(*RS*)-α-Hydroxy-α-(methoxymethyl)-α-phenylacetate (7a). A solution of 5.12 g (0.04 mol) of (*RS*)-3-quinuclidinol (6) in 200 mL of anhydrous benzene was heated at reflux for 1 h (Dean-Stark trap used to remove traces of water), and then 0.4 g of sodium was added and the mixture was refluxed with stirring for 1 h. After removal of the remaining sodium, 6 g (0.027 mol) of 5a was added and the reaction mixture was heated again at reflux for 24 h. After the solvent was removed, the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water, and dried over magnesium sulfate. After removal of the solvent, an oil remained. The oxalate salt was recrystallized from ethanol/petroleum ether to yield 4.2 g (39%). Mp: 142–152 °C. TLC [oxalate; silica gel, MeOH/NH_4OH (98:2)]: R_f 0.4. IR (oxalate, KBr): 3424, 2940, 1737 cm^{-1}. Anal. ($C_{19}H_{25}NO_8$) C, H, N.

Tissue Preparation. The muscarinic acetylcholine receptor was prepared as previously described.[22] Brains were removed from freshly decapitated male Sprague–Dawley rats (200–250 g) and immediately placed on ice. The corpus striatum (CS) was dissected, immediately frozen, and stored at −80 °C until used. Receptors prepared from tissue stored up to 1 year exhibit the same binding properties as that of freshly prepared samples. Samples of 0.15–0.2 fg of CS were homogenized in 20 mL of ice-cold 0.9% saline containing 10 mM Tris buffer (pH 7.4) and 10% sucrose (buffer I), using a Polytron PC-U (medium speed, two bursts of 15 s each). The membranes containing receptors were used without further purification. The 10% sucrose aids in maintaining a uniform suspension of the homogenate while sampling. The concentration of m-AChR was ca. 1 nM. Upon diluting in the assay system, the final concentration of receptor was approximately 20 pM.

Determination of Apparent Equilibrium Association Constants. The apparent equilibrium association constants (K_A) for the muscarinic ligands presented in Table I were determined by competitive ligand binding assay using [^{3}H]QNB as the radiotracer.[13] The compounds were dissolved in 100% EtOH and added to 4 mL of Tris-buffered (10 mM, pH 7.4) 0.9% saline containing 2.5×10^{-10} M [^{3}H]QNB at a final concentration of 0.5% EtOH. Concentrations of EtOH <2% do not affect the binding parameters of QNB to the m-AChR. Competition curves were generated by using 12 concentrations of unlabeled compound from 10^{-12} to 10^{-6} M for (±)-QNB and compounds that exhibited affinities within 5-fold of QNB, and from 10^{-10} to 10^{-5} M for compounds with affinities that differed from that of QNB by greater than 5-fold. Aliquots of 0.1 mL of tissue preparation were added, and the mixture was vortexed and incubated at room temperature for 2 h. The incubation mixture was rapidly filtered on a GF/C filter paper, washed with 10 mL of ice-cold saline, air-dried, placed in Ecoscint A (National Diagnostics) scintillation cocktail, and counted for 5 min each. Data were analyzed by using the LIGAND program of Munson and Rodbard.[23] K_A values were obtained by using pooled data of at least five determinations in duplicate on separate days.

Acknowledgment. This work was supported in part by Grant NS22215 from the National Institutes of Health, DHEW, and by Contract DE FG05 82ER60649 from the Department of Energy. NMR spectra were obtained by Mrs. Biyun Jin.

Supplementary Material Available: Tables listing IR data of compounds **4a–g** and IR and NMR data of compounds **5a–g** and **7a–g** (3 pages). Ordering information is given on any current masthead page.

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2-Phenyl-3*H*-imidazo[4,5-*b*]pyridine-3-acetamides as Non-Benzodiazepine Anticonvulsants and Anxiolytics†

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A series of 2-phenyl-3*H*-imidazo[4,5-*b*]pyridine-3-acetamides were designed and synthesized as non-benzodiazepine anxiolytics based on a molecular disconnection of a typical 1,4-benzodiazepine (BZD). A number of these compounds showed submicromolar potency in a [^{3}H]benzodiazepine binding assay in vitro and good potency in protecting rodents against pentylenetetrazole-induced seizures. Compound **84** appears to be a selective anticonvulsant (pentylenetetrazole) agent when tested against a profile of chemically and electrically induced seizures in mice. In addition, compound **148** appears to be a selective anxiolytic/hypnotic agent on the basis of biochemical and pharmacological characterization. It appears to be a full BZD agonist as assessed by GABA shift ratio and to be effective in punishment and nonpunishment animal models of anxiety. In addition, it shows a lower side-effect profile than diazepam as assessed by rotorod neurotoxicity and potentiation of ethanol-induced sleep time in mice. The chemistry and structure-activity relationships of this series is discussed.

The benzodiazepines (BZDs) are currently the agents of choice in the clinical treatment of anxiety, but undesirable side effects such as ataxia, sedation, psychological dependence, and a synergistic effect with central nervous system depressants has prompted a search for a non-benzodiazepine anxiolytic which would be free of these effects. Several compounds have shown potential for activity during the last decade including CL-218,872 (I),[1] zopiclone (II),[2] zolpidem (III),[3] CGS-9896 (IV),[4] and bu-

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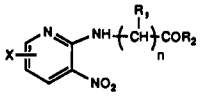
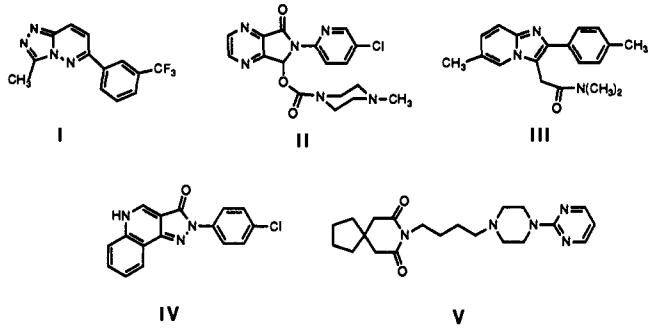
Table I. 3-Nitropyridyl Intermediates



no.	X	R_1	R_2	n	mp, °C (solv)[a]	% yield[b]	formula[c]
1	H	H	OC_2H_5	1	38–39	90	$C_8H_{11}N_3O_4$
2	H	CH_3 (*S*)	OC_2H_5	1	oil	79	$C_{10}H_{13}N_3O_4$
3	H	CH_3 (*R*)	OCH_3	1	39–40	85	$C_8H_{11}N_3O_4$
4	H	H	OC_2H_5	2	137–140 (k)	83	$C_{10}H_{13}N_3O_4 \cdot HCl$
5	6-Cl	H	OC_2H_5	1	78–80 (l)	45	$C_8H_{10}N_3O_4Cl$
6	5-Cl	H	OC_2H_5	1	73–75 (m)	47	$C_8H_{10}N_3O_4Cl$
7	H	H	NH_2	1	250–251	73	$C_7H_8N_4O_3$

[a] k = acetonitrile, l = isopropyl ether, m = petroleum ether. [b] The general method of preparation was method A, described in the Experimental Section. [c] Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values.

Chart I

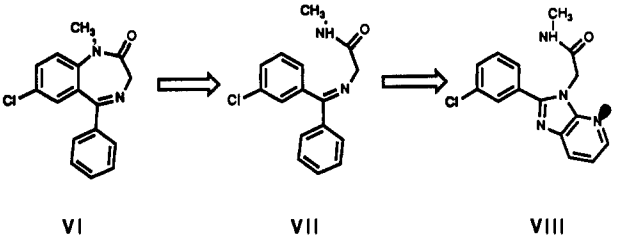


I II III IV V

spirone (V)[5] (Chart I), as well as others that are less defined pharmacologically. With the exception of V, all of these compounds exert potency against pentylenetetrazole (PTZ) induced seizures, nanomolar potency in a tritiated-BZD binding assay, and activity in a punishment-type conflict procedure.

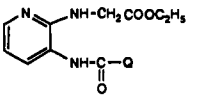
Compounds II and III have found more use as sedative–hypnotics than as anxiolytics and have received market approval in a number of countries.[6,7] Clinical trials are underway with IV, while V, whose mechanism of action does not involve BZD-receptor phenomena, has received wide market approval. However, none of these agents have displaced the BZDs as the agents of choice in the treatment of anxiety. Thus, the discovery of an anxioselective agent which does not produce side effects is still an unfulfilled goal. In 1980, before the disclosure of the imidazo[1,2-*a*]pyridine zolpidem (III), we designed a series of imidazo[4,5-*b*]pyridine-3-acetic acid esters and amides as potential anxiolytics on the basis of perceived structural requirements of the BZD binding site(s), which had been identified earlier.[8,9] This project culminated in the synthesis of over 300 compounds and the structure–activity relationships of this series are the subject of this paper.

Chart II



VI VII VIII

Table II. 3-Benzamidopyridyl Intermediates



no.	Q	mp, °C (solv)[a]	% yield[b]	formula[c]
9	C_6H_5	115–116 (n)	83	$C_{16}H_{17}N_3O_3$
10	$3\text{-}ClC_6H_4$	143–144 (wx)	73	$C_{16}H_{16}N_3O_3Cl$
11	$4\text{-}ClC_6H_4$	133–134 (wz)	87	$C_{16}H_{16}N_3O_3Cl$
12	$4\text{-}BrC_6H_4$	153–154 (s)	80	$C_{16}H_{16}N_3O_3Br$
13	$4\text{-}CH_3OC_6H_4$	130–131 (wx)	30	$C_{17}H_{18}N_3O_4$
14	$4\text{-}FC_6H_4$	127–129 (rz)	64	$C_{16}H_{16}N_3O_3F$
15	$4\text{-}NO_2C_6H_4$	151–154 (r)	95	$C_{16}H_{16}N_4O_5$
16	$4\text{-}CF_3C_6H_4$	155–156 (rt)	93	$C_{17}H_{16}N_3O_3F_3$
17	$4\text{-}CH_3C_6H_4$	131–132 (rz)	20	$C_{17}H_{19}N_3O_3$
18	$2,4\text{-}Cl_2C_6H_3$	124–126 (rz)	68	$C_{16}H_{15}N_3O_3Cl_2$
19	$2\text{-}C_5H_4N$[d]	95–96 (z)	35	$C_{16}H_{16}N_4O_3$
20	$5\text{-}Cl\text{-}2\text{-}C_5H_4N$[e]	78–80 (p)	24	$C_{15}H_{15}N_4O_3Cl$
21	$2\text{-}C_4H_3O$[f]	91–100 (rz)	80	$C_{14}H_{15}N_3O_4 \cdot H_2O$
22	$5\text{-}Br\text{-}2\text{-}C_4H_2O$[g]	78–81 (rz)	62	$C_{14}H_{14}N_3O_4Br \cdot H_2O$
23	$5\text{-}Br\text{-}2\text{-}C_4H_2S$[h]	95–105 (qx)	20	$C_{14}H_{14}N_3O_3SBr$
24	$3\text{-}C_4H_3S$[i]	131–134 (rz)	64	$C_{14}H_{15}N_3O_3S$
25	$3\text{-}C_4H_3O$[j]	125–127 (rz)	53	$C_{14}H_{15}N_3O_4$
26	$CH_2\text{-}4\text{-}ClC_6H_4$	141–143 (r)	95	$C_{17}H_{18}N_3O_3Cl$

[a] n = ether, o = acetone, p = hexane, q = ethyl acetate, r = isopropyl alcohol, s = ethanol, t = isopropyl ether, w = tetrahydrofuran, x = petroleum ether, y = methanol, z = water. [b] The general method of preparation was method B, described in the Experimental Section. [c] Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [d] 2-Pyridyl group. [e] 5-Chloro-2-pyridyl group. [f] 2-Furanyl group. [g] 5-Bromo-2-furanyl group. [h] 5-Bromo-2-thienyl group. [i] 3-Thienyl group. [j] 3-Furanyl group.

