Phe (13)]. Each of these cyclized free peptides (2–13) was obtained from their respective protected precursors II–XIII by the procedure followed in obtaining the free peptide 1 from the protected intermediate I. (For some of the more insoluble peptides, it was necessary to use up to 100 mL of 50% aqueous acetic acid to dissolve the residue following evaporation of NH_3.) Their physiochemical properties are given in Table IV. The pharmacological properties of peptides 1–13 are presented in Tables I and II.

[1-(β-Mercapto-β,β-pentamethylenepropionic acid)-2-D-phenylalanine,4-amino acid X]arginine-vasopressin [d-(CH_2)$_5$D-Phe^2,X^4,AVP] (14 and 15) [X = Ser (14), Orn (15)]. Both of these cyclized free peptides (14 and 15) were obtained from their respective protected precursors XIV and XV by the procedure followed in obtaining the free peptide 1 from the protected intermediate I. Their physiochemical properties are given in Table IV. The pharmacological properties of both peptides are presented in Tables I and II.

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Registry No. 1, 88686-52-6; 2, 88686-53-7; 3, 88686-54-8; 4, 88686-55-9; 5, 88686-56-0; 6, 88686-57-1; 7, 88686-58-2; 8, 88703-08-6; 9, 88686-59-3; 10, 88686-60-6; 11, 88686-61-7; 12, 88686-62-8; 13, 88686-63-9; 14, 88686-64-0; 15, 88686-65-1; I, 88686-66-2; II, 88686-67-3; III, 88686-68-4; IV, 88703-09-7; V, 88686-69-5; VI, 88703-10-0; VII, 88686-70-8; VIII, 88686-71-9; IX, 88686-72-0; X, 88686-73-1; XI, 88686-74-2; XII, 88686-75-3; XIII, 88686-76-4; XIV, 88686-77-5; XV, 88686-78-6; Boc-Arg(Tos), 13836-37-8; Boc-Pro, 15761-39-4; Boc-Cys(Bzl), 5068-28-0; Boc-Asn NPE, 4587-33-1; Boc-L-Abu, 34306-42-8; Boc-L-Phe, 13734-34-4; Boc-D-Phe, 18942-49-9; *p*-nitrophenyl β-(benzylmercapto)-β,β-cyclopentamethylenepropionate, 55154-81-9; oxytocin, 50-56-6.

Inhibitors of Inosinic Acid Dehydrogenase. 2-Substituted Inosinic Acids

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A series of 2-substituted inosine monophosphate (IMP) and inosine derivatives were synthesized and tested for inhibitory activity against IMP dehydrogenase from *Escherichia coli*. All of the IMP analogues that possessed electron-withdrawing substituents on the phenyl ring of a benzylthio group placed at the 2-position of IMP showed strong inhibition, which was competitive with IMP. No evidence of hydrophobic interactions of the 2-substituent with the enzyme was observed.

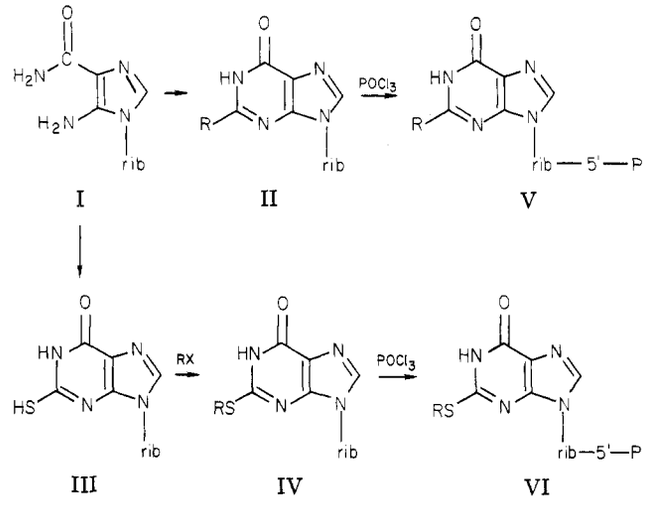
In a previous paper,[1] we discussed the importance of inosinic acid dehydrogenase (IMP dehydrogenase, EC 1.2.1.14) to rapidly growing cells. Most compounds that inhibit IMP dehydrogenase have anticancer activity. We presented some results on inhibition of that enzyme by a series of 8-substituted purine nucleotides.[1] In particular, we found that there is an apparent electron-rich binding site near the IMP site that can bind electron-deficient phenyl substituents on the 8-position of IMP or AMP. We now report the effect of various substituents at the 2-position of IMP on the ability of these compounds to inhibit IMP dehydrogenase, as well as the inhibition of this enzyme by the corresponding 2-substituted inosines.

