4,5-*c*]pyridine used was 79%; mp 226 °C (lit.[1,2a] mp 225–231 °C, lit.[2b,c] mp 229–231 °C); $[\alpha]^{20}_D$ −63.4° (*c* 1, DMF); UV λ_{max} nm ($\epsilon \times 10^{-3}$) at pH 1, 261 (10.4), at pH 13, 266 (10.4) [lit.[1,2] λ_{max} at pH 1, 262 (10–11.6); at pH 13, 265 (10.7)]; NMR δ 8.29 (s, 1 H, H_2), 7.68 (d, 1 H, $J_{6,7}$ = 5.8 Hz, H_6), 6.92 (d, 1 H, $J_{6,7}$ = 5.8 Hz, H_7), 6.13 (s, 2 H, NH_2), 5.76 (d, 1 H, J = 6.0 Hz, $H_{1'}$). Anal. ($C_{11}H_{14}N_4O_4$) C, H, N.

4-(Benzylamino)-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]-pyridine (29). 4-(Benzylamino)-1*H*-imidazo[4,5-*c*]pyridine (**13**; 0.68 g, 3.03 mmol) was dissolved in 10 mL of 1-propanol. An aqueous solution (206 mL) containing uridine (4.1 g, 16.8 mmol), KH_2PO_4 (1.2 mmol), Na_2EDTA (0.078 mmol), and KN_3 (0.0018 mmol) was adjusted to pH 7 with KOH. The aqueous solution was added to the 1-propanol solution. Uridine phosphorylase (390 units) and purine nucleoside phosphorylase (2800 units) were then added to the suspension. After 5 days at 37 °C, the reaction mixture was filtered and the volume of the filtrate was decreased to one-half its original volume by evaporation in vacuo. The resulting suspension was placed at 3 °C for 18 h. The precipitate was collected by filtration and washed with water, yielding 389 mg of product after drying. A second crop (428 mg) was obtained from the wash and mother liquor by further evaporation and cooling. Both crops analyzed for the monohydrate after drying. On the basis of the amount of acceptor base used, the overall yield was 72%; mp, softens at 124 °C, decomposes at 172 °C; $[\alpha]^{20}_D$ −41.8° (*c* 0.5, DMF); UV in methanol, λ_{max} nm ($\epsilon \times 10^{-3}$), 274 (16.6); NMR δ 8.31 (s, 1 H, H_2), 7.71 (d, 1 H, $J_{6,7}$ = 5.8 Hz, H_6), 7.27 (m, 5 H, Ar H), 6.92 (d, 1 H, $J_{6,7}$ = 5.8 Hz, H_7), 5.77 (d, 1 H, J = 6.0 Hz, $H_{1'}$), 4.70 (m, 2 H, $NArCH_2$). Anal. ($C_{18}H_{20}N_4O_4 \cdot H_2O$) C, H, N.

4-Chloro-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (34).[18] An aqueous suspension (355 mL) containing 4-chloro-1*H*-imidazo[4,5-*c*]pyridine[12,13] (**3**; 20 g, 130 mmol), thymidine (65 g, 268 mmol), KH_2PO_4 (64 mmol), and Na_2EDTA (1.6

mmol) was adjusted to pH 7.4 with KOH. Thymidine phosphorylase (11 200 units) and purine nucleoside phosphorylase (12 700 units) were then added. After 5 days at 37 °C, the reaction mixture was filtered. The volume of the filtrate was reduced to one-half by evaporation in vacuo. The precipitate that formed was removed by filtration and the filtrate was lyophilized. The lyophilizate was triturated at 24 °C sequentially with 200, 250, and 400 mL portions of tetrahydrofuran. The filtrate extracts were stored at 3 °C for 18 h. The precipitates that formed were combined with the trituration residue. These solids were dissolved in 200 mL of a 1-propanol–water mixture (30/70, v/v) and applied to a column packed with polyacrylamide gel (P-2; 7.5 × 90 cm) that had been equilibrated with the mixture of 1-propanol and water. After elution of the product with this mixture and removal of the solvents, 21.88 g of product was obtained. A second crop was obtained from the tetrahydrofuran extracts by evaporating them in vacuo to a thick syrup and diluting this syrup with the mixture of 1-propanol and water. Chromatography on polyacrylamide gel (P-2) gave 8.09 g of product. On the basis of the amount of acceptor heterocycle used, the overall yield was 85%; mp 162–165 °C (lit.[18b] mp 173–175 °C); $[\alpha]^{20}_D$ −24.62° (c 1, DMF); UV λ_{max} nm ($\epsilon \times 10^{-3}$), at pH 1, 273, 266 (6.1, 6.0), at pH 13, 273, 265, 255 (4.5, 5.7, 6.2); NMR δ 8.70 (s, 1 H, H_2), 8.17 (d, 1 H, $J_{6,7}$ = 5.6 Hz, H_6), 7.87 (d, 1 H, $J_{6,7}$ = 5.6 Hz, H_7), 6.42 (t, 1 H, $H_{1'}$). Anal. ($C_{11}H_{12}ClN_3O_3$) C, H, Cl, N.