The initial hypothesis for the design of the imidazo-[4,5-*b*]pyridine-3-acetamides involved first, a disconnection of the 1,4-benzodiazepine structure between the benzo ring and N-1 nitrogen (VII) (Chart II). Structures similar to VII have been reported to have anticonvulsant and antianxiety properties.[10] Second, fusion of the imidazo[4,5-*b*]pyridine system to the open chain (VII) at the C–N imine

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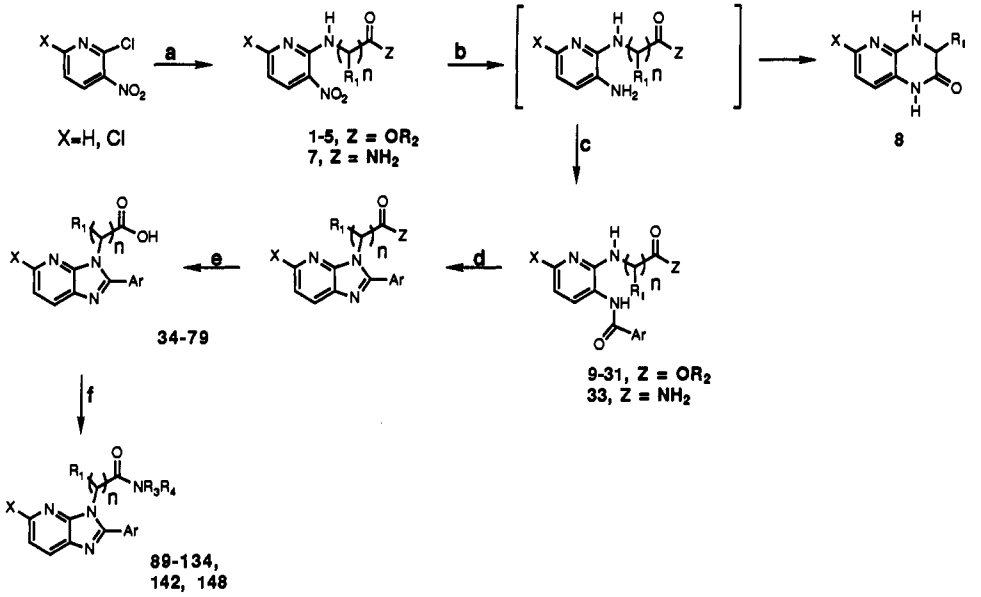
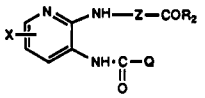
Scheme I[a]



[a] Reagents: (a) $NH_2(CHR_1)_nCO_2R_2 \cdot HCl$, NEt_3, Δ; (b) 5% Pd/C or PtO_2, THF, room temperature; (c) ArCOCl, NEt_3; (d) Δ, neat or ethylene glycol; (e) NaOH, H_2O/EtOH; (f) $ClCO_2C_2H_5$, $SOCl_2$, or 1,1′-carbonyldiimidazole, NEt_3, HNR_3R_4.

Table III. 3-Benzamidopyridyl Intermediates



no.	X	Z	R_2	Q	mp, °C (solv)[a]	% yield[b]	formula[c]
27	H	(*S*)-CH(CH$_3$)	OC_2H_5	4-ClC_6H_4	172–177 (rt)	66 (C)	$C_{17}H_{18}N_3O_3Cl \cdot HCl$
28	H	(*R*)-CH(CH$_3$)	OCH_3	4-ClC_6H_4	125–134 (rx)	19 (C)	$C_{16}H_{16}N_3O_3Cl$
29	6′-Cl	CH_2	OC_2H_5	4-ClC_6H_4	157–159 (rz)	23 (D)	$C_{16}H_{15}N_3O_3Cl_2$
30	6′-Cl	CH_2	OC_2H_5	4-$CH_3C_6H_4$	155–158 (rt)	30 (D)	$C_{17}H_{18}N_3O_3Cl$
31	H	C_2H_4	OC_2H_5	4-ClC_6H_4	124–125 (wx)	22 (B)	$C_{17}H_{18}N_3O_3Cl$
32	5′-Cl	CH_2	OC_2H_5	4-ClC_6H_4	157–158 (rz)	35 (D)	$C_{16}H_{15}N_3O_3Cl_2$
33	H	CH_2	NH_2	4-ClC_6H_4	194–195 (s)	44 (E)	$C_{14}H_{13}N_4O_2Cl \cdot 0.5H_2O$

[a] r = isopropyl alcohol, s = methanol, t = isopropyl ether, w = tetrahydrofuran, x = petroleum ether, z = water. [b] Letters refer to methods of preparation described in the Experimental Section. [c] Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values.

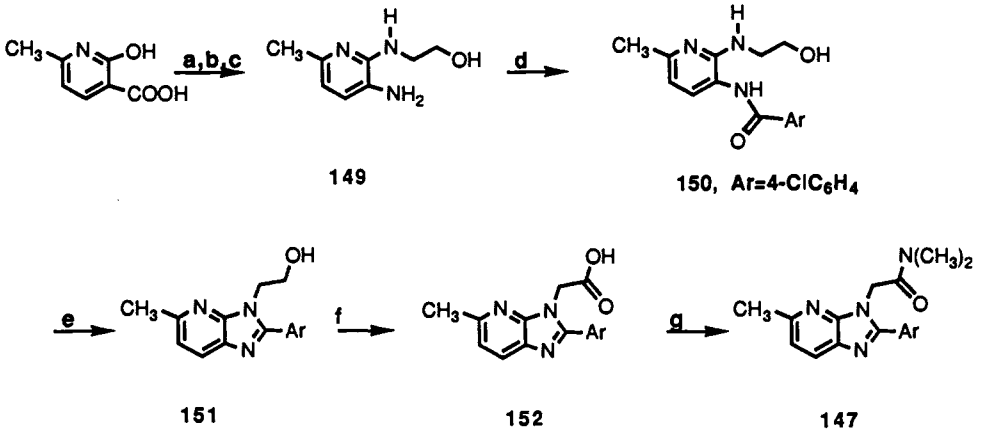
system would provide an electron-rich mimicry of the imine moiety (VIII). In addition, the lone pair on the pyrido nitrogen of the imidazo[4,5-*b*]pyridine system may provide dipolar hindrance to free rotation of the acetamide moiety to mimic the conformation of the azepine ring system of VI. A phenyl substituent at position 2 of the imidazo[4,5-*b*]pyridine system should overlap the benzo ring of the BZDs.

Chemistry

The target 3*H*-imidazo[4,5-*b*]pyridine-3-acetamides were prepared by the regiospecific synthesis outlined in Scheme I. The reaction of 2-chloro-3-nitropyridine with amino acid esters or amides gave key intermediates **1–5** and **7** (Table I). Reduction of the nitro group with either 5% Pd/C or PtO_2 (to avoid dehalogenation where X = Cl) gave the intermediate amines, which tended to cyclize to **8**. It was expedient, therefore, to acylate immediately to obtain 3-benzamidopyridyl intermediates **9–33** (Tables II and III). The cyclization to the imidazo[4,5-*b*]pyridine ring system was effected by heating **9–33** neat in a Wood's metal bath at 180–240 °C or by refluxing in ethylene glycol. The imidazo[4,5-*b*]pyridine-3-acetic acids, listed in Tables IV and V, were prepared by basic hydrolysis. The majority of the amides (**89–134**, **142**, and **148**) (Tables VI, VII, and IX) were prepared from the corresponding carboxylic acids by one of several activation methods, such as 1,1′-carbonyldiimidazole, thionyl chloride, or mixed anhydride. The isomeric imidazo[4,5-*c*]pyridine-1-acetamide **139** was prepared as outlined in Scheme VI, by the reaction of 4-chloro-3-nitropyridine with ethyl glycinate to give **157**. Because of the tendency to cyclize immediately upon reduction, intermediate amide **158** was prepared. Reduction and acylation of **158** produced diacylated product **159**, which could be cyclized to **139** upon heating.

The 5-chloroimidazo[4,5-*b*]pyridines were prepared from the 2,6-dichloro-3-nitropyridine by the methods shown in Scheme I. The 5-methyl-substituted compounds were prepared as shown in Scheme II. The reaction of commercially available 2-hydroxy-6-methylpyridine-3-carboxylic acid with phosphoryl chloride and phosphorus pentachloride followed by ammonium hydroxide gave 2-chloro-6-methylpyridine-3-carboxamide, which underwent a Hoffman rearrangement with bromine and sodium hydroxide to give 2-chloro-6-methyl-3-pyridinamine. The pyridinamine was reacted with 2-aminoethanol to give diamine **149**, which was converted to the 2-substituted phenylimidazo[4,5-*b*]pyridine-3-ethanol **151** according to the previously outlined steps. 5-Methyl acid **152** was prepared by $KMnO_4$ oxidation of the alcohol and converted to amide **147** by the procedures already outlined. 6-Chloro target was prepared as shown in Scheme III.

Scheme II[a]

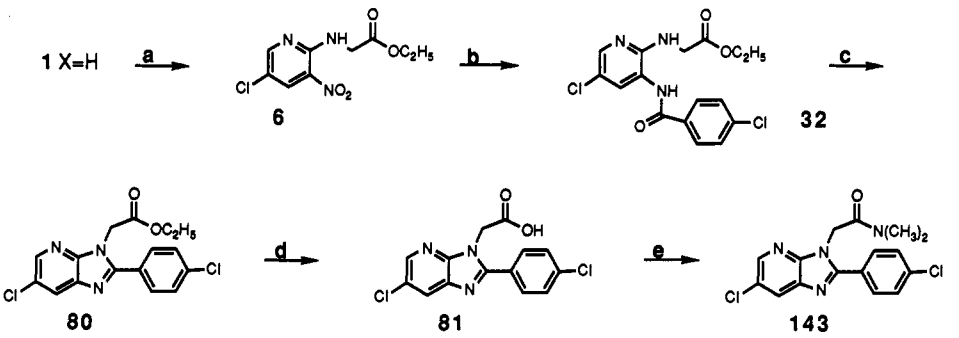


149 150, Ar=4-ClC$_6$H$_4$

151 152 147

[a] Reagents: (a) $POCl_3$, PCl_5, NH_2OH; (b) Br_2, NaOH; (c) $H_2NCH_2CH_2OH$, neat, Δ; (d) 4-ClC_6H_4COCl, NEt_3; (e) ethylene glycol, CH_3SO_3H, Δ; (f) $KMnO_4$; (g) $SO(Cl)_2$, $NH(CH_3)_2$.

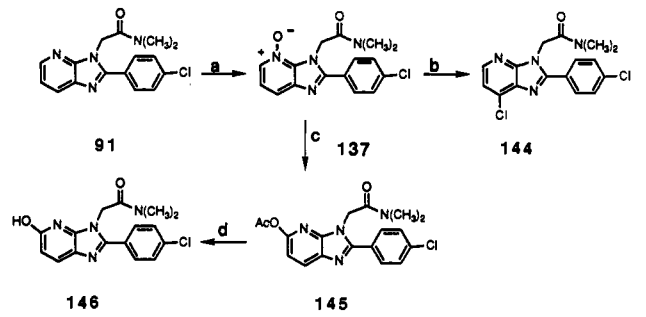
Scheme III[a]



6 32 80 81 143

[a] Reagents: (a) NaOCl; (b) PtO_2, then 4-chlorobenzoyl chloride; NEt_3; (c) Δ, ethylene glycol; (d) NaOH, H_2O/EtOH; (e) 1,1′-carbonyldiimidazole, NEt_3, $HN(CH_3)_2$.

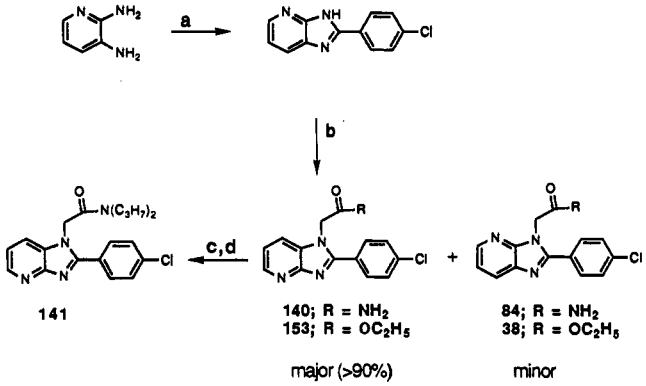
Scheme IV[a]



91 137 144 146 145

[a] Reagents: (a) *m*-ClPBA; (b) $POCl_3$; (c) $(CH_3CO)_2O$, Δ; (d) NaOH, H_2O.

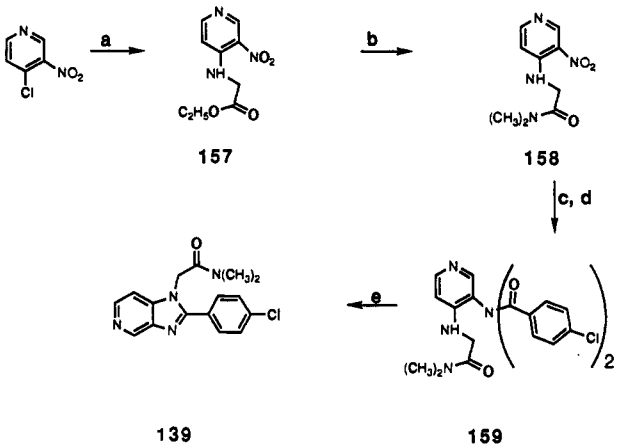
Scheme V[a]



141 140; R = NH_2; 153; R = OC_2H_5 (major (>90%)) 84; R = NH_2; 38; R = OC_2H_5 (minor)

[a] Reagents: (a) 4-ClC_6H_4COOH, PPA, Δ; (b) NaH, $BrCH_2COOC_2H_5$; (c) NaOH, H_2O; (d) $ClCO_2C_2H_5$, NEt_3, $HN(C_3H_7)_2$.

Scheme VI[a]



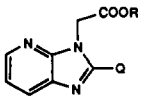
157 158 139 159

[a] Reagents: (a) $NH_2CH_2CO_2C_2H_5$·HCl, NEt_3; (b) $HN(CH_3)_2$; (c) 5% Pd/C, THF, 50 °C; (d) ArCOCl, NEt_3; (e) Δ, neat.

Nitro intermediate **1**, when treated with sodium hypochlorite, chlorinated in position 5. This intermediate was carried through the reactions outlined in Scheme I to give compound **143**. 7-Chloro compound **144** was the only chlorinated product isolated in a low yield from the reaction of phosphoryl chloride with *N*-oxide **137** outlined in Scheme IV. Reaction of *N*-oxide **137** with acetic anhydride gave 5-acetate **145**, which gave 5-hydroxy compound **146** upon basic hydrolysis.