Results

Synthesis. All of the nucleosides and nucleotides in this report were prepared from 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA riboside, I). Those inosines with alkyl or aryl 2-substituents (II) were prepared by sodium ethoxide catalyzed ring closure of I with the appropriate ester.[2,3] When this method was used in an attempt to prepare nitro-substituted 2-aryl or aralkyl analogues, however, only intractable mixtures were obtained. Treatment of 2-mercaptoinosine[3] (III) with the appropriate benzyl halide gave a series of 2-[(substituted-benzyl)thio]inosines (IV). These methods are summarized in Scheme I, and the physical properties of all new nucleosides are given in Table I.

All of the inosine analogues were converted to the corresponding IMP analogues (V and VI) by phosphorylation

Scheme I



in phosphoryl chloride/trimethyl phosphate. The nucleotides were purified either by preparative HPLC or by chromatography on boric acid gel. Physical properties of all the new IMP analogues are given in Table II. Substantial difficulty was encountered in obtaining correct combustion analyses (especially nitrogen) on the nucleo-

(1) Skibo, E. S.; Meyer, Jr., R. B. *J. Med. Chem.* **1981**, *24*, 1155.
(2) Yamasaki, A.; Kumashiro, I.; Takenishi, T. *J. Org. Chem.* **1967**, *32*, 3258.
(3) Imai, K.; Marumoto, R.; Kobayashi, K.; Yoshioko, Y.; Toda, J.; Honjo, M. *Chem. Pharm. Bull.* **1971**, *19*, 576.
(4) Yoshikawa, M.; Hata, T.; Takenishi, T. *Tetrahedron Lett.* **1967**, 5056.

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Table I. Preparation and Physical Properties of 2-Substituted Analogues of Inosine

2-substituent	method	yield,[a] %	mp, °C	λ_{max}, nm ($\epsilon \times 10^{-4}$) pH 1	pH 7	pH 13	formula[b]
n-$CH_3(CH_2)_4$	A	35	167	252 (1.24)	250 (1.26)	256 (1.28)	$C_{15}H_{22}N_4O_5 \cdot 1.25H_2O$
$CH_3(CH_2)_5$	A	61	184	250 (1.24)	251 (1.26)	255 (1.35)	$C_{16}H_{24}N_4O_5$
$CH_3(CH_2)_6$	A	67	185	250 (1.26)	250 (1.17)	255 (1.25)	$C_{17}H_{26}N_4O_5 \cdot 0.25H_2O$
$CH_3(CH_2)_7$	A	95	194	251 (1.21)	251 (1.21)	253 (1.43)	$C_{18}H_{28}N_4O_5$
$CH_3(CH_2)_8$	A	36	199	251 (1.24)	251 (1.27)	256 (1.29)	$C_{19}H_{30}N_4O_5 \cdot 0.25H_2O$
$CH_3(CH_2)_9$	A	90	205	250 (0.87)	251 (0.86)	253 (0.93)	$C_{20}H_{32}N_4O_5$
$PhCH_2$	A	57	133	251 (1.26)	251 (1.24)	256 (1.26)	$C_{17}H_{18}N_4O_5 \cdot 0.50H_2O$
3-c-NC_5H_4	A	67	166–168	306 (0.87), 249 (1.18)	293 (1.13), 258 (1.03)	289 (0.95), 259 (1.14)	$C_{15}H_{15}N_5O_5 \cdot 0.50H_2O$
p-OCH_3Ph	A	21	229–231 dec	302 (2.09), 265 (1.22, sh)	300 (1.90), 260 (1.35)	290 (1.71), 270 (1.56), 249 (2.04)	$C_{17}H_{18}N_4O_6 \cdot 0.50H_2O$
$PhCH{=}CH$	A	62	223–225	326 (2.22)	329 (2.09), 269 (1.19)	318 (2.09), 288 (1.71), 261 (1.57)	$C_{18}H_{18}N_4O_5$
p-OCH_3PhCH_2	A	89	198	253 (1.39)	251 (1.33)	256 (1.46)	$C_{18}H_{20}N_4O_6 \cdot 0.25H_2O$
3-c-$NC_5H_4CH_2$	A	87	140	256 (1.72), 260 (1.70, sh)	251 (1.49), 260 (1.27, sh)	256 (1.66), 260 (1.63, sh)	$C_{16}H_{17}N_5O_5 \cdot 0.25H_2O$
m-NO_2PhCH_2S	B	69	194	267 (2.28)	262 (2.35)	270 (2.23)	$C_{17}H_{17}N_5O_7S \cdot 0.25H_2O$
2-Cl-4-NO_2PhCH_2S	B	67	167	270 (1.96)	265 (1.80)	271 (1.98)	$C_{17}H_{16}ClN_5O_7S \cdot 0.25H_2O$[c]
p-NO_2PhCH_2S	B	92	232	270 (1.39)	263 (1.64), 275 (1.63, sh)	271 (2.89)	$C_{17}H_{17}N_5O_7S \cdot 0.75H_2O$
o-$ClPhCH_2S$	B	64	133	269 (2.86)	263 (2.52), 280 (2.14, sh)	265 (2.95)	$C_{17}H_{17}ClN_4O_5S \cdot 1.0H_2O$
3,5-$(NO_2)_2PhCH_2S$	B	88	145	250 (2.87)	250 (2.67)	250 (2.74)	$C_{17}H_{16}N_6O_9S \cdot 0.75H_2O$[d]