4-(Methylthio)-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (36). An aqueous suspension (10.5 mL) containing 4-(methylthio)-1*H*-imidazo[4,5-*c*]pyridine (**5**; 0.6 g, 3.6 mmol), thymidine (1.4 g, 5.8 mmol), KH_2PO_4 (1.0 mmol), and KN_3 (0.17 mmol) was adjusted to pH 7.4 with KOH. Thymidine phosphorylase (14 units) and purine nucleoside phosphorylase (317 units) were added. After 17 days at 37 °C, the reaction mixture was filtered. The large beige crystals in the filter cake were picked out manually and washed with water. They were then resuspended in 75 mL of boiling water. After removal of the insoluble material by filtration, the product was recrystallized at 3 °C. After washing and drying, 0.63 g of white crystalline product as the monohydrate was obtained. The yield calculated on the basis of the acceptor base used was 58%; mp 179–183 °C; $[\alpha]^{20}_D$ −23.47° (c 1, DMF); UV λ_{max} nm ($\epsilon \times 10^{-3}$), at pH 1, 307 (16.8), 238 (sh) (10.4), 223 (15.1), at pH 13, 285 (13.3); NMR δ 8.54 (s, 1 H, H_2), 8.24 (d, 1 H, $J_{6,7}$ = 5.7 Hz, H_6), 7.54 (d, 1 H, $J_{6,7}$ = 5.7 Hz, H_7), 6.35 (t, 1 H, $H_{1'}$). Anal. ($C_{12}H_{15}N_3O_3S \cdot H_2O$) C, H, N, S.

4-Amino-1-(2-deoxy-β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (38).[10c,18b] An aqueous suspension (200 mL) containing 4-amino-1*H*-imidazo[4,5-*c*]pyridine hydrochloride hemihydrate (1.3 g, 7.2 mmol), thymidine (2.2 g, 9.1 mmol), and K_2HPO_4 (1 mmol) was adjusted to pH 7.0 with KOH. Thymidine phosphorylase (5000 units) and purine nucleoside phosphorylase (8200 units) were added. After 6 days at 37 °C, the reaction mixture was filtered. The filtrate was dried in vacuo. The resulting solid was suspended by vigorous stirring in 20 mL of water. This suspension was filtered and the filter cake washed with water. The combined filtrate and washes had a volume of 23 mL to which 7 mL of methanol was added. This solution was applied to an AG-1 column (2.8 × 10 cm) which was preequilibrated with a mixture of water and methanol (70/30, v/v). The product was eluted with this solvent mixture. The volume of the product-containing eluate was decreased to 20 mL by evaporation in vacuo. Product that crystallized from this solution upon standing at 24 °C was reserved. The mother liquor was applied to a column (2.5 × 72 cm) containing Sephadex G-10. The product was eluted with water. Fractions which contained only product by TLC on cellulose in water (R_f 0.47) were combined with the reserved crystals. After evaporation of solvent in vacuo, 1.3 g of the partially hydrated (0.3 H_2O) product was obtained. The yield on the basis of the 4-amino-1*H*-imidazo[4,5-*c*]pyridine used was 70%; mp 206 °C; decomposes at 215 °C (lit.[18b] mp 209–211 °C); $[\alpha]^{20}_D$ −25.5° (c 1, DMF); UV λ_{max} nm ($\epsilon \times 10^{-3}$), at pH 1, 262 (10.6), at pH 13, 266 (10.6); NMR δ 8.29 (s, 1 H, H_2), 7.67 (d, 1 H, $J_{6,7}$ = 5.8 Hz, H_6), 6.90 (d, 1 H, $J_{6,7}$ = 5.8 Hz, H_7), 6.25 (t, 1 H, J = 6.4, $H_{1'}$), 6.12 (s, 2 H, NH_2). Anal. ($C_{11}H_{14}N_4O_3 \cdot 0.3H_2O$) C, H, N.