Isomeric imidazo[4,5-*b*]pyridine-1-acetamides **140** and **141** and imidazo[4,5-*c*]pyridine-3-acetamide **138** were prepared as shown in Scheme V. Commercially available 2,3- and 3,4-diaminopyridines were reacted with substi-

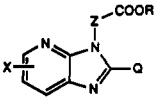
Table IV. 3*H*-Imidazo[4,5-*b*]pyridine-3-acetic Acids and Esters



no.	R	Q	mp, °C (solv)[a]	% yield[b]	formula[c]
34	C_2H_5	C_6H_5	93–95 (p)	38 (F)	$C_{16}H_{15}N_3O_2$
35	H	C_6H_5	239–245 (y)	50 (I)	$C_{14}H_{11}N_3O_2$
36	C_2H_5	3-ClC_6H_4	98–100 (wx)	44 (F)	$C_{16}H_{14}N_3O_2Cl$
37	H	3-ClC_6H_4	214–216 (s)	80 (H)	$C_{14}H_{10}N_3O_2Cl$
38	C_2H_5	4-ClC_6H_4	123–124 (wx)	48 (F)	$C_{16}H_{14}N_3O_2Cl$
39	H	4-ClC_6H_4	271–273 (yz)	58 (H)	$C_{14}H_{10}N_3O_2Cl$
40	C_2H_5	4-BrC_6H_4	138–139 (st)	49 (F)	$C_{16}H_{14}N_3O_2Br$
41	H	4-BrC_6H_4	264–269 (wy)	84 (H)	$C_{14}H_{10}N_3O_2Br$
42	C_2H_5	4-$CH_3OC_6H_4$	107–108 (wx)	30 (F)	$C_{17}H_{17}N_3O_3$
43	H	4-$CH_3OC_6H_4$	260–263 (s)	85 (I)	$C_{15}H_{13}N_3O_3$
44	C_2H_5	4-FC_6H_4	126–128 (rt)	19 (G)	$C_{16}H_{14}N_3O_2F$
45	H	4-FC_6H_4	266–268 (wy)	87 (H)	$C_{14}H_{10}N_3O_2F$
46	C_2H_5	4-$NO_2C_6H_4$	192–194 (sz)	71 (G)	$C_{16}H_{14}N_4O_4$
47	H	4-$NO_2C_6H_4$	249–251 (y)	24 (I)	$C_{14}H_{10}N_4O_5 \cdot H_2O$
48	C_2H_5	4-$CF_3C_6H_4$	147–148 (o)	15 (F)	$C_{17}H_{14}N_3O_2F_3$
49	H	4-$CF_3C_6H_4$	240–241 (sz)	75 (H)	$C_{15}H_{10}N_3O_2F_3$
50	C_2H_5	4-$CH_3C_6H_4$	117–119 (rz)	29 (G)	$C_{17}H_{17}N_3O_2$
51	H	4-$CH_3C_6H_4$	262–264 (y)	44 (I)	$C_{15}H_{13}N_3O_2$
52	C_2H_5	2,4-$Cl_2C_6H_3$	76–80 (tx)	18 (G)	$C_{16}H_{13}N_3O_2Cl_2$
53	H	2,4-$Cl_2C_6H_3$	186–188 (yz)	77 (I)	$C_{14}H_9N_3O_2Cl_2 \cdot 0.5H_2O$
54	C_2H_5	2-C_5H_4N[d]	109–110 (sz)	31 (F)	$C_{15}H_{14}N_4O_2$
55	H	2-C_5H_4N[d]	224–225 (sz)	72 (H)	$C_{13}H_{10}N_4O_2$
56	C_2H_5	5-Cl-2-C_5H_3N[e]	99–101 (yz)	21 (F)	$C_{15}H_{13}N_4O_2Cl$
57	H	5-Cl-2-C_5H_3N[e]	218–220 (z)	58 (H)	$C_{13}H_9N_4O_2Cl$
58	C_2H_5	2-C_4H_3O[f]	102–105 (t)	52 (G)	$C_{17}H_{16}N_3O_4 \cdot 0.5$hydroquinone
59	H	2-C_4H_3O[f]	>250 (y)	48 (I)	$C_{12}H_9N_3O_3$
60	C_2H_5	5-Br-2-C_4H_2O[g]	123–125 (rz)	26 (G)	$C_{14}H_{12}N_3O_3Br$
61	H	5-Br-2-C_4H_2O[g]	229–231 (yz)	43 (I)	$C_{12}H_8N_3O_3Br$
62	C_2H_5	5-Br-2-C_4H_2S[h]	148–150 (rt)	21 (G)	$C_{14}H_{12}N_3O_2BrS$
63	H	5-Br-2-C_4H_2S[h]	255–258 (yz)	51 (I)	$C_{12}H_8N_3O_2BrS$
64	C_2H_5	3-C_4H_3S[i]	129–131 (rt)	19 (G)	$C_{14}H_{13}N_3O_2S$
65	H	3-C_4H_3S[i]	>250 (y)	41 (I)	$C_{12}H_9N_3O_2S$
66	C_2H_5	3-C_4H_3O[j]	117–120 (rt)	22 (G)	$C_{14}H_{13}N_3O_3$
67	H	3-C_4H_3O[j]	>250 (y)	22 (I)	$C_{12}H_9N_3O_3$
68	C_2H_5	CH_2-4-ClC_6H_4	150–151 (y)	28 (G)	$C_{17}H_{16}N_3O_2Cl$
69	H	CH_2-4-ClC_6H_4	>250 (y)	35 (I)	$C_{15}H_{12}N_3O_2Cl \cdot CH_3OH$

[a] p = sublimates, q = hexane, r = isopropyl alcohol, s = ethanol, t = isopropyl ether, w = tetrahydrofuran, x = petroleum ether, y = methanol, z = water. [b] Letters refer to methods of preparation described in the Experimental Section. [c] Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [d] 2-Pyridyl group. [e] 5-Chloro-2-pyridyl group. [f] 2-Furanyl group. [g] 5-Bromo-2-furanyl group. [h] 5-Bromo-2-thienyl group. [i] 3-Thienyl group. [j] 3-Furanyl group.

Table V. 3*H*-Imidazo[4,5-*b*]pyridine-3-acetic Acids and Esters



no.	X	Z	R_2	Q	mp, °C (solv)[a]	% yield[b]	formula[c]
70	H	(*S*)-CH(CH_3)	C_2H_5	4-ClC_6H_4	103–106 (sz)	68 (G)	$C_{17}H_{16}N_3O_2Cl$
71	H	(*S*)-CH(CH_3)	H	4-ClC_6H_4	226–229 (yz)	64 (I)	$C_{15}H_{12}N_3O_2Cl$
72	H	(*R*)-CH(CH_3)	C_2H_4OH	4-ClC_6H_4	110–113 (rt)	40 (G)	$C_{17}H_{16}N_3O_3Cl$
73	H	(*R*)-CH(CH_3)	H	4-ClC_6H_4	225–227 (rz)	39 (I)	$C_{15}H_{12}N_3O_2Cl$
74	5′-Cl	CH_2	C_2H_5	4-ClC_6H_4	144–146 (t)	23 (F)	$C_{16}H_{13}N_3O_2Cl_2$
75	5′-Cl	CH_2	H	4-ClC_6H_4	>250 (yz)	54 (I)	$C_{14}H_9N_3O_2Cl_2$
76	5′-Cl	CH_2	C_2H_5	4-$CH_3C_6H_4$	148–152 (rz)	12 (F)	$C_{17}H_{16}N_3O_2Cl$
77	5′-Cl	CH_2	H	4-$CH_3C_6H_4$	>250 (y)	14 (H)	$C_{15}H_{12}N_3O_2Cl$
78	H	C_2H_4	C_2H_5	4-ClC_6H_4	57–59 (yz)	37 (F)	$C_{17}H_{16}N_3O_2Cl$
79	H	C_2H_4	H	4-ClC_6H_4	232–234 (y)	37 (F)	$C_{15}H_{12}N_3O_2Cl$
80	6′-Cl	CH_2	C_2H_5	4-ClC_6H_4	160–162 (rt)	16 (G)	$C_{16}H_{13}N_3O_2Cl_2$
81	6′-Cl	CH_2	H	4-ClC_6H_4	>250 (yz)	21 (I)	$C_{14}H_9N_3O_2Cl_2$

[a] r = isopropyl alcohol, s = ethanol, t = isopropyl ether, y = methanol, z = water. [b] Letters refer to methods of preparation described in the Experimental Section. [c] Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values.

tuted benzoic acids in polyphosphoric acid to give imidazopyridines.[11] Alkylation of the imidazo[4,5-*b*]pyridine with ethyl bromoacetate or ethyl bromoacetamide and a base gave 1-alkylated acetic acid ester **153** or amide **140** as the major product, isolated in greater than 90% yield. Acetic acid ester **153** was converted to amide **141** by the methods discussed earlier. The corresponding imidazo-

(11) Garmaise, D. L.; Komlossy, J. The preparation of 2-arylimidazo[4,5-*b*]pyridines. *J. Org. Chem.* **1964**, *29*, 3403–3405.

[4,5-c]pyridine gave ester 154 as the major product, which was hydrolyzed to acid 155 and converted to dimethylamide 138 by the methods discussed earlier.

Results and Discussion

The primary screening data for the target compounds are compiled in Tables VI–IX. The biochemical data consisted of the inhibition of [^{3}H]benzodiazepine binding with either diazepam or flunitrazepam as the tritiated ligand and reported as an IC_{50} (nM). The pharmacological data measured the ability of the test compound to protect against convulsions induced chemically by pentylenetetrazole (PTZ). Anticonvulsant activity, especially against PTZ, is a hallmark of BZD agonists, such as the 1,4-benzodiazepines.[12] While there is evidence that there is a correlation between BZD receptor occupancy and anti-PTZ activity, there are several factors, such as solubility, agonism/antagonism, and efficacy, as well as absorption, distribution, metabolism, and excretion (ADME), which could provide noncorrelation between the in vitro and in vivo data.[13] Thus, the discussion of structure–activity relationships (SAR) will focus on the receptor binding potencies, while the anticonvulsant activity will be discussed in congeneric series.

The initial hypothesis involved a molecular disconnection which would predict that amides should be the active moiety. Indeed, in the 4-chlorophenyl series, the corresponding carboxylic acid and esters [OC_2H_5, OCH_3, O-*t*-Bu, and O-*i*-Pr] were inactive at the BZD receptor. Only the amides in the 4-chlorophenyl series showed low micromolar potencies (e.g. 84, 91, and 107).

The molecular disconnection hypothesis would predict a 3-substituted phenyl group at the 2-position of the imidazopyridine ring system. However, the observed SAR is ambiguous. A comparison of the 3 vs 4-chlorophenyl compounds indicates micromolar binding activity for the 4-chlorophenyl in the primary amide series (83 vs 84) and for both the 3- and 4-chlorophenyl in the dimethyl amide series (90 and 91). However, in both the primary and tertiary amide series, the unsubstituted phenyl 82 and 89 were inactive. The weak anticonvulsant activity of both of the 3-chlorophenyl derivatives 83 and 90 mitigated against additional 3-substituted analogues. Among the 4-substituents, the following rank-order was observed in BZD binding potency: $CH_3 > Cl = Br = CF_3 > OCH_3 > H = F = NO_2$. This pattern may suggest an importance for a positive π contribution ($1 > \pi > 0.5$) and a smaller influence of electronic factors ($0.5 > \sigma_p > -0.2$). The 2,4-dichloro-substituted compound 98 was inactive in the BZD binding and anticonvulsant assays.

The heterocyclic variations in the 2-position 99–105 show dramatic binding differences. For example, 2-furanyl 101 was inactive, while the 5-bromo-2-furanyl 102 had an IC_{50} of 200 nM. The corresponding 5-bromo-2-thienyl 103 was 10-fold less potent than 102 in the binding assay. Despite its submicromolar potency, 102 was inactive as an anticonvulsant. A study of the antagonism of the protection by diazepam (5.62 mg/kg, ip) from maximal electroshock convulsions in mice indicated that 102 may be a BZD antagonist.[14] As expected, the 4-chloro benzyl analogue 106, which inserts a methylene moiety between the imidazopyridine ring system and the aromatic group, was inactive at the BZD receptor.