[a] Yields are from AICA riboside (method A) or 2-mercaptoinosine (method B). [b] All compounds gave satisfactory analyses for C, H, and N within ±0.4%, except where indicated. [c] N: calcd, 14.76; found, 14.21. [d] N: calcd, 17.05; found, 16.52.

tides, a problem we have encountered before. All compounds, however, were verified as being pure on both TLC and HPLC immediately before enzyme assay.

Enzyme Inhibition Studies. All of the compounds in Tables I and II were screened as inhibitors of IMP dehydrogenase from *E. coli*. The 2-[(substituted-benzyl)thio]inosines were only very weak inhibitors, giving I_{50} values at substrate concentrations of 50 μM (the K_m of IMP is 12 μM) of greater than 0.8 mM. All other inosines did not inhibit the enzyme at 1 mM concentration.

The inhibition of IMP dehydrogenase by the phosphorylated analogues (V and VI, the 2-substituted IMP's) is shown in Table III. Most of the values in the table reflect the concentration (I_{50}) necessary for 50% inhibition at an IMP concentration of 50 μM. Certain entries in the Table are K_i values, determined by nonlinear fitting of the velocities to the competitive inhibition equation, as described by Cleland.[5] K_i values were determined for some of the analogues, whose I_{50}'s are reported, and in all cases competitive inhibition was observed as judged by the pattern of lines intersecting on the Y axis of a plot of $1/[IMP]$ vs. $1/V$. In particular, one of the most potent inhibitors, 2-(2-Cl-4-$O_2NC_6H_3CH_2S$)-IMP (**22**, Table III) showed competitive kinetics and a K_i value of 6.3 μM. Under the conditions of our assay, the I_{50}'s determined are very approximately 4-fold greater than the K_i.[6] Since our compounds all had substituents on the 2-position, they were not tested as substrates of IMP dehydrogenase.

Discussion

The realization that extra active site binding areas, particularly hydrophobic areas, can be exploited has led to the design and synthesis of tight binding inhibitors of many enzymes, including dihydrofolate reductase,[7] xanthine oxidase,[8] and adenosine deaminase.[9] In this work, we have investigated 2-substituents on inosinic acid as probes for such binding areas, adjacent to the active site, on IMP dehydrogenase. As can be seen from Table III, none of the straight-chain aliphatic substituents (**5–11**) contributed in a significant manner to inhibitor binding, indicating no significant hydrophobic area on the enzyme adjacent to the 2-position binding site. As can be seen in **2–5**, addition of even small groups to the 2-position of IMP resulted in loss of binding.