4-Amino-1-(5-deoxy-β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (43). 4-Amino-1*H*-imidazo[4,5-*c*]pyridine (**7**; 0.40 g, 3.0 mmol), 5′-deoxyuridine[20] (0.73 g, 3.2 mmol), and KH_2PO_4 (0.06 mmol) were dissolved in 35 mL of water. The pH was adjusted to 8.3 with KOH. Uridine phosphorylase (420 units) and purine nucleoside phosphorylase (8200 units) were added to the solution. After 8 days at 37 °C, the reaction mixture was filtered. Methanol (27 mL) was added to the filtrate and this solution was applied to a column packed with an anion exchange resin (AG-1; 2.5 × 17 cm) that had been preequilibrated with a water–methanol mixture (70/30, v/v). After the sample was applied, the column was washed as described above for **23**. Product was eluted with the formic acid/methanol/water mixture. It had an R_f value of 0.45 on TLC (cellulose/H_2O). After removal of the solvents in vacuo, the product was recrystallized from water. After drying, product (180 mg, 22%) was obtained as the monohydrate; mp 140 °C; $[\alpha]^{20}_D$ −31.9° (c 0.5, DMF); UV λ_{max} nm ($\epsilon \times 10^{-3}$), at pH 1, 263 (9.1), at pH 13, 267 (8.3); NMR δ 8.19 (s, 1 H, H_2), 7.68 (d, 1 H, $J_{6,7}$ = 5.9 Hz, H_6), 6.77 (d, 1 H, $J_{6,7}$ = 5.9 Hz, H_7), 6.15 (br s, 2 H, NH_2), 5.71 (d, 1 H, J = 5.6 Hz, $H_{1'}$), 4.26 (m, 1 H, $H_{2'}$), 4.04 (mm, 2 H, $H_{3'}$, $H_{4'}$), 1.33 (d, 3 H, J = 6.2 Hz, 5′-CH_3). Anal. ($C_{11}H_{14}N_4O_3 \cdot H_2O$) C, H, N.

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Registry No. 1, 272-97-9; **2**, 91184-02-0; **3**, 2770-01-6; **4**, 34550-51-1; **5**, 34550-50-0; **6**, 98858-04-9; **7**, 6811-77-4; **7**·2HCl, 80639-85-6; **8**, 98858-10-7; **9**, 98858-11-8; 10, 98858-12-9; 11, 98858-13-0; **12**, 98858-14-1; **13**, 80443-18-1; 14, 98858-05-0; **15**·HCl, 98858-07-2; **16**, 6703-46-4; **17**, 7465-42-1; 18, 98858-15-2; 18 (3-Ribo), 98858-31-2; **19**, 6736-60-3; 20, 7103-34-6; **21**, 3483-02-1; **22**, 98858-16-3; **23**, 6736-58-9; **24**, 22566-92-3; **25**, 98858-17-4; **26**, 98858-18-5; **27**, 98858-19-6; **28**, 98858-20-9; **29**, 80443-17-0; 30, 98858-21-0; **31**, 98858-22-1; **32**, 98858-23-2; **32** (3-2′-dRibo), 98858-32-3; **33**, 98858-24-3; **33** (3-2′-dRibo), 98858-33-4; **34**, 78582-15-7; **35**, 98858-25-4; **36**, 98858-08-3; **37**, 98858-26-5; **38**, 78582-17-9; **39**, 80639-84-5; 40, 98858-27-6; **41**, 98858-28-7; **42**, 98858-29-8; **43**, 98858-09-4; **44**, 98858-30-1; benzylamine, 100-46-9; dimethylamine, 124-40-3; uridine, 58-96-8; uridine phosphorylase, 9030-22-2; purine nucleoside phosphorylase, 9030-21-1; thymidine, 50-89-5; thymidine phosphorylase, 9030-23-3; 5′-deoxyuridine, 15958-99-3; 1*H*-imidazo[4,5-*c*]tetrazolo[1,5-*a*]pyridine, 98858-06-1.