The nature of the amide moiety would not be predicted by the molecular disconnection hypothesis since both diazepam and *N*-desmethyldiazepam are active. A comparison of primary amides 84–88 indicates a large discrepancy between in vitro binding potency and in vivo anticonvulsant activity. This apparent discrepancy may be due to poor in vitro solubility of these primary amides, but adequate bioavailability via the intraperitoneal route. The high anti-PTZ potency of 84 prompted us to pursue this compound as an anticonvulsant through the NINDS epilepsy program. The anticonvulsant profile of 84 obtained from this program is shown in Table X, together with a comparison to known anticonvulsant agents. The profile of 84 indicated a selective anticonvulsant potency against PTZ similar to, but more potent than, valproic acid. Neurotoxicity, as defined by rotorod activity, was negligible. These results have provided the basis for further studies of 84, especially against absence epilepsy.[15]

In the 4-chlorophenyl series, secondary amides 107 and 108 were more potent at the BZD receptor than primary amide 84. However, tertiary amides varied considerably in BZD binding potency with di-*n*-propyl (110) (IC_{50} = 380 nM) > di-*n*-butyl (112) > diethyl (109) > dimethyl (91) (IC_{50} = 3600 nM). There is an apparent steric bulk limitation since the diisopropyl amide has no measurable BZD binding. Cyclic (114, 115), morpholine (116), and acylated piperazine amides (118, 119) provided moderate binding potency, while *N*-methylpiperazinamide 117 was inactive. This observation was also apparent in 100, which has structural similarities to zopiclone (II). Since 100 is inactive in the BZD binding assay, while zopiclone (II) has a reported IC_{50} of 50 nM, it probably indicates a mechanism of action for this series different from that of II.[16]

A series of secondary and tertiary amides were prepared, which incorporate basic, acidic, or aromatic groups, in order to probe for additional receptor binding interactions. All of the isomeric pyridyl amides (121–123) showed very potent binding compared to the inactive aniline 120. However, all of these had marginal anticonvulsant activity. These compounds were extremely insoluble and may not have been bioavailable. The series of carboxylate (124–127) and amino (128–131) alkyl analogues were inactive at the BZD receptor.

The molecular disconnection hypothesis would predict that side-chain variations would not be tolerated. Indeed, as shown in Table VII, the homologation to propionamide 134 significantly decreases the binding affinity compared to that of acetamide 91. Likewise, the side-chain should be sensitive to α-methyl substitution since this corresponds to the C-3 position of a typical 1,4-BZD. This position can tolerate heteroatom substituents such as SCH_3 and OH, but cannot tolerate alkyl substitution such as methyl.[17] In this series, both α-methyl enantiomers 132 and 133 were devoid of measurable binding activity.

The design of this series included the fusion of the imidazo[4,5-*b*]pyridine ring system in order to provide an

(12) Harvey, Stewart, C. Hypnotics and Sedatives. In *The Pharmacological Basis of Therapeutics*, 6th Ed.; Gilman, A. G., Goodman, L. S., Gilman, A., Macmillan Publishing Co: New York, 1980; pp 339–375.

(13) Igari, Y.; Sugiyama, Y.; Sawada, Y.; Iga, T.; Hanano, M. Kinetics of Receptor Occupation and Anticonvulsant Effects of Diazepam in Rats. *Drug Metab. Dispos.* 1985, *13*, 102–106.

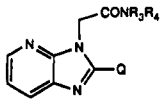
(14) Johnson, D. N., unpublished results.

(15) Johnson, D. N.; White, H. S.; Swinyard, E. A.; Albertson, T. E.; Kilpatrick, B. F. AHR-12245: A potential anti-absence drug. *Epilepsy Res.* 1991, *8*, 64–70.

(16) Trifiletti, R. R.; Snyder, S. H. Anxiolytic cyclopyrrolones zopiclone and suriclone bind to a novel site linked allosterically to benzodiazepine receptors. *Mol. Pharmacol.* 1984, *26*, 458–469.

(17) Sternbach, L. H.; Randall, L. O.; Banziger, R.; Lehr, H. Drugs Affecting the Central Nervous System. *Medicinal Research Series*; Burger, A., Ed.; Marcel Dekker: New York, 1968; Vol. 2, pp 237–264.

Table VI. 3H-Imidazo[4,5-b]pyridine-3-acetamides

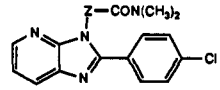


no.	R_3	R_4	Q	mp, °C (solv)[a]	% yield[b]	formula[c]	[^{3}H]Flu IC_{50},[d] nM	PTZ ED_{50},[e] mg/kg ip
82	H	H	C_6H_5	226–227 (z)	45 (N)	$C_{14}H_{12}N_4O$	(>10000)	na
83	H	H	3-ClC_6H_4	245–247 (yz)	66 (O)	$C_{14}H_{11}N_4OCl$	(<10000)	na
84	H	H	4-ClC_6H_4	270–271 (y)	53 (N)	$C_{14}H_{11}N_4OCl$	8000 [3]	4.1 [1.4–12.3]
85	H	H	4-BrC_6H_4	>250 (y)	54	$C_{14}H_{11}N_4OBr$	>10000	17.8 [11.1–28.5]
86	H	H	4-FC_6H_4	246–248 (yz)	68	$C_{14}H_{11}N_4OF\cdot H_2O$	>10000	na
87	H	H	4-$CF_3C_6H_4$	>250 (rt)	46	$C_{15}H_{11}N_4OF_3$	>10000	11.7 [5.9–23.4]
88	H	H	4-$CH_3C_6H_4$	252–253 (r)	61	$C_{15}H_{14}N_4O$	4500	~50.5
89	CH_3	CH_3	C_6H_5	160–163 (rt)	64	$C_{16}H_{16}N_4O$	>10000	35.0 [17.2–71.1]
90	CH_3	CH_3	3-ClC_6H_4	125–126 (z)	22	$C_{16}H_{15}N_4OCl\cdot 0.5H_2O$	(2400)	>56.2 <100
91	CH_3	CH_3	4-ClC_6H_4	216–220 (nw)	73 (J)	$C_{16}H_{15}N_4OCl\cdot HCl$	3600 [3]	4.1 [2.9–5.7]
92	CH_3	CH_3	4-BrC_6H_4	224–225 (s)	63	$C_{16}H_{15}N_4OBr$	(3000)	7.0 [5.3–9.3]
93	CH_3	CH_3	4-$CH_3OC_6H_4$	156–158 (r)	25	$C_{17}H_{16}N_4O_2$	8500	39.5 [24.7–55.3]
94	CH_3	CH_3	4-FC_6H_4	180–183 (rt)	39	$C_{16}H_{15}N_4OF$	>10000	40.5 [22.6–72.5]
95	CH_3	CH_3	4-$NO_2C_6H_4$	204–207 (r)	38	$C_{16}H_{15}N_5O_3$	>10000	47.0 [30.3–72.9]
96	CH_3	CH_3	4-$CF_3C_6H_4$	183–185 (mr)	37	$C_{17}H_{15}N_4OF_3$	(3700)	3.2 [1.3–5.1]
97	CH_3	CH_3	4-$CH_3C_6H_4$	182–183 (r)	53	$C_{17}H_{18}N_4O$	1750 [2]	4.7 [2.1–10.3]
98	CH_3	CH_3	2,4-$Cl_2C_6H_3$	192–199 (rt)	30	$C_{16}H_{14}N_4OCl_2\cdot HCl$	>10000	conv[f]
99	CH_3	CH_3	2-C_6H_4N[g]	171–174 (wy)	45	$C_{15}H_{15}N_5O\cdot HCl\cdot 0.5H_2O$	(2600)	
100	*N*-methylpiperazino		5-Cl-2-C_5H_3N[h]	174–175 (ox)	24 (M)	$C_{18}H_{19}N_6OCl$	(>10000)	na
101	CH_3	CH_3	2-C_4H_3O[i]	176–178 (rt)	64	$C_{14}H_{14}N_4O_2$	>10000	>100
102	CH_3	CH_3	5-Br-2-C_4H_2O[j]	182–185 (rz)	58	$C_{14}H_{13}N_4O_2Br\cdot 0.5H_2O$	200 [2]	100 (62.5%)
103	CH_3	CH_3	5-Br-2-C_4H_2S[k]	207–210 (rt)	47	$C_{14}H_{13}N_4OSBr$	2500	100 (62.5%)
104	CH_3	CH_3	3-C_4H_3S[l]	204–205 (rt)	49	$C_{14}H_{14}N_4OS$	>10000	38.0 [19.0–76.0]
105	CH_3	CH_3	3-C_4H_3O[m]	165–168 (rt)	57	$C_{14}H_{14}N_4O_2$	>10000	na
106	CH_3	CH_3	CH_2-4-ClC_6H_4	173–174 (r)	46	$C_{17}H_{17}N_4OCl$	>10000	*n*
107	H	CH_3	4-ClC_6H_4	239–240 (r)	54	$C_{15}H_{13}N_4OCl$	1600 [2]	12.5 [9.3–16.8]
108	H	*n*-C_3H_7	4-ClC_6H_4	220–221 (rz)	63	$C_{17}H_{17}N_4OCl$	(1260)	37.5 [26.8–52.5]
109	C_2H_5	C_2H_5	4-ClC_6H_4	155–156 (rz)	57	$C_{16}H_{19}N_4OCl\cdot 0.5H_2O$	1800 [2]	16.0 [13.3–19.2]
110	*n*-C_3H_7	*n*-C_3H_7	4-ClC_6H_4	156–157 (rz)	59	$C_{20}H_{23}N_4OCl$	380 [6]	11.0 [5.5–22.0]
111	*i*-C_3H_7	*i*-C_3H_7	4-ClC_6H_4	218–220 (rz)	12	$C_{20}H_{23}N_4OCl$[o]	>10000	na
112	*n*-C_4H_9	*n*-C_4H_9	4-ClC_6H_4	99–101 (rz)	48	$C_{22}H_{27}N_4OCl$	900 [4]	38.0 [25.7–56.2]
113	CH_3	*n*-C_3H_7	4-ClC_6H_4	130–133 (rz)	60	$C_{18}H_{19}N_4OCl$	680 [2]	11.6 [7.0–19.1]
114		$-(CH_2)_4-$	4-ClC_6H_4	224–225 (r)	60	$C_{18}H_{17}N_4OCl$	2700	17.9 [10.5–30.4]
115		$-(CH_2)_5-$	4-ClC_6H_4	176–178 (r)	59	$C_{19}H_{19}N_4OCl$	2700	56.2 [34.1–92.7]
116	morpholino		4-ClC_6H_4	213–216 (s)	72	$C_{18}H_{17}N_4O_2Cl$	4100	11.5 [7.2–18.3]
117	*N*-methylpiperazino		4-ClC_6H_4	190–196 (rt)	63	$C_{19}H_{20}N_5OCl\cdot 1.0C_4H_4O_4$	>10000	100 (62.5%)
118	*N*-acetylpiperazino		4-ClC_6H_4	217–219 (r)	57	$C_{20}H_{20}N_5O_2Cl$	6400	100 (50%)
119	*N*-carbethoxypiperazino		4-ClC_6H_4	184–186 (rz)	63	$C_{21}H_{22}N_5O_3Cl$	5100	na
120	H	C_6H_5	4-ClC_6H_4	240–242 (r)	66 (L)	$C_{20}H_{15}N_4OCl$	>10000	na
121	H	2-C_5H_4N[g]	4-ClC_6H_4	190–191 (lz)	20 (L)	$C_{16}H_{14}N_5OCl$	310 [4]	100 (75%)
122	H	3-C_5H_4N[q]	4-ClC_6H_4	217–218 (rt)	49 (L)	$C_{16}H_{14}N_5OCl\cdot 0.5H_2O$	(230) [2]	~100
123	H	4-C_5H_4N[r]	4-ClC_6H_4	>250 (r)	19 (L)	$C_{19}H_{14}N_5OCl\cdot 0.5H_2O$	470 [2]	na
124	H	$CH_2CO_2C_2H_5$	4-ClC_6H_4	191–192 (sz)	43	$C_{16}H_{17}N_4O_3Cl$	(9100)	na
125	H	CH_2COOK	4-ClC_6H_4	>300 (s)	55 (P)	$C_{16}H_{12}N_4O_3ClK\cdot 2.0H_2O$	(7100)	na
126	H	$(CH_2)_3CO_2C_2H_5$	4-ClC_6H_4	169–171 (s)	56	$C_{20}H_{21}N_4O_3Cl$	>10000	100 (62.5%)
127	H	$(CH_2)_3COOK$	4-ClC_6H_4	263–264 (k)	35 (P)	$C_{13}H_{16}N_4O_3ClK\cdot 1.0H_2O$	>10000	na
128	H	$(CH_2)_2N(CH_3)_2$	4-ClC_6H_4	195–197 (r)	62	$C_{18}H_{20}N_5OCl$	>10000	62.0 [38.8–99.2]
129	CH_3	$(CH_2)_2N(CH_3)_2$	4-ClC_6H_4	120–122 (t)	34	$C_{19}H_{22}N_5O_2Cl$	>10000	100 (62.5%)
130	H	$(CH_2)_2NHCOCH_3$	4-ClC_6H_4	247–249 (r)	18	$C_{18}H_{18}N_5O_2Cl\cdot 0.5H_2O$	7200	na
131	H	$(CH_2)_3N(CH_3)_2$	4-ClC_6H_4	188–190 (r)	35	$C_{19}H_{22}N_5OCl\cdot 2.0HCl\cdot 1.0H_2O$	>10000	64.0 [44.4–79.4]
diazepam							7	0.9
chlordiazepoxide							710	1.9