In earlier work investigating the binding of 8-substituted purine nucleotides to IMP dehydrogenase,[1] we found that aromatic rings bearing a strong electron-withdrawing group could give significant increases in binding, although the nucleotide no longer bound in the same conformation as the substrate. We had found a good correlation between the inductive effect of the substituent on an 8-(benzylthio) group on IMP or AMP with the K_i of the inhibitors. We felt that one possible explanation for this increased binding was a charge-transfer complex between the electron-deficient phenyl ring in the substituent and an electron donor on the enzyme. In this work, we find a similar phenomenon. Compounds **18–22** (Table III), the 2-(benzylthio) analogues, are easily the best inhibitors of the group. Although the lack of a substantial spread in the data substantially diminishes the utility of examining quantitative structure–activity relationships, it would appear that

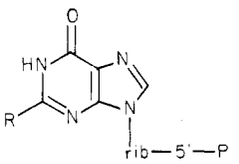
(5) Cleland, W. W. *Adv. Enzymol.* **1967**, *29*, 1.
(6) Baker, B. R. "Design of Active-Site-Directed Irreversible Enzyme Inhibitors"; Wiley: New York, 1967; p 203.
(7) (a) Baker, B. R. Ref 6, p 203. (b) Baker, B. R. *J. Med. Chem.* **1968**, *11*, 483.
(8) Baker, B. R.; Wood, W. F. *J. Med. Chem.* **1968**, *11*, 644.
(9) Schaeffer, H. J.; Schwander, C. F. *J. Med. Chem.* **1974**, *17*, 6.

Table II. Preparation and Physical Properties of 2-Substituted Analogues of IMP (V and VI)

2-substituent	purification method	yield, %	R_f A	R_f B	formula	anal.[a]
$CH_3(CH_2)_4$	D	12	0.23	0.13	$C_{15}H_{29}N_6O_8P$	C, H; P[b]
$CH_3(CH_2)_5$	D	18	0.24	0.15	$C_{16}H_{31}N_6O_8P\cdot H_2O$	P
$CH_3(CH_2)_6$	D	10	0.24	0.17	$C_{17}H_{33}N_6O_8P$	
$CH_3(CH_2)_7$	D	15	0.24	0.21	$C_{18}H_{35}N_6O_8P$	P
$CH_3(CH_2)_8$	D	15	0.25	0.25	$C_{19}H_{37}N_6O_8P\cdot H_2O$	P
$CH_3(CH_2)_9$	D	17	0.25	0.29	$C_{20}H_{39}N_6O_8P\cdot 0.25H_2O$	C, H, P
$PhCH_2$	C	54	0.16	0.24	$C_{17}H_{26}N_6O_8P$	C, H, P
3-c-NC_5H_4	C	35	0.42	0.00	$C_{15}H_{22}N_7O_8P\cdot H_2O$	C, H; N[c]
p-OCH_3Ph	C	24	0.12	0.16	$C_{17}H_{25}N_6O_9P\cdot 0.5H_2O$	C, H, P
PhCH=CH	C	45	0.17	0.25	$C_{18}H_{25}N_6O_8P$	H, P; C[d]
p-OCH_3PhCH_2	C	53	0.12	0.17	$C_{18}H_{28}N_6O_9P\cdot 0.5H_2O$	C, H, P
3-c-NC_5H_4-CH_2	D	17	0.39	0.00	$C_{16}H_{24}N_7O_8P\cdot 2H_2O$	C, H; P[e]
m-NO_2PhCH_2S	D	10	0.32	0.33	$C_{17}H_{24}N_7O_{10}PS$	P
2-Cl-4-NO_2PhCH_2S	D	17	0.53	0.25	$C_{17}H_{23}ClN_7O_{10}PS\cdot 16.5H_2O$	C, H; N[f]
p-NO_2PhCH_2S	D	14	0.19	0.42	$C_{17}H_{21}N_6O_{10}PS$	C; H, N[g,h]
o-$ClPhCH_2S$	D	20	0.46	0.25	$C_{17}H_{24}N_6O_8PSCl$	C, H, P
3,5-$(NO_2)_2PhCH_2S$	D	11	0.42	0.37	$C_{17}H_{20}N_7O_{12}PS\cdot 4H_2O$	C, H, N[h]

[a] All compounds gave satisfactory analyses within ±0.4%, except where indicated. All compounds were isolated as diammonium salts, except where indicated. [b] P: calcd, 6.85; found, 7.40. [c] N: calcd, 20.54; found, 18.02. [d] C: calcd, 44.63; found, 45.33. [e] P: calcd, 6.08; found, 5.00. [f] N: calcd, 11.13; found, 10.20. [g] H: calcd, 4.82; found, 3.87. N: calcd, 13.90; found, 12.24. [h] Monoammonium salt.

