than compensates the polarity of the M3 minimum.

Thus, if we postulate that β_2 affinity and selectivity are based on specific hydrophobic interactions, a validation of this hypothesis cannot be found in MEP patterns. Clearly a variety of approaches are necessary for a relevant pharmacophore to be obtained.

Experimental Section

Molecular Electrostatic Computations. In order to reduce computing times the calculations were carried out on the primary amine analogues of all compounds. Although adrenoceptor ligands bind and act as in their protonated form, the MEP in thus study were calculated for the neutral molecules. This renders the MEP details much more visible while not altering the difference in electrostatic potential between local minima and maxima.[20]

Standard bond lengths and angles were used as input for the calculations. Wave functions and electronic densities were calculated with the ab initio Monstergauss 81 program as reported previously[20] and with the Gaussian 82 program,[51] using a minimal STO-3G basis set. MEPs were obtained with a slightly modified version of DENPOT (QCPE 360) and were generated in planes parallel to the aromatic ring, viewing compounds from above and below the aromatic ring plane for each compound. All calculations were performed on the CDC CYBER 170/855 and CRAY 1S computers of the Federal Institute of Technology in Lausanne and a Norsk Data ND 560 computer of the University of Lausanne.

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Supplementary Material Available: Plots of the MEP of compounds 1, 3, 5–9, 11–13, 15, 16, 18, 29–32 in a plane parallel to, and 2 Å above, the plane of the aromatic ring (9 pages). Ordering information is given on any current masthead page.

Radioiodinated Benzodiazepines: Agents for Mapping Glial Tumors

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Two isomeric iodinated analogues of the peripheral benzodiazepine binding site (PBS) ligand Ro5-4864 have been synthesized and labeled in high specific activity with iodine-125. Competitive binding assays conducted with the unlabeled analogues indicate high affinity for PBS. Tissue biodistribution studies in rats with these ^{125}I-labeled ligands indicate high uptake of radioactivity in the adrenals, heart, and kidney—tissues known to have high concentrations of PBS. Preadministration of the potent PBS antagonist PK 11195 blocked in vivo uptake in adrenal tissue by over 75%, but to a lesser degree in other normal tissues. In vivo binding autoradiography in brain conducted in C_6 glioma bearing rats showed dense, PBS-mediated accumulation of radioactivity in the tumor. Ligand **6** labeled with ^{123}I may have potential for scintigraphic localization of intracranial glioma.

Since their discovery in the late 1950s, the 1,4-benzodiazepines have constituted a class of widely used anxiolytic and anticonvulsant drugs.[1] In the last several years, the presence of two pharmacologically distinct subclasses of benzodiazepine binding sites have been demonstrated. One class, the central benzodiazepine receptor, is localized in central neuronal tissue and represents the site at which benzodiazepine ligands exert their anxiolytic effects.[2,3] In addition, binding sites for benzodiazepines have been identified in peripheral tissues including the adrenal cortex, nasal epithelium, kidney, and heart; these sites were subsequently termed the peripheral benzodiazepine binding sites (PBS).[4,5] It has also been shown, however, that these sites though present mainly in the periphery are also located in the olfactory bulb as well as glial cells in brain.[6,7] The pharmacological distinction between these two types of binding sites can be demonstrated by the use of highly selective ligands. The clinically potent anticonvulsant clonazepam binds with nanomolar affinity to the central benzodiazepine receptor but with less than micromolar affinity to PBS.[8] Conversely Ro5-4864, which exhibits no anxiolytic activity, has nanomolar affinity for the peripheral binding site but a 1000-fold lower affinity

Table I. Radiosynthetic Data

ligand	isolated radiochemical yield, %	sp. act., Ci/mmol	radiochemical purity, %
5	18–31 (N = 3)	117–128 (N = 3)	>98
6	21–43 (N = 6)	103–145 (N = 3)	>98