[a] k = *tert*-butanyl alcohol, l = dimethylformamide, m = cyclohexane, n = ether, o = acetone, r = isopropyl alcohol, s = ethanol, t = isopropyl ether, w = tetrahydrofuran, x = petroleum ether, y = methanol, z = water. [b] The general method of preparation was method K, unless stated otherwise, which is described in the Experimental Section. [c] Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [d] The IC_{50} values in parentheses designate that [^{3}H]diazepam was used as the ligand; otherwise the ligand was [^{3}H]flunitrazepam. The IC_{50} value is the mean of the number of replications shown in brackets, except when $n = 1$. [e] The pentylene tetrazole (PTZ) data are reported as na (not active) for those compounds which gave 0% protection at 100 mg/kg, or 100 (*x*%) for those showing *x*% protection at 100 mg/kg, or an ED_{50} value (95% confidence limits are in brackets). [f] Convulsant at 10 mg/kg. [g] 2-Pyridyl group. [h] 5-Chloro-2-pyridyl group. [i] 2-Furanyl group. [j] 5-Bromo-2-furanyl group. [k] 5-Bromo-2-thienyl group. [l] 3-Thienyl group. [m] 3-Furanyl group. [n] 0% protection at 31.6 mg/kg. [o] Calcd, 64.77; found, 67.26. [p] Fumarate salt. [q] 3-Pyridyl group. [r] 4-Pyridyl group.

electron-rich mimicry of the imine moiety in a typical 1,4-BZD as well as the dipolar hindrance to free rotation of the acetamide side chain. The importance of this particular heterocyclic fusion is demonstrated in Tables VIII and IX. The corresponding benzimidazole **135** containing an *N,N*-dipropylacetamide (IC_{50} = 2700 nM), was considerably less potent than **110** (IC_{50} = 380 nM). In addition, the oxidation of the imidazopyridine nitrogen, such as derivatives **136** and **137**, abolishes binding activity. The isomeric pyridyl derivatives **138–141**, in which the pyrido nitrogen occupies other ring positions, indicates a marked preference for the 3*H*-imidazo[4,5-*b*]pyridine ring system (e.g., **91** and **110**). The effect of substitution on the imidazopyridine ring in the dimethylacetamide series is demonstrated by the analogues in Table IX. As exemplified by **142**, **147**, and **148**, a 5′-chloro or 5′-methyl sub-

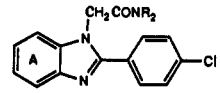
Table VII. 3*H*-Imidazo[4,5-*b*]pyridine-3-acetamides



no.	Z	mp, °C (solv)[a]	% yield[b]	formula[c]	[^{3}H]Flu IC_{50},[d] nM	PTZ ED_{50},[e] mg/kg ip
132	(*S*)-CH(CH_3)	124–126 (t)	59	$C_{17}H_{17}N_4OCl$	>10000	31.6 [18.6–53.7]
133	(*R*)-CH(CH_3)	124–127 (t)	48	$C_{17}H_{17}N_4OCl$	>10000	43.5 [27.2–69.6]
134	CH_2CH_2	203–205 (rt)	30	$C_{17}H_{17}N_4OCl \cdot 1.0HCl$	9300	29.4 [20.3–42.6]

[a–e] See Table VI for definition of footnotes.

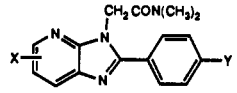
Table VIII. 2-Phenylimidazopyridines and Benzimidazoles



no.	A	R	mp, °C (solv)[a]	% yield[b]	formula[c]	[^{3}H]Flu IC_{50},[d] nM	PTZ ED_{50},[e] mg/kg ip
135		*n*-C_3H_7	180–162 (rz)	63 (J)	$C_{21}H_{24}N_3OCl$[f]	2700	na
136		H	272–275	52 (Q)	$C_{14}H_{11}N_4O_2Cl$	>10000	52.1 [38.9–69.8]
137		CH_3	200–202 (pw)	88 (Q)	$C_{16}H_{15}N_4O_2Cl$	>10000	20.0 [12.5–32.0]
138		CH_3	236–238 (rt)	16 (J)	$C_{16}H_{15}N_4OCl$	>10000	na
139		CH_3	236–238 (rt)	24 (R)	$C_{16}H_{15}N_4OCl$[g]	>10000	na
140		H	280–283 (jz)	37 (S)	$C_{14}H_{11}N_4OCl$	>10000	na
141		*n*-C_3H_7	139–141 (rz)	19 (J)	$C_{20}H_{23}N_4OCl \cdot 0.5H_2O$	>10000	na

[a–e] See Table VI for definition of footnotes. [f] Calcd 68.19, found, 67.50. [g] Calcd, 61.05; found, 60.61.

Table IX. Substituted 3*H*-Imidazo[4,5-*b*]pyridine-3-acetamides



no.	X	Y	mp, °C (solv)[a]	% yield[b]	formula[c]	[^{3}H]Flu IC_{50},[d] nM	PTZ ED_{50},[e] mg/kg ip
142	5-Cl	Cl	202–205 (rz)	73	$C_{16}H_{14}N_4OCl_2$	460 [7]	1.1 [0.58–2.0]
143	6-Cl	Cl	224–226 (rz)	63	$C_{16}H_{14}N_4OCl_2$[f]	>10000	56.2 [28.4–111.3]
144	7-Cl	Cl	201–203 (rt)	18 (T)	$C_{16}H_{14}N_4OCl_2$	>10000	na
145	5-$OCOCH_3$	Cl	190–193 (qx)	40 (U)	$C_{16}H_{17}N_4O_3Cl$	>10000	~100
146	5-OH	Cl	>250 (rz)	34 (V)	$C_{16}H_{15}N_4O_2Cl$	5200	na
147	5-CH_3	Cl	192–194 (rz)	54 (K)	$C_{17}H_{17}N_4OCl$	880 [3]	2.5 [1.1–5.4]
148	5-Cl	CH_3	182–185 (rz)	71 (K)	$C_{17}H_{17}N_4OCl$	150 [6]	2.7 [1.4–4.3]

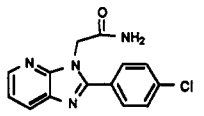
[a–e] See Table VI for definition of footnotes. [f] Calcd, 55.03; found, 54.59.

stituent produced marked increases in both binding and anticonvulsant potencies. However, the 5′-acetoxy (145) and 5′-hydroxy (146) derivatives were much less potent at the BZD receptor. In addition, halogenation at the 6′- and 7′-positions (143, 144) gave compounds devoid of measurable binding activity.

The observed SAR lends general support to the proposed hypothesis, which involved a molecular disconnection of a typical 1,4-benzodiazepine and a heterocyclic fusion. Thus, the binding activity of amides compared to the inactive esters and carboxylic acid would be expected by the molecular disconnection of a 1,4-benzodiazepine molecule. In addition, only the acetamide, and not the propionamide moiety, possesses receptor binding affinity. Perhaps the most convincing observations is the detrimental effect of α-methyl substitution. The corresponding position on a typical 1,4-benzodiazepine (C-3) is very sensitive to alkyl substitution.[17] On the other hand, this disconnection would predict a meta-substituted phenyl ring to be preferred, which is not apparent from the SAR. The observation that a para substituent is actually preferred may be due to the inherent greater degrees of freedom of the target molecules to interact with particular binding domains within the BZD receptor. Thus, the amide functionality may be pushed/pulled in the direction of a cationic domain of the receptor more than the inherently rigid amide of a typical 1,4-benzodiazepine.[18] This could result in the substituted phenyl moiety inter-

(18) Lowe, G. H.; Nienow, J. R.; Poulsen, M. Theoretical Structure–Activity Studies of Benzodiazepines. *Mol. Pharmacol.* 1984, *26*, 19–34.

Table X. Anticonvulsant Profile of 84[a]



compound	ED_{50}, mg/kg ip				rotorod TD_{50}/
	PTZ[b]	MES[c]	BIC[d]	PIC[e]	
84	28.9	329.2	173.9	210.6	>1000
phenytoin	>300	9.5	>100[g]	>100[g]	65.5
phenobarbitol	13.2	21.8	37.7	27.5	69.0
ethosuximide	130.4	>1000	459.0	242.7	440.8
valproic acid	148.6	271.6	359.9	387.2	425.8

[a] Reference 14. [b] Pentylenetetrazole, 80 mg/kg sc. [c] Maximal electroshock, 50 mA, 200 ms. [d] Bicuculline, 2.7 mg/kg sc. [e] Picrotoxin, 3.2 mg/kg sc. [f] Rotorod activity is defined as neurotoxicity; TD_{50} = dose that is toxic to 50% of the animals (mg/kg ip). [g] No protection at 100 mg/kg ip.

acting in a slightly different manner with a hydrophobic domain of the receptor. As additional support, the corresponding benzyl derivative is inactive at the BZD receptor.

The observed heterocyclic SAR supports the hypothesis regarding the fusion of an imidazo[4,5-*b*]pyridine ring system. The position and integrity of the lone-pair on the pyrido nitrogen is essential to retain binding activity. It is hoped that this series of compounds will enhance the definition of an anxiolytic/hypnotic pharmacophore. The SAR of the imidazo[1,2-*a*]pyridine zolpidem has not been disclosed. However, an overlap of the heterocyclic frameworks of zolpidem and the current series indicates that it possesses many of the same optimal features of the current series. These include a 2-phenyl group with a para substituent, an acetamide side chain, and a 5′-methyl substituent on the imidazopyridine ring.

Several compounds in this series possess both in vitro BZD receptor binding and in vivo anticonvulsant potencies comparable to or better than that of chlordiazepoxide but less than that of diazepam. In particular, **148** showed an encouraging profile to warrant further evaluation as an anxiolytic or hypnotic. As summarized in Table XI, **148** was evaluated as an agonist/antagonist/inverse agonist by the GABA (γ-aminobutyric acid) shift ratio.[19] This is the ratio of IC_{50} values for the displacement of [^{3}H]Ro 15-1788 in the absence and presence of excess GABA. According to the model of the BZD receptor proposed by Ehlert et al., GABA would act as a positive alloster by stabilization of an activated (agonist) conformation of the receptor.[20] The absence of GABA would allow for the stabilization of the ground (inverse agonist) conformation, while antagonists would have equal affinity for both states of the receptor. Thus, a full agonist, such as diazepam, should give a ratio greater than 1.0, while an antagonist would be 1.0, and an inverse agonist would have a ratio of less than 1.0. By this criteria, **148** behaves as a full agonist (3.06) comparable to diazepam (3.14). The evaluation of **148** as an anxiolytic agent was assessed by the Vogel paradigm (shock-induced suppression of drinking) and the light/dark exploratory behavioral test, which involves a nonpunishment regimen and no food reward.[21,22] In the Vogel test, **148** was 2-fold less potent than diazepam, although it was 10-fold more potent by the light/dark behavioral criteria. Typical side-effects of 1,4-benzodiazepines, such as neurotoxicity and the synergism with other CNS depressants, was assessed for **148**. Neurotoxicity was assessed by the rotorod assay and synergy was assessed by ethanol-induced sleep time in mice. In both test systems, **148** was less potent than diazepam. Because of the increased separation of side effects from anxiolytic effects, **148** was singled out as a potential selective anxiolytic and/or hypnotic in a novel structural class.

Experimental Section

Biological Methods. Benzodiazepine Receptor Binding Assay in Rat Cerebral Cortex Membranes. The IC_{50} values for the displacement of tritiated flunitrazepam binding in vitro to rat cerebral cortex membrane preparations was determined by using a modification of procedure described by Chiu et al.[23] The membrane preparation consisted of P_2 and P_3 fractions prepared from fresh rat cerebral cortex homogenates with two freeze–thaw–wash cycles to remove endogenous GABA (γ-aminobutyric acid). Six concentrations ($1 \times 10^{-5} - 1 \times 10^{-10}$ M) of the test compounds were incubated with 1 nM tritiated flunitrazepam and 0.4 mg of membrane protein for 30 min at 4 °C. The reaction was stopped when the assay mixture was washed with cold buffer and filtered through GF/b glass-fiber filters using a cell harvester. The filters were then transferred to vials, scintillation cocktail was added, and the radioactivity in each vial was determined by liquid scintillation counting. Nonspecific binding was determined in the presence of 10^{-5} M diazepam.

The IC_{50} values were calculated by means of regression analysis of the logits of the percent control binding versus the log of the molar test compound concentration (log–logit transformation).

The procedure for the displacement of tritiated diazepam binding was essentially identical with the procedure for tritiated flunitrazepam binding except that the membrane preparation lacked the freeze–thaw–wash cycles to remove endogenous GABA and the concentration of tritiated diazepam in the assay was 1.5 nM.

The effect of GABA (GABA shift ratios) on the IC_{50} values of **148** and diazepam for [^{3}H]Ro 15-1788 binding in rat cerebral cortex was determined as a measure of agonist efficacy.[19]

Anticonvulsant Activity. Anticonvulsant activity was assessed against subcutaneous pentylenetetrazole-induced clonic seizures in mice.[24] For these studies, groups of eight adult, female ICR mice (Dominion Labs, Dublin, VA) were pretreated with 100 mg/kg ip of the test compound (dissolved or suspended in 0.5% aqueous methylcellulose) or with an identical volume (10 mg/kg) of the vehicle. Each animal was challenged (subcutaneous pentylenetetrazole, 80 mg/kg) 30 min later. Active compounds were studied further with a minimum of three logarithmically spaced doses. Protective ED_{50} values were calculated.[25]

Anxiolytic Activity. A modification of the Vogel method was used to determine anticonflict activity in rats.[21] The rats were deprived of water for 48 h prior to testing, although food was available during this time. After randomization into treatment groups, the animals were given **148** or diazepam 30 min prior to being placed in individual cages equipped with an electrifiable

(19) Wood, P. L.; Loo, P.; Braunwalder, A.; Yokoyama, N.; Chenoy, D. L. In vitro characterization of benzodiazepine receptor agonsts, antagonists, inverse agonists, and agonist/antagonists. *J. Pharmacol. Exp. Ther.* **1984**, *231*, 572–576.