Table III. Inhibition of IMP Dehydrogenase by 2-Substituted IMP's[d]



no.	R	I_{50},[a] μM
1	H	12 (k_m)
2	Cl[c]	120[b]
3	NH_2 (GMP)	38[b]
4	OCH_3[c]	46[b]
5	CH_3[c]	600[b]
6	$(CH_2)_2CH_3$[c]	190[b]
7	$(CH_2)_5CH_3$	580
8	$(CH_2)_6CH_3$	270
9	$(CH_2)_7CH_3$	680
10	$(CH_2)_8CH_3$	160
11	$(CH_2)_9CH_3$	150
12	$CH_2C_6H_5$	770
13	C_6H_5	>1000
14	$C_6H_4OCH_3$-*p*	810
15	$CH{=}CHC_6H_5$	69
16	3-c-NC_5H_4	>1000
17	CH_2-3-c-NC_5H_4	430
18	$SCH_2C_6H_4NO_2$-*m*	33
19	$SCH_2C_6H_4NO_2$-*p*	56
20	$SCH_2C_6H_3$-3,5-$(NO_2)_2$	85
21	$SCH_2C_6H_4$Cl-*o*	72
22	$SCH_2C_6H_3$-2-Cl-4-NO_2	23

[a] Concentration for 50% inhibition; [IMP] = 50 μM. Determined as described in Experimental Section. [b] K_i values. [c] Supplied by Dr. A. Yamasaki. [d] All values represent averages of at least two determinations, for which the range is within ±30%.

the 2-substituted compounds in this report are not only experiencing the same type of binding interaction that we found earlier in the 8-substituted series (where increasingly electron-deficient substituents gave correspondingly tighter binding) but that the strength of the interactions are about the same. The loss of binding due to placement of the 2-substituent (see **2–17**, Table III) is regained by the interaction of the electron-deficient 2-substituent with the enzyme. It is possible, although not conclusive, that these 2-substituents are binding to the same site as the 8-substituents in our prior study. The possibility that this binding is due to charge-transfer complex formation is one that we shall explore.

Experimental Section

Isolation of IMP Dehydrogenase. This enzyme was isolated from *E. coli*, obtained as a frozen paste form Grain Processing, Inc., Mustcaine, IA, according to the method of Streeter et al.[10] Crude enzyme preparation from a 0–40% $(NH_4)_2SO_4$ precipitate was used for the assays. No NAD^+ reductase activity was detected, and no evidence of IMP degradation was observed upon prolonged incubation. NADH oxidase activity was negligible; initial velocities obtained by monitoring the formation of either NADH or XMP correlated within experimental error.

Assay of IMP Dehydrogenase. The 40% $(NH_4)_2SO_4$ precipitate was redissolved in a minimal amount of 0.05 M Tris buffer, pH 8.5. For K_i values, the standard assay in a 1-mL volume consists of 0.05 M Tris buffer, pH 8.5; 33.4 mM KCl; 1.5 mM glutathione; 0.8 mM NAD^+; 0.002 unit of crude enzyme; and concentrations of 10, 12.5, 20, 25, and 75 μM IMP for each concentration of inhibitor. Reactions were started at 25 °C with the addition of enzyme. Initial velocities were measured by following the increase in absorbance of NADH at 340 nm (ϵ 6.22 mM^{-1} cm^{-1}) on a Gilford 2600. In the determination of K_m, the formation of XMP was monitored at 290 nm in order to check the velocities obtained from following the formation of NADH at 340 nm. At least two inhibitor concentrations were used to determine K_i. For this enzyme preparation, the K_m of IMP and NAD^+ were 1.2×10^{-5} and 4.5×10^{-4} M, respectively. For I_{50}'s, IMP was present at 50 μM, and inhibitor concentrations were chosen to bracket 50% inhibition. I_{50}'s were then determined graphically. In duplicate determinations, values within 20% were routinely obtained.