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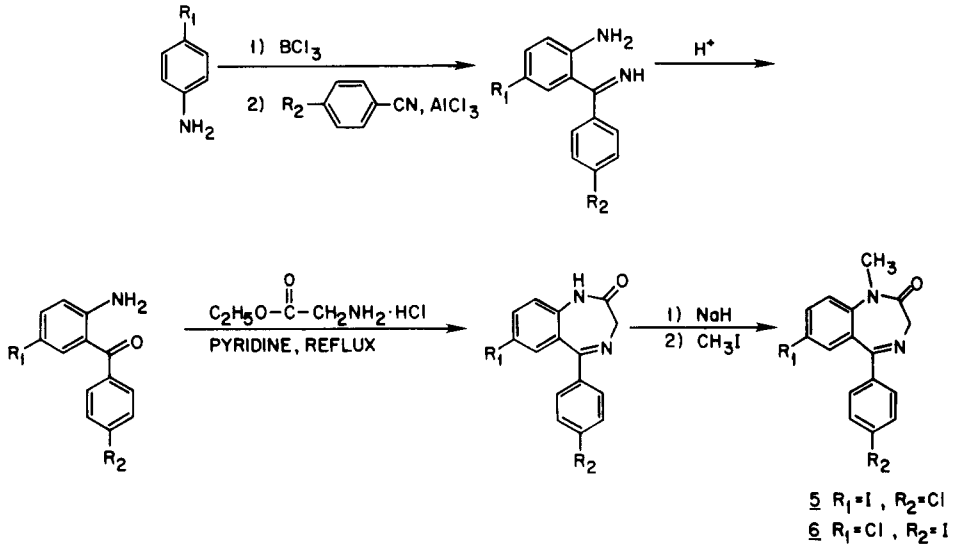
Scheme I



for the central receptor.[8] Moreover, benzodiazepine binding to PBS is unaffected by the presence of γ-aminobutyric acid unlike the case of central receptors.[7,9]

Recent studies have indicated the presence of high concentrations of PBS in human glial tumors.[10] Successful delineation of intracranial C_6 gliomas in rats has been achieved by in vivo binding autoradiography using the peripheral binding site specific ligand [^{3}H]Ro5-4864.[11] This observation indicated that an analogue of Ro5-4864 radiolabeled with a γ-emitting radionuclide could have clinical utility for scintigraphic localization of intracranial human glioma. We report here the synthesis of two ^{125}I-labeled analogues of Ro5-4864, their in vitro affinity for PBS, and preliminary brain autoradiographic studies conducted in C_6 glioma bearing rats.

Chemistry

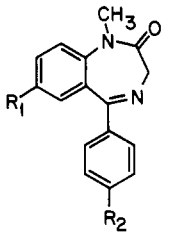
The novel approach of Sugasawa and co-workers[12] was used with minor modifications to prepare the halogenated 2-aminobenzophenone precursors (Scheme I). Condensation of the aniline–boron trichloride complex with the appropriate benzonitrile analogue in the presence of $AlCl_3$ gave the corresponding ketimine which afforded the 2-aminobenzophenone analogue on acid hydrolysis. The latter underwent smooth cyclization with glycine ethyl ester hydrochloride in pyridine to give the corresponding 1,4-benzodiazepine analogue by the procedure of Sternbach et al.[13] N-Methylation was carried out in excellent yields by treatment of the corresponding sodium salt with methyl iodide.[13]

Radioiodide labeling was performed by slight modification of the ammonium sulfate catalyzed solid-phase exchange technique described previously.[14] The results are summarized in Table I. Exchanges were conducted in 3-mL septum-sealed multidose vials vented with a disposable 10-cm^3 plastic syringe which served as the distillate condenser. During the heating process, charcoal and aqueous sodium thiosulfate traps were connected in series with the condenser to trap any volatile radioiodine that might be released.

Radiochemical purity was assessed by radio-TLC and by reversed-phase high-performance liquid chromatography (radio-HPLC) using systems A and B. In all cases the retention times of the labeled materials were in agreement with that of the unlabeled ligands.

The chemical purity of the ligands following the isotope-exchange procedures was assessed by an indirect approach. The exact exchange procedure and workup described above was repeated for each ligand with Na^{127}I instead of Na^{125}I, and the reaction products were analyzed by normal phase HPLC (system A). These studies demonstrated that the labeling conditions resulted in ligands with greater than 95% chemical purity.