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(21) Vogel, J. R.; Beer, B.; Clody, D. E. A simple and reliable conflict procedure for testing anti-anxiety agents. *Psychopharmacologia* **1971**, *21*, 1–7.

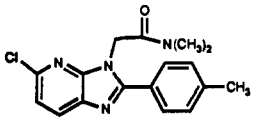
(22) Crawley, J. N. Neuropharmacologic Specificty of a Simple Animal Model for the Behavioral Actions of Benzodiazepines. *Pharmacol. Biochem. Behav.* **1981**, *15*, 695–699.

(23) Chiu, T. H.; Dryden, D. M.; Rosenberg, H. C. Kinetics of [^{3}H]flunitrazepam binding to membrane-bound benzodiazepine receptors. *Mol. Pharmacol.* **1982**, *21*, 57–65.

(24) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. Antiepileptic drug development: II. Anticonvulsant drug screening. *Epilepsia* **1978**, *19*, 409–428.

(25) Litchfield, J. T., Jr.; Wilcoxon, F. A simplified method of evaluating dose–effect experiments. *J. Pharmacol. Exp. Ther.* **1949**, *99*, 96–113.

Table XI. Anxiolytic/Hypnotic Profile of 148



compound	[3H]Flu[a]	GABA[b] shift	PTZ[c]	Vogel[d]	L/D[e] med	L/D[e] sedn	rotorod[f]	EtOH[g]
148	150	3.06	2.7	1.0	0.1	5.62	15.2	10.0
diazepam	11	3.14	0.2	1.0	1.0	10.0	1.7	2.5

[a] [3H]flunitrazepam, IC_{50} (nM). [b] Ratio of IC_{50} values for [3H]Ro 15-1788 binding in absence and presence of added GABA (rat cerebral cortex). [c] Pentylenetetrazole, 80 mg/kg sc; ED_{50} (mg/kg ip). [d] Vogel punished drinking test in rats; MED (mg/kg ip). [e] Light/dark exploratory behavioral test in mice; MED (mg/kg ip) and sedative dose (mg/kg ip). [f] Rotorod neurotoxicity; ED_{50} (mg/kg ip). [g] Potentiation of ethanol (EtOH) induced sleep time in mice; MED (mg/kg po).

grid floor and a water bottle with an electrifiable metal drinking tube. A drinkometer circuit (Omnitech Electronics, Inc., Columbus, OH) was used to count the number of licks and deliver a shock (0.65 mA) after every 20th lick during a 3-min test session, which started with the first shock. Total shocks delivered were counted and compared with the shocks delivered to the vehicle-treated rats. Statistical evaluation was based on Dunn's multiple comparison test.[26]

A modification of the light/dark Exploratory behavioral test was used for anxiolytic testing in mice.[22] The effects of 148 and diazepam on the aversive properties of a brightly lit chamber (280 lx) were investigated. After administration of test compound 30 min prior to testing, the mice were placed individually in a two-compartment light/dark activity chamber (Omnitech Digiscan Model RXYZCM16) and left undisturbed for 5 min. The amount of time the mice spent in each compartment was recorded and compared with those of vehicle-treated mice. Statistical analyses were based on the Dunnett's t test.

Rotorod Test. Groups of five randomly selected mice were treated with 148 or diazepam 30 min prior to being placed on a knurled rod (28 mm in diameter) which rotated at 6 rpm. The inability of the animal to remain on the rod for 1 min was scored as a neurotoxic effect, and the ED_{50} values and confidence limits were determined from a computer-generated probit-analysis program.

General Procedures. Melting points were determined in open capillary tubes in a Thomas-Hoover melting point apparatus and are uncorrected; ^1H NMR spectra were obtained in $CDCl_3$ or $(CD_3)_2SO$ with tetramethylsilane as internal standard on a Varian A-60 or on a Varian EM-360L spectrometer; mass spectra were determined on a Varian MAT-44 mass spectrometer; IR spectra were run as KBr pellets on a Beckman IR8 or on a Perkin-Elmer 297 IR spectrophotometer; purification by HPLC, where indicated, utilized a Waters Prep LC-500A apparatus with a PrepPAK-500 silica cartridge; analytical results for compounds followed by elemental symbols are within +/−0.4% of the theoretical values and were determined on a Perkin-Elmer Model 240 or a Control Equipment Model 240XA CHN analyzer. Spectral data for all reported compounds were consistent with assigned structures. The 2-chloro-3-nitropyridine was purchased from Ruetgers-Nease Co., New York, NY, and 2,6-dichloro-3-nitropyridine, 4-chloro-3-nitropyridine, 2-hydroxy-6-methylpyridine-3-carboxylic acid, and 2,3- and 3,4-diaminopyridines were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI.

Method A. *N*-(3-Nitro-2-pyridinyl)glycine Ethyl Ester (1). A solution of 2-chloro-3-nitropyridine (167 g, 1.06 mol), glycine ethyl ester hydrochloride (221.3 g, 1.58 mol) and triethylamine (406 mL, 2.92 mol) in 1.5 L of absolute ethanol was heated at reflux for 1.75 h. The ethanol was evaporated from the reaction mixture and the residue was partitioned between ethyl acetate (1 L) and water (0.5 L). The separated aqueous layer was extracted with ethyl acetate (0.2 L). The organic layers were combined and washed with water (3 × 250 mL) and saturated brine. The organic layer was separated, dried (Na_2SO_4), and concentrated to an oil (quantitative). A 124-g portion was purified by column chromatography on silica gel (500 g) using isopropyl ether as the eluting solvent. The pure fractions were combined, evaporated, and dried under high vacuum to give 112.2 g (90%) of 1 as a yellow solid, mp 38–39 °C. Anal. ($C_9H_{11}N_3O_4$) C, H, N.

***N*-(5-Chloro-3-nitro-2-pyridinyl)glycine Ethyl Ester (6).** A solution of 1 (1.0 g, 44 mmol) in 3 N HCl (20 mL) was added to a 5.25% commercial bleach solution (89 mmol). The resulting suspension was stirred at room temperature overnight, the solid was collected by filtration, and rinsed with water to give 0.95 g of crude 6. Recrystallization of the yellow solid from a mixture of isopropyl ether and petroleum ether gave 0.56 g (47%), mp 73–75 °C. Anal. ($C_9H_{10}N_3O_4Cl$) C, H, N.

2-[(3-Nitro-2-pyridinyl)amino]acetamide (7). A mixture of 2-chloro-3-nitropyridine (1.0 g, 6.3 mmol), glycinamide hydrochloride (3.1 g, 28 mmol), and triethylamine (3.1 g, 31 mmol) in 35 mL of acetonitrile was heated at reflux for 16.5 h. The yellow precipitate was collected by filtration, washed sequentially with water, methanol, and acetone. The solid was dried under high vacuum to give 0.9 g (73%), mp 250–251 °C. Anal. ($C_9H_8N_4O_3$) C, H, N.

3,4-Dihydro-3-methylpyrido[2,3-*b*]pyrazin-2(1*H*)-one (8). A solution of 2 (103.7 g, 0.43 mol) in dry tetrahydrofuran (460 mL) was hydrogenated over 5% palladium on carbon (10 g) for 1 h. The reaction mixture was dried ($MgSO_4$) and filtered through a Celite pad, which was washed with hot methanol. The solid, which formed upon cooling, was collected by filtration and dried under high vacuum to give 1.7 g (2.4%) of 8, mp >250 °C. Anal. ($C_9H_9N_3O$) C, H, N.

Method B. *N*-[3-[(4-Chlorobenzoyl)amino]-2-pyridinyl]glycine Ethyl Ester (11). A solution of 1 (64.4 g, 0.29 mol) in tetrahydrofuran (900 mL) was hydrogenated in a 2-L Parr bottle over 5% palladium on carbon (6.5 g) at room temperature for 1 h. The reaction mixture was dried ($MgSO_4$) and filtered through a Celite pad. A 235-mL portion (0.057 mol) of the filtrate was used in the reaction. Under a nitrogen atmosphere, 4-chlorobenzoyl chloride (9.97 g, 0.0257 mol) and triethylamine (6.33 g, 0.063 mol) were added dropwise simultaneously to the above stirred filtrate at room temperature and allowed to stir overnight. The reaction mixture was filtered and the filtrate was treated with Florisil. The Florisil was removed by filtration and the filtrate was evaporated to a solid, which was recrystallized from a mixture of tetrahydrofuran and water to give 16.5 g (87%), mp 133–134 °C. Anal. ($C_{16}H_{16}N_3O_3Cl$) C, H, N.

Method C. (*R*)-*N*-[3-[(4-Chlorobenzoyl)amino]-2-pyridinyl]alanine Methyl Ester (28). This compound was prepared according to method B, except that the temperature of the hydrogenation reaction was carefully maintained at room temperature. The solution of the reduction product was cooled in an ice water bath and dried ($MgSO_4$). Upon stirring with 4-chlorobenzoyl chloride and triethylamine overnight, the solvents were removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The aqueous layer was separated and extracted with ethyl acetate. The combined organic layers were washed with water, dried ($MgSO_4$), treated with charcoal, filtered, and evaporated to give crude solid (99%). A 3.5-g portion of the solid was dissolved in hot isopropyl alcohol, treated with charcoal, and filtered. Petroleum ether (bp 50–110 °C) was added to the cool solution to initiate crystallization. The solid was collected by filtration and dried to give 0.68 g (19%),

(26) Dunn, O. J. Multiple comparisons using rank sums. *Technometrics* 1964, *6*, 241–252.

mp 125–134 °C. Anal. ($C_{16}H_{16}N_3O_3Cl$) C, H, N.

Method D. *N*-[5-Chloro-3-[(4-chlorobenzoyl)amino]-2-pyridyl]glycine Ethyl Ester (32). This compound was prepared according to method C, except that platinum oxide (PtO_2) was used as the hydrogenation catalyst to give 83% of crude **32**. A 1.5-g sample was recrystallized twice from a mixture of isopropyl alcohol and water, then dried under high vacuum to give 0.63 g (35%), mp 157–158 °C. Anal. ($C_{16}H_{15}N_3O_3Cl$) C, H, N.

Method E. *N*-[2-[(2-Amino-2-oxoethyl)amino]-3-pyridyl]-4-chlorobenzamide Hemihydrate (33). A mixture of intermediate 3-amino-2-[(2-amino-2-oxoethyl)amino]pyridine (0.133 mol) in acetone (1 L) was treated dropwise simultaneously with 4-chlorobenzoyl chloride (23.28 g, 0.15 mol) and triethylamine (14.75 g, 0.15 mol). The reaction mixture was allowed to stir overnight at room temperature. The precipitate was collected by filtration, washed with acetone, and dried. The solid was dissolved in hot methanol, filtered, and concentrated to produce a crystalline crop. The solid was collected by filtration, washed with cold methanol and water, and dried under high vacuum at 60 °C to give 18.36 g (44%), mp 194–195 °C dec. Anal. ($C_{14}H_{13}N_4O_2Cl\cdot0.5H_2O$) C, H, N.

Method F. 2-(4-Chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-3-acetic Acid Ethyl Ester (38). The solid **11** (15.5 g, 0.046 mol) was heated at 210–220 °C in a flask with a Wood's metal bath for 8 min. The residue was dissolved in methylene chloride, treated with charcoal, and filtered through a Celite pad. The filtrate was treated with Florisil to give a colorless filtrate, which was evaporated to a solid. The solid was recrystallized from tetrahydrofuran and petroleum ether to give 7.0 g (48%), mp 123–124 °C. Anal. ($C_{16}H_{14}N_3O_2Cl$) C, H, N.

Method G. 2-(4-Methylphenyl)-3*H*-imidazo[4,5-*b*]pyridine-3-acetic Acid Ethyl Ester (50). A mixture of **17** (63.5 g, 0.2 mol) in ethylene glycol (500 mL) was heated at reflux for 1 h. A 50-mL aliquot of the reaction mixture was poured into water and refrigerated overnight. The precipitate was collected by filtration, washed with water, and dried under high vacuum at 50 °C. The mixture of esters (ethyl and ethylene glycol ester) was purified on a silica gel column (75 g) by eluting with 1:1 methylene chloride and ethyl acetate to collect the ethyl ester. The appropriate fractions (TLC) were combined and evaporated to dryness. The solid was recrystallized from isopropyl alcohol and water and dried under high vacuum at 60 °C overnight to give 1.73 (29%), mp 117–119 °C. Anal. ($C_{17}H_{17}N_3O_2$) C, H, N.

Method H. 2-(4-Chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-3-acetic Acid (39). A mixture of **38** (14.6 g, 0.04 mol), sodium hydroxide pellets (2.9 g, 0.07 mol), and a 1:1 mixture of ethanol/water (150 mL) was heated at reflux for 0.75 h. The reaction mixture was poured into water (250 mL) and acidified with concentrated hydrochloric acid, and the solid was collected by filtration. A 2-g portion was recrystallized from methanol and water to give 1.2 g (58%), mp 271–273 °C dec. Anal. ($C_{14}H_{10}N_3O_2Cl$) C, H, N.