Kinetic Data Analysis. Kinetic constants were determined by a weighted fit of the data to the linear competitive velocity equation using the nonlinear fitting routine of Cleland.[5] Also, K_m(app) and V_{max}(app) were calculated for each set of velocities and substrate concentrations at constant inhibitor concentrations by using the weighted fit to $V = S\cdot V_{max}/(K_m + S)$. These lines were then plotted on a graph with coordinates of $1/V$ vs. $1/S$. From this method the pattern of intersection of the lines determined the type of inhibition.

Synthetic Methods. UV spectra were determined on a Cary 118 or Gilford 2600. TLC were run on EM Laboratories silica gel 60 F-254 plates with either solvent system A (*i*-PrOH–concentrated NH_4OH–H_2O, 7:1:2) or system B (*n*-BuOH–AcOH–H_2O, 5:1:2). Elemental analyses were performed by the Microanalytical

(10) Streeter, D. G.; Witkowski, J. T.; Khare, G. P., Sidwell, R. W.; Bauer, R. J.; Robins, R. K.; Simon, L. N. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 1174.

Laboratory, Department of Chemistry, University of California, Berkeley. Proton NMR spectra of the compounds were taken on a Varian FT-80 at 80 MHz in either D_2O or Me_2SO-d_6 and were consistent with assigned structures. All the chromatographic, spectral, and analytical data of inosine derivatives are in Table I, and all the data of IMP analogues are in Table II. Melting points were corrected and were done on a Fisher-Johns apparatus.

2-Alkyl- and 2-Arylinosines (II). Method A. In 50 mL of 1 N NaOEt (prepared from sodium metal and anhydrous ethanol) was added 1 g (3.99 mmol) of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (Sigma Chemical Co., St. Louis, MO). The solution was heated and stirred until clear before the addition of the appropriate ester. After refluxing for 4 h, when TLC in 4:1 chloroform–methanol indicated completion of reaction, the hot ethanolic solution was evaporated to dryness and redissolved in water. The pH was adjusted to 3.0 with concentrated HCl, and the solution was extracted four to six times with either ether or chloroform. The aqueous layer was neutralized with NaOH and concentrated in vacuo until the product precipitated. If the product did not precipitate, chilling or addition of ethanol caused it to do so. Recrystallization was from either water or water–ethanol. Compounds prepared in this manner are shown in Table I.

2-(Alkylthio)inosines (IV). Method B. One equivalent of 2-mercaptoinosine[3] was dissolved in 5 mL of dry dimethylformamide, and 1 equiv of anhydrous K_2CO_3 was added. To this stirred solution was added 1 equiv of the appropriate aryl halide. After stirring at room temperature for 2 h, the solution was poured into 30 mL of water, the pH was adjusted to 6 with dilute HCl, and the solution was concentrated in vacuo and cooled if necessary. Filtration and recrystallization of the product from H_2O/EtOH gave the products shown in Table I.

Synthesis of IMP Analogues (V and VI). Inosine analogues were phosphorylated by the method of Yoshikawa et al.[4] To 0.25 mmol of the appropriate nucleoside in 1.2 mL of freshly distilled trimethyl phosphate at 0 °C was added dropwise $POCl_3$ (1 mmol) in approximately 100 μL of trimethyl phosphate and 4.5 μL (0.25 mmol) of H_2O (precooled to 0 °C). After stirring at 0 °C for 5 h, the solution was allowed to stir at room temperature for another 2 h or longer, until reaction was complete as monitored by TLC in BuOH–AcOH–H_2O, 5:1:2. The solution was poured onto ice and neutralized with concentrated NH_4OH. This solution was stored overnight in the refrigerator, extracted with ether to remove trimethyl phosphate, evaporated in vacuo, and then redissolved in 5 mL water. Two methods then were used to purify the resulting diammonium salt of the nucleotide, which are shown in Table II.

Method C. The solution was filtered, and if necessary, the pH was adjusted to 7.0 with dilute HCl. Then, 500-μL aliquots of the mixture were injected into a reverse-phase column (Lichrosorb RP-18, particle size 10 μm, 1 × 30 cm) using as the eluant water containing 0.5% methanol. Evaporation of the appropriate fractions in vacuo, followed by lyophilization, yielded pure analytical product.