Table II. Affinity of Ligands for Central Receptor and Peripheral Benzodiazepine Binding Site



ligand	R_1	R_2	K_i,[a] nM ± SD PBS, nM	K_i,[a] nM ± SD CBR, nM
Ro5-4864	Cl	Cl	7.3 ± 2.3	>1000
5	I	Cl	13.3 ± 4.8	>1000
6	Cl	I	13.6 ± 4.5	>1000

[a] N = 3 for each ligand per assay; PBS is peripheral benzodiazepine binding site; CBR is central benzodiazepine receptor.

Results and Discussion

The affinity of the ligands for PBS was evaluated in vitro by their ability to displace [^{3}H]flunitrazepam (5 nM) binding in rat brain and kidney tissue sections. These results are summarized in Table II. As seen from this data, both **5** and **6** display high affinity for PBS with K_i values of 13.3 and 13.6 nM, respectively. The K_i for Ro5-4864 under the same conditions was determined to be 7.3 nM. Thus, substitution of iodine for chlorine at

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Table III. Concentration of Radioactivity at Various Time Intervals after Intravenous Administration of ^{125}I-Labeled Ligands[a,b]

time	ligand	adrenal	brain	blood	heart	kidney	thyroid[c]
5 min	**5**	4.85 ± 0.54	0.75 ± 0.06	0.20 ± 0.01	1.15 ± 0.06	1.10 ± 0.03	1.50 ± 0.43
	6	7.10 ± 1.85	1.00 ± 0.10	0.22 ± 0.01	2.18 ± 0.11	1.78 ± 0.13	3.27 ± 0.58
30 min	**5**	3.63 ± 0.53	0.46 ± 0.03	0.15 ± 0.02	0.77 ± 0.10	0.74 ± 0.06	4.42 ± 0.48
	6	7.85 ± 0.32	0.60 ± 0.05	0.11 ± 0.04	1.13 ± 0.10	1.05 ± 0.12	2.25 ± 0.14
2 h	**5**	1.84 ± 0.22	0.41 ± 0.01	0.12 ± 0.00	0.63 ± 0.04	0.58 ± 0.03	5.57 ± 0.74
	6	2.38 ± 0.25	0.35 ± 0.02	0.10 ± 0.01	0.50 ± 0.05	0.51 ± 0.03	4.26 ± 0.34 (0.49 ± 0.07)
6 h	**5**	1.30 ± 0.10	0.46 ± 0.04	0.12 ± 0.02	0.57 ± 0.04	0.59 ± 0.04	17.28 ± 4.90[d]
	6	0.60 ± 0.05	0.14 ± 0.04	0.05 ± 0.01	0.19 ± 0.02	0.21 ± 0.03	11.85 ± 1.88[d]
24 h	**5**	0.42 ± 0.16	0.21 ± 0.06	0.05 ± 0.02	0.25 ± 0.07	0.25 ± 0.08	17.20 ± 2.65
	6	0.05 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	10.85 ± 5.05

[a] Concentrations, normalized to a 200-g rat, are percent of injected dose/gram of tissue (wet weight). [b] Male Sprague–Dawley rats (N = 5 for each ligand per time point) weighing 160–265 g were used. [c] Value in parentheses was obtained with rats on oral KI solution. [d] The mean concentration of radioactivity in the thyroid of two rats administered Na^{125}I (20 μCi) was 360.5% dose/g, suggesting that <5% of ligands **5** and **6** have undergone radioiodeiodination at 6 h postinjection.

either the 7- or the 4′- positions of Ro5-4864 results in only a small decrease in binding affinity for PBS. The affinities of **5** and **6** for the central benzodiazepine receptor were nearly a 100-fold lower than that observed for the peripheral binding site.