Method I. 2-(4-Methylphenyl)-3*H*-imidazo[4,5-*b*]pyridine-3-acetic Acid (51). A mixture of **17** (63.5 g, 0.2 mol) in ethylene glycol (500 mL) was heated at reflux for 1 h. The reaction mixture was cooled and a solution of potassium hydroxide (11.4 g, 0.2 mol) in water (100 mL) was added. The mixture was heated at reflux for 1 h and then filtered into ice/water (2 L). The pH was adjusted to 2 with 10% hydrochloric acid, and the precipitate was collected by filtration, washed with water, and dried at 50 °C under high vacuum to give 42.5 g. A 3-g portion was recrystallized from methanol and dried under high vacuum at 50 °C overnight to give 1.55 g (44%), mp 262–264 °C. Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

Method J. 2-(4-Chlorophenyl)-*N*,*N*-dimethyl-3*H*-imidazo[4,5-*b*]pyridine-3-acetamide Hydrochloride (91). Under a nitrogen atmosphere, a mixture of **39** (6.0 g, 0.02 mol) and 1,1'-carbonyldiimidazole (3.39 g, 0.02 mol) in tetrahydrofuran (150 mL) was stirred at room temperature for 3 h. A solution of dimethylamine (84 mL of a 1 M solution in tetrahydrofuran, 0.042 mol) was added dropwise at room temperature to the stirred solution and the reaction mixture was allowed to stir overnight. The solvent was evaporated and the solid residue was treated with water. The insoluble material was collected by filtration and dried under high vacuum overnight. The solid was dissolved in tetrahydrofuran and acidified with saturated ethereal hydrochloric acid to produce a crystalline precipitate, which was collected by filtration, washed with tetrahydrofuran, and dried under high vacuum overnight to give 5.3 g (73%), mp 216–220 °C. Anal. ($C_{16}H_{15}N_4OCl\cdot HCl$) C, H, N.

Method K. 5-Chloro-*N*,*N*-dimethyl-2-(4-methylphenyl)-3*H*-imidazo[4,5-*b*]pyridine-3-acetamide (148). Under a nitrogen atmosphere, a slurry of **77** (2.8 g, 9.3 mmol) in tetrahydrofuran (11 mL) and dimethylformamide (0.68 g, 9.3 mmol) was cooled in an ice/water bath. Thionyl chloride (1.22 g, 10.2 mmol) was added dropwise to the stirred suspension. After stirring at room temperature for 20 min, the solution was cooled again in an ice/water bath and a solution of dimethylamine in tetrahydrofuran (18.9 mL of a 2.95 M solution, 56 mmol) was added dropwise. The suspension was stirred at room temperature for 1 h, and the solvents were removed under reduced pressure. The residue was treated with water (100 mL) and the insoluble material was collected by filtration and washed with water. The solid was dissolved in hot isopropyl alcohol, filtered while hot, and diluted with water. The solid, which formed upon cooling, was collected by filtration, washed with water, and dried under high vacuum at 70 °C to give 2.17 g (71%), mp 182–185 °C. Anal. ($C_{17}H_{17}N_4OCl$) C, H, N.

Method L. 2-(4-Chlorophenyl)-*N*-phenyl-3*H*-imidazo[4,5-*b*]pyridine-3-acetamide (120). Under a nitrogen atmosphere, oxalyl chloride (1.73 g, 13.7 mmol) was added dropwise to a stirred, chilled (10–15 °C) suspension of **39** (3.7 g, 13 mmol) in dimethylformamide (50 mL). The reaction mixture was heated at 60 °C for 5 h. The solution of the acyl chloride was added dropwise to a stirred and chilled (10–15 °C) solution of aniline (1.31 g, 14 mmol), triethylamine (1.42 g, 14 mmol), and dimethylformamide (75 mL). The reaction mixture was stirred at room temperature overnight, then poured into water (200 mL). The insoluble material was collected by filtration, washed with water, and dried under high vacuum. The solid was recrystallized from isopropyl alcohol with refrigeration overnight, collected by filtration, washed with water, and dried under high vacuum at 70 °C overnight to give 3.11 (66%), mp 240–242 °C. Anal. ($C_{20}H_{15}N_4OCl$) C, H, N.

Method M. 2-(5-Chloro-2-pyridinyl)-3-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-3*H*-imidazo[4,5-*b*]pyridine (100). A solution of ethyl chloroformate (0.66 g, 6.1 mmol) in methylene chloride (20 mL) was added slowly to a solution of **57** (1.75 g, 6.0 mmol) and triethylamine (0.68 g, 6.7 mmol) in methylene chloride (75 mL). After the solution was allowed to stir at room temperature for 2 h, a solution of *N*-methylpiperazine (0.61 g, 6.1 mmol) in methylene chloride (20 mL) was added slowly and allowed to stir overnight. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was partitioned between dilute aqueous hydrochloric acid and ether. The aqueous layer was separated and extracted with ether (2×) and methylene chloride (3×). The aqueous layer was adjusted with dilute aqueous sodium hydroxide, first to pH 7.0 and filtered, then to pH 9–10. The aqueous layer was extracted with methylene chloride (3×), which were combined and evaporated to a gum. The residue was purified on a alumina column (Brockman, activity 1, 80–20 mesh) by elution with 1:4 mixture of acetone and benzene. The appropriate fractions were combined and evaporated. The residue was recrystallized from acetone and petroleum ether and collected to give 0.54 g (24%), mp 174–175 °C. Anal. ($C_{18}H_{19}N_6OCl$) C, H, N.

Method N. 2-(4-Chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-3-acetamide (84). Solid **33** (7.62 g, 0.028 mol) in a glass flask was heated in a Wood's metal bath at 200 °C for 7 min. The residue was treated with methanol and filtered. The filtrate was concentrated and the solid was collected by filtration and recrystallized from methanol to give 3.19 g (53%), mp 270–271 °C. Anal. ($C_{14}H_{11}N_4OCl$) C, H, N.

Method O. 2-(3-Chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-3-acetamide (83). A suspension of *N*-[2-[(2-amino-2-oxoethyl)amino]-3-pyridinyl]-3-chlorobenzamide (8.7 g, 0.03 mol) in ethylene glycol (200 mL) was heated at 190 °C (oil bath) for 40 min. The reaction mixture was filtered when cool and diluted with water to produce a solid, which was collected by filtration and dried. The solid was recrystallized from methanol and water to give 5.4 g (66%) after drying under high vacuum at 60 °C overnight, mp 245–247 °C. Anal. ($C_{14}H_{11}N_4OCl$) C, H, N.

Method P. N-[[2-(4-Chlorophenyl)-3H-imidazo[4,5-b]pyridinyl]acetyl]glycine Potassium Salt Dihydrate (125). A mixture of 124 (4.0 g, 0.011 mol), potassium hydroxide pellets (0.62 g, 0.011 mol), and 190-proof ethanol (100 mL) was heated at reflux for 2 h. The hot solution was filtered and allowed to cool. The solid, which formed upon cooling, was collected by filtration, washed with a minimum of 190-proof ethanol, and dried under high vacuum to give 2.5 g (55%), mp >300 °C Anal. ($C_{16}H_{12}N_4O_3KCl\cdot 2.0H_2O$) C, H, N.

Method Q. 2-(4-Chlorophenyl)-N,N-dimethyl-3H-imidazo[4,5-b]pyridine-3-acetamide 4-Oxide (137). A mixture of 91 (0.3 g, 0.96 mmol), glacial acetic acid (2.5 mL), and *m*-chloroperbenzoic acid (0.66 g, 3.84 mmol) was heated at 60 °C for 4 h. The reaction mixture was diluted with water and the organic acid which precipitated was filtered off. The aqueous filtrate was neutralized for peroxide with 10% sodium sulfite. The aqueous solution was evaporated to dryness and the residue was dissolved in methylene chloride and extracted with a minimum of saturated sodium bicarbonate solution. The organic layer was dried and evaporated to a solid, which was recrystallized from tetrahydrofuran and hexane with refrigeration overnight to give 0.28 g (88%), mp 200–202 °C. Anal. ($C_{16}H_{15}N_4O_2Cl$) C, H, N.

Method R. 2-(4-Chlorophenyl)-N,N-dimethyl-1H-imidazo[4,5-c]pyridine-1-acetamide (139). The solid 159 (9.24 g, 0.02 mol) was heated in a glass flask with a Wood's metal bath at 180–190 °C for 10 min. The residue was dissolved in methylene chloride and extracted with dilute aqueous sodium hydroxide (3×). The aqueous basic layers were combined and extracted with methylene chloride. The methylene chloride layers were combined, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography on silica gel eluting first with ethyl acetate (5.5 L), followed by ethyl acetate/methanol (9:1) and finally ethyl acetate/methanol (8:2).[27] The desired product was contained in the latter fractions which were combined and evaporated. The solid was recrystallized from isopropyl alcohol/isopropyl ether to give 1.4 g (24%), mp 236–238 °C. Anal. ($C_{16}H_{15}N_4OCl$) H, N; C: calcd, 61.05; found, 60.61.

Method S. 2-(4-Chlorophenyl)-1H-imidazo[4,5-b]pyridine-1-acetamide (140). Under a flow of nitrogen, sodium hydride (0.96 g, 0.0234 mol, 60% in oil) was washed with hexanes (70 mL) and decanted. *N,N*-Dimethylformamide (100 mL) and the 2-(4-chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine[11] (5.0 g, 0.022 mol) was added in portions. The reaction mixture was heated at 70–85 °C for 1.5 h. 2-Chloroacetamide (2.06 g, 0.022 mol) was added and the reaction mixture was stirred overnight at room temperature. The mixture was added to water (600 mL) and the precipitate was collected by filtration and dried under high vacuum. ^1H NMR indicated a ratio of 14% of 84 and 86% of 140. The solid was dissolved in pyridine and filtered, and the filtrate was diluted with water to produce crystals with refrigeration. The solid was collected by filtration and dried under high vacuum at 90 °C overnight to give 2.3 g (37%) of 140, mp 280–283 °C. Anal. ($C_{14}H_{11}N_4OCl$) C, H, N.

Method T. 7-Chloro-2-(4-chlorophenyl)-N,N-dimethyl-3H-imidazo[4,5-b]pyridine-3-acetamide Hemihydrate (144). Phosphorus oxychloride (27.4 g, 0.18 mol) was added to solid 137 (5.5 g, 0.017 mol). The resulting solution was refluxed for a few minutes and then evaporated under reduced pressure to an oil. The oil was partitioned between ethyl acetate and saturated sodium bicarbonate solution. An insoluble, crystalline solid was collected by filtration, dissolved in hot isopropyl alcohol, and filtered while hot. The filtrate was diluted with isopropyl ether to initiate crystallization. The solid was collected by filtration, washed with isopropyl ether, and dried under high vacuum at 60 °C to give 1.05 g (18%), mp 201–203 °C. Anal. ($C_{16}H_{14}N_4OCl_2\cdot 0.5H_2O$) C, H, N.

Method U. 5-(Acetyloxy)-2-(4-chlorophenyl)-N,N-dimethyl-3H-imidazo[4,5-b]pyridine-3-acetamide (145). A suspension of 137 (1.0 g, 3 mmol) and acetic anhydride (0.29 mL, 3 mmol) in tetrahydrofuran (40 mL) was heated at reflux under a nitrogen atmosphere for 2 days. An additional aliquot of acetic anhydride (0.29 mL) was added and the reaction mixture was heated at reflux overnight. Water was added and the solvent was evaporated under reduced pressure until only an aqueous suspension remained. Ethyl acetate and solid potassium carbonate were added and the aqueous layer was separated. The aqueous layer was extracted with ethyl acetate. The organic layers were combined, extracted with saturated sodium chloride solution, dried (Na_2SO_4), and evaporated. The solid was recrystallized twice from ethyl acetate and petroleum ether to give 0.48 g (40%), mp 190–193 °C. Anal. ($C_{18}H_{17}N_4O_3Cl$) C, H, N.

Method V. 2-(4-Chlorophenyl)-5-hydroxy-N,N-dimethyl-3H-imidazo[4,5-b]pyridine-3-acetamide (146). A solution of 145 (3.0 g, 8 mmol) and potassium hydroxide (0.5 g, 9 mmol) in methanol (100 mL) and water (5 mL) was stirred at room temperature overnight. The solvent was evaporated and the residue was partitioned between saturated sodium chloride solution and ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate. Upon standing, a solid precipitate formed in the aqueous layer. The solid was collected by filtration, washed with water, dissolved in hot isopropyl alcohol, and diluted with water to initiate crystallization. The solid was collected by filtration, washed with water, and dried under high vacuum at 60 °C to give 0.91 g (34%), mp >250 °C. Anal. ($C_{16}H_{15}N_4O_2Cl$) C, H, N.

2-[(3-Amino-6-methyl-2-pyridinyl)amino]ethanol Monohydrochloride (149). A suspension of 2-hydroxy-6-methylpyridine-3-carboxylic acid (24.8 g, 0.162 mol) and phosphoryl chloride (92.33 g, 0.602 mol) was heated to 75 °C under a nitrogen atmosphere. Solid phosphorus pentachloride (26.65 g, 0.128 mol) was added in portions to the hot solution, and the reaction mixture was heated at reflux for 3 h, then stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was azeotroped twice with benzene. The residue was triturated three times with chloroform, and the combined organic layers were dried and filtered. The filtrate was cooled in an ice/water bath, and treated with concentrated ammonium hydroxide. The yellow precipitate was collected by filtration, washed with water, and dried to give 10.9 g (39%) of 2-chloro-6-methyl-3-pyridinecarboxamide.