Method D. The solution was adjusted to pH 8.2 with NaOH and passed through a column of boric acid gel (5 mL, Sigma Chemical Co.). Compounds with cis diols will form a borate complex with the column, while salts and nucleosides phosphorylated at either the 2′- or 3′-position on the sugar will wash through. After the column was washed with 300 mL of 1 N $(NH_4)_2CO_3$, pH 9.5, the nucleotide was eluted with distilled, deionized water (measured pH, 6.2). The product emerged after approximately 20 mL of eluant. This solution was evaporated in vacuo, then redissolved in water, and either lyophilized or precipitated by the addition of acetone; it was then filtered, and the filtrate was dried.

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Registry No. 1, 131-99-7; 2, 26550-86-7; 3, 85-32-5; 4, 88868-94-4; 5, 15000-04-1; 6, 88868-95-5; 7·2NH_3, 88868-78-4; 8·2NH_3, 88868-79-5; 9·2NH_3, 88868-80-8; 10·2NH_3, 88868-81-9; 11·2NH_3, 88868-82-0; 12·2NH_3, 88868-83-1; 14·2NH_3, 88868-85-3; 15·2NH_3, 88868-86-4; 16·2NH_3, 88868-84-2; 17·2NH_3, 88868-88-6; 18·2NH_3, 88868-89-7; 19·NH_3, 88868-91-1; 20·NH_3, 88868-93-3; 21·2NH_3, 88868-92-2; 22·2NH_3, 88868-90-0; II (R = $CH_3(CH_2)_4$), 88868-61-5; II (R = $CH_3(CH_2)_5$), 88868-62-6; II (R = $CH_3(CH_2)_6$), 88868-63-7; II (R = $CH_3(CH_2)_7$), 88868-64-8; II (R = $CH_3(CH_2)_8$), 88868-65-9; II (R = $CH_3(CH_2)_9$), 88868-66-0; II (R = $PhCH_2$), 88868-67-1; II (R = 3-c-NC_5H_4), 88868-68-2; II (R = p-OCH_3Ph), 56489-60-2; II (R = PhCH=CH), 88868-69-3; II (p-OCH_3PhCH_2), 88868-70-6; II (R = 3-c-NC_5H_4-CH_2), 88868-71-7; IV (R = m-NO_2PhCH_2S), 88868-72-8; IV (R = 2-Cl-4-NO_2PhCH_2S), 88868-73-9; IV (R = p-NO_2PhCH_2S), 88868-74-0; IV (R = o-$ClPhCH_2S$), 88868-75-1; IV (R = 3,5-$(NO_2)_2PhCH_2S$), 88868-76-2; V (R = $CH_3(CH_2)_4$), 88868-77-3; V (R = p-OCH_3PhCH_2), 88868-87-5; 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide, 2627-69-2; 2-mercaptoinosine, 6544-32-7; IMP dehydrogenase, 9028-93-7.

2,3,4,4a,5,9b-Hexahydro-1*H*-indeno[1,2-*b*]pyridines: Potential Antidepressants

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The synthesis of various diastereoisomeric H_{4a},H_5-*cis*,H_{4a},H_{9b}-*cis*- and H_{4a},H_5-*trans*,H_{4a},H_{9b}-*cis*-2,3,4,4a,5,9b-hexahydro-1*H*-indeno[1,2-*b*]pyridines is described, as well as the evaluation of their antidepressant potency. Elucidation of structure–activity relationships revealed the H_{4a},H_5-trans compounds as being by far the more active of the two series of diastereoisomers. Pharmacological and biochemical data suggest that these compounds are potential antidepressants with central stimulating properties, which are characterized by strong norepinephrine and dopamine reuptake inhibition.

The antidepressant activity of the unsubstituted 5-phenyl-2,3,4,4a,5,9b-hexahydro-1*H*-indeno[1,2-*b*]pyridines has been reported, but no data on structure–activity relationships in this series have been available so far.[1] This structure was chosen as a lead in our effort to find new antidepressants with improved tolerability and potency and minimized side effects.

The lack of a suitable synthesis for 1*H*-indeno[1,2-*b*]-pyridines, allowing a broad variation of substituents, prompted us to look for a more versatile synthetic procedure. After the development of an appropriate method,[2]

(1) Augstein, J.; Ham, A. L.; Leeming, P. R. *J. Med. Chem.* **1972**, *15*, 466.

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