Bromine (4.6 g, 0.029 mol) was added to a cooled solution of sodium hydroxide (3.6 g, 0.09 mol) in water (65 mL). The solution was cooled to 0 °C in a salt/ice bath and treated with 2-chloro-6-methyl-3-pyridinecarboxamide (4.0 g, 0.023 mol). The colorless solution was stirred at 0 °C for 15 min followed by heating to 75 °C for 45 min. The reaction mixture was stirred overnight, then treated with diethyl ether and sodium chloride. The organic layer was separated, and the aqueous layer was extracted with ether. The combined organic layers were dried, filtered, and evaporated to give 3.3 g of crude product (99%). The solid was dissolved in hot isopropyl ether, filtered while hot, and diluted with petroleum ether. The precipitated solid was collected by filtration and dried under high vacuum to give 1.4 g of 3-amino-2-chloro-6-methyl-3-pyridinamine, mp 76–78 °C.

A solution of 2-chloro-6-methyl-3-pyridinamine (80.0 g, 0.561 mol) in ethanolamine (136.9 g, 2.24 mol) was refluxed overnight under nitrogen. To a portion (10.0 mL) of the cooled solution was added tetrahydrofuran and the solution was decanted from the resulting sludge. Carbon dioxide was bubbled through the solution and the solution was again decanted, dried, filtered, and evaporated under reduced pressure to a solid. The solid was dissolved in hot 2-propanol and acidified with hydrogen chloride in 2-propanol. Addition of isopropyl ether gave a solid which was collected by filtration, redissolved in hot 2-propanol, treated with charcoal, and filtered while hot. The solution was brought to the cloud point with isopropyl ether. After cooling to room temperature, the solid was collected by filtration, washed with isopropyl ether, and dried under high vacuum to give 1.61 g (31%), mp 193–196 °C. Anal. ($C_8H_{13}N_3O\cdot HCl$) C, H, N.

4-Chloro-N-[2-[(2-hydroxyethyl)amino]-6-methyl-3-pyridinyl]benzamide (150). A solution of 149 (83.6 g, 0.412 mol) in *N*-methyl-2-pyrrolidinone was treated simultaneously, dropwise, with triethylamine (87.2 g, 0.862 mol) and 4-chlorobenzoyl chloride (72.03 g, 0.412 mol), and the mixture was stirred at room temperature overnight. The reaction mixture was poured into water, and the resulting solid was collected by filtration. The solid was treated with 5% aqueous potassium hydroxide solution and ethyl

(27) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* 1978, *43*, 2923–2925.

acetate. Insoluble material was removed by filtration, and the filtrate was evaporated under reduced pressure to give 43.8 g (73%) of crude **150**. A 2-g portion of the solid was dissolved in hot isopropyl ether (50 mL) and 2-propanol (3 mL) and filtered while hot. The filtrate was evaporated to half volume and cooled. The resulting solid was collected by filtration and dried under high vacuum at 60 °C to give 0.10 g of pure **150**, mp 150–153 °C. Anal. ($C_{15}H_{16}N_3O_2Cl$) H, N; C: calcd, 58.92; found, 58.48.

2-(4-Chlorophenyl)-5-methyl-3*H*-imidazo[4,5-*b*]pyridine-3-ethanol (151). A solution of **150** (30.0 g, 0.098 mol) in ethylene glycol (300 mL) was heated at reflux under nitrogen for 1.25 h and cooled to room temperature. The suspension was poured into water (2L) and the resulting solid was collected by filtration and washed with water to give 26.31 g. A 1-g portion of the solid was dissolved in hot 2-propanol, filtered while hot, and cooled to room temperature. Addition of water gave a solid which was collected by filtration and dried under high vacuum at 60 °C to give 0.81 g of **151**, mp 190–192 °C. Anal. ($C_{15}H_{14}N_3OCl$), C, H, N.

2-(4-Chlorophenyl)-5-methyl-3*H*-imidazo[4,5-*b*]pyridine-3-acetic Acid (152). A mixture of glacial acetic acid (36.9 mL) in water (74 mL) was heated to 60 °C, while 7 mL of a solution of **151** (20.5 g, 0.071 mol) in glacial acetic acid (55 mL) was added with mechanical stirring. The reaction mixture was maintained at 60 °C while potassium permanganate (36.9 g, 0.234 mol) was added. The remainder of the solution of **151** was added dropwise while an ice/water bath was used to maintain the reaction temperature at 60 °C. The reaction mixture was stirred in the ice/water bath for 15 min, then warmed to room temperature for 40 min. The mixture was cooled to 5 °C and a solution of sodium bisulfite (32.8 g) in water (133 mL) was added slowly while the temperature was maintained below 20 °C. The suspension was stirred in an ice-bath for 3.25 h. The solid was collected by filtration, washed with water, and dissolved in 5% potassium hydroxide. The insoluble material was removed by filtration, and the filtrate was acidified with 3 N hydrochloric acid. The precipitate was collected by filtration and dried under high vacuum at 60 °C to give 15.0 g (70%). Recrystallization of a 1.15-g portion from methanol and drying under high vacuum at 60 °C gave 0.63 g of pure **152**, mp >250 °C. Anal. ($C_{15}H_{12}N_3O_2Cl$) C, H, N.

2-(4-Chlorophenyl)-1*H*-imidazo[4,5-*b*]pyridine-1-acetic Acid Ethyl Ester Monohydrochloride (153). Under a nitrogen atmosphere, 2-(4-chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine[11] (9.16 g, 0.04 mol) was added to a stirred suspension of sodium hydride (1.76 g, 0.044 mol, 60% in oil, washed with hexane) in dimethylformamide (150 mL) and the reaction mixture was heated at 70–85 °C for 1.5 h. Ethyl bromoacetate (6.68 g, 0.04 mol) was added dropwise, and the reaction mixture was allowed to stir at room temperature over the weekend. The reaction mixture was poured into water and the precipitate was collected by filtration, washed with water, and dried under high vacuum at 50 °C overnight (79% crude yield). A 1.5-g portion was dissolved in ethyl acetate and acidified with ethereal hydrogen chloride. The crystalline precipitate was collected by filtration, washed with ethyl acetate, and dried under high vacuum to give 1.55 g, which was recrystallized from acetonitrile to give 1.16 g (59%), mp 199–200 °C dec. Anal. ($C_{16}H_{14}N_3O_2Cl$·HCl) C, H, N.

2-(4-Chlorophenyl)-1*H*-imidazo[4,5-*b*]pyridine-1-acetic Acid Monohydrate (154). A mixture of **153** (8.4 g, 0.027 mol) and potassium hydroxide (2.25 g, 0.04 mol) in ethanol (75 mL) and water (5 mL) was heated at reflux for 2.5 h. The mixture was evaporated to dryness. The residue was dissolved in water and acidified with glacial acetic acid and the resulting precipitate was collected by filtration, washed with water, and dried under high vacuum overnight at 60 °C to give 7.4 g (95%). A 1.4-g portion was dissolved in hot methanol (450 mL), treated with Florisil, and filtered. The filtrate was concentrated and diluted with water. A crystalline precipitate was collected by filtration, washed with water, and dried under high vacuum at 60 °C to give 0.16 g, mp 194–196 °C. Anal. ($C_{14}H_{10}N_3O_2Cl$·H_2O) C, H, N.

2-(4-Chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine-3-acetic Acid Ethyl Ester (155). A suspension of 2-(4-chlorophenyl)-3*H*-imidazo[4,5-*b*]pyridine[11] (3.0 g, 0.013 mol) and sodium hydride (0.52 g of 60% in oil) in *N*,*N*-dimethylformamide (65 mL) was heated at 70 °C under nitrogen for 1.5 h, then cooled to room temperature. Ethyl bromoacetate (2.18 g, 0.013 mol) was added and the reaction mixture was stirred at room temperature overnight. The mixture was poured into water and the resulting precipitate was collected by filtration. The solid was recrystallized from 2-propanol and water and dried under high vacuum at 70 °C to give 1.24 g (30%) of **155**, mp 250–252 °C. Anal. ($C_{16}H_{14}N_3O_2Cl$) C, H, N.

2-(4-Chlorophenyl)-3*H*-imidazo[4,5-*c*]pyridine-3-acetic Acid Monohydrochloride Hemihydrate (156). A solution of **155** (1.0 g, 3.2 mmol), water (2.6 mL), and solid potassium hydroxide (0.28 g, 4.9 mmol) in 95% ethanol (10 mL) was heated at reflux for 0.75 h. The reaction mixture was evaporated, dissolved in water, and acidified with 3 N HCl. The resulting solid was collected by filtration, washed with water, and dried under high vacuum at 70 °C to give 0.5 g (47%), mp 246–247 °C. Anal. ($C_{14}H_{10}N_3O_2Cl$·HCl·0.5H_2O) C, H, N.

***N*-(3-Nitro-4-pyridinyl)glycine Ethyl Ester (157).** This compound was prepared according to method A starting from 4-chloro-3-nitropyridine, except that dioxane was used as the solvent at room temperature overnight. The crude oil was purified by HPLC on silica gel using ethyl acetate/hexane (1:1) as the eluting solvent. The pure fractions were combined, evaporated, and dried under high vacuum to give 1.1 g (69%), mp 82–83 °C. Anal. ($C_9H_{11}N_3O_4$) C, H, N.

***N*,*N*-Dimethyl-2-[(3-nitro-4-pyridinyl)amino]acetamide (158).** Condensed dimethylamine (20 mL) was added in portions to a solution of **157** (4.0 g, 18 mmol) in absolute ethanol (10 mL). The reaction mixture was allowed to stir at room temperature over a weekend. The reaction mixture was diluted with petroleum ether and the resulting solid was collected by filtration. The solid was dissolved in tetrahydrofuran, filtered, and diluted with isopropyl ether to produce a dark solid that was removed by filtration. The filtrate was further diluted with petroleum ether and was placed in the freezer. The crystalline solid was collected by filtration, redissolved in methylene chloride, and treated with Florisil, then evaporated to give 1.8 g (45%), mp 142–143 °C. Anal. ($C_9H_{12}N_4O_3$) C, H, N.

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Synthesis and Antiviral Activity of Certain Guanosine Analogues in the Thiazolo[4,5-*d*]pyrimidine Ring System

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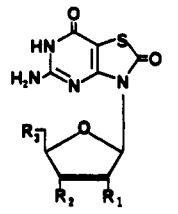
Several sugar-modified nucleoside derivatives of the purine analogue 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]-pyrimidine-2,7-dione (1) were synthesized. Phosphorylation of 1 using $POCl_3$ resulted in 5′-monophosphate 2, which was subsequently converted to 3′,5′-cyclic phosphate 3, by reported methods. 5′-Sulfamoyl derivative 4 was synthesized by treatment of the 2,3-*O*-isopropylidene derivative of 1 with chlorosulfonamide followed by acid deprotection. Compounds 5–7, the 5′-deoxy, the tri-O-acetyl, and the 2′-deoxy derivatives of 1, respectively, were synthesized by glycosylation of 5-aminothiazolo[4,5-*d*]pyrimidine-2,7-dione, the aglycon of 1, with the appropriate sugar moieties, utilizing the Vorbruggen procedure. Oxidative cleavage of the C_2–C_3 bond in 1 followed by reduction with sodium borohydride led to "seco" analogue 8. Nucleosides 2–8 were evaluated for antiviral activity in vivo against the Semliki Forest virus. The activity of compounds 2, 5, and 7 were similar to that of 1. Cyclic phosphate 3 was toxic at the high dose and weakly active at the lower dose. Compounds 4, 6, and 8 were inactive in this system.

Introduction

A recent report from our laboratories detailed the synthesis and immunopotentiating properties of purine nucleoside analogues in the thiazolo[4,5-*d*]pyrimidine ring system.[1] The guanosine analogue (1), in particular, was shown to possess excellent in vivo activity against a variety of DNA and RNA viruses.[1-4] While 1 exhibited a stimulatory effect on both cellular and humoral components of the immune response, the observed antiviral effect has been attributed primarily to the induction of α-interferon.[5] Other guanosine analogues and derivatives have been studied over the years for their ability to activate the immune system,[6] such as 8-bromo-, 8-mercapto-, and 7-methyl-8-oxoguanosine. These guanosines, including 1, have all been modified in the heterocyclic moiety relative to guanosine itself. It is evident from our own studies[1,7,8] that there is a good deal of "structural permissiveness" with regard to the structure–activity relationship and requirements for good in vivo antiviral activity. When the exception of one report dealing with B-cell activation,[9] to the best of our knowledge, no other reports have appeared in the literature which address the contribution and struc-



1 : $R_1 = R_2 = R_3 = OH$

2 : $R_1 = R_2 = OH$, $R_3 = -O-P(=O)(OH)OH$

3 : $R_1 = OH$, R_2, $R_3 = -O-P(=O)(OH)-O-$

4 : $R_1 = R_2 = OH$, $R_3 = -OSO_2NH_2$

5 : $R_1 = R_2 = R_3 = -OCOCH_3$

6 : $R_1 = R_2 = OH$, $R_3 = H$

7 : $R_1 = H$, $R_2 = R_3 = OH$

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