The concentration of radioactivity in various tissues was determined following intravenous administration of the ^{125}I-labeled ligands to rats. Of the 20 different tissues analyzed, only six are presented in Table III. The adrenal gland and kidney, organs known to have high concentrations of PBS,[4] gave high concentrations of radioactivity. In general [^{125}I]-**6** gave higher radioactivity concentrations in PBS-rich tissues such as adrenal, heart, and kidney than [^{125}I]-**5** at early time intervals (i.e. 5 and 30 min); the reverse pattern was observed at longer intervals (i.e. 6 and 24 h). Both ligands showed similar tendencies to undergo in vivo deiodination as reflected in their thyroid radioactivity concentrations. As noted in Table III, approximately 90% of the thyroid radioactivity uptake from [^{125}I]-**6** could be blocked by preadministration of oral potassium iodide. On the basis of thyroid radioactivity levels obtained from iv injections of Na^{125}I in rats, the 6-h thyroid values for [^{125}I]-**5** and [^{125}I]-**6** in Table III represent less than 5% in vivo deiodination of these ligands. Metabolic studies of **6** in rats 30 min after iv injection show that approximately 95% of the radioactivity in the heart is unchanged **6**; desmethyl **6** (i.e. [^{125}I]-**4**) was the only metabolite of **6** detected in the heart. Radio-TLC analysis of the blood revealed 78% of the radioactivity as unchanged **6**. The desmethyl analogues of benzodiazepines such as **5** and **6** are known to have low affinity for PBS.[8]

Ligand **6** was chosen for further studies, including in vivo blocking studies in normal rats and autoradiographic analyses in C_6 glioma bearing rats. As shown in Table IV, preadministration of the potent PBS antagonist PK 11195 lowered the in vivo accumulation of radioactivity in the adrenal gland by 75%; kidney and heart were less affected, a result which suggests that a large component of the radioactivity in these tissues is not PBS-mediated 30 min after tracer injection.

As discussed above, desmethyl **6** is formed in vivo from **6** and sequestered to a small extent by the heart by a mechanism that is likely not PBS mediated. The presence of this metabolite may account in part for the radioactivity in other tissues that is not blocked by PK 11195. Metabolic studies of **6** in other organs and in PK 11195 treated animals should answer this question. Whether specific binding increases at later time points remains to be determined. Uptake in brain, a tissue poor in PBS, showed no statistical lowering on blocking with PK 11195. In vivo binding of [^{125}I]-**6** to PBS located in C_6 glioma tissue was used to image the tumor by autoradiography (Figure 1).

Table IV Effect of PK 11195 Preadministration on Tissue Distribution of [^{125}I]-**6**[a–c]

tissue	control	PK 11195 pretreated	% change
adrenal	6.89 ± 0.54	1.74 ± 0.29	−75
heart[d]	1.15 ± 0.08	0.76 ± 0.13	−34
kidney	1.13 ± 0.06	0.74 ± 0.14	−35
lung	1.08 ± 0.08	0.58 ± 0.18	−46
submaxillary gland	1.44 ± 0.06	0.74 ± 0.17	−49
brain[e]	0.56 ± 0.05	0.69 ± 0.13	
blood	0.14 ± 0.03	0.16 ± 0.02	

[a] Rats were given either vehicle (0.1 mL/kg, 1:1 DMSO/EtOH) or PK 11195 (10 mg/kg, iv) 30 min prior to tracer administration; animals were sacrificed 30 min after tracer. [b] Concentrations are in percent of injected dose/gram of tissue (wet weight) normalized to a 200-g rat. [c] Male Sprague–Dawley rats (N = 5 per group) weighing 179–302 g. [d] Left ventricle. [e] Cerebral cortex.

Dense accumulation of ^{125}I was observed in the tumor region; the outline of radioactive accumulation was very similar to the histological outline of the glioma (compare panels A and B in Figure 1). Animals which received a predose of PK 11195 (5 mg/kg, iv) showed decreased accumulation of radioactivity in tumor tissue, similar to background binding in normal brain, throughout the time course studied. Nearly two-thirds of tumor binding at 10 min was not blocked by PK 11195. At 30 min, the specific to nonspecific binding ratio (i.e. the ratio of radioactivity concentration in tumor to noninvolved cortical tissue) was approximately 2:1 and the absolute amount of radioactivity in tumor increased. At 120 min, the binding ratio showed no change although the absolute levels of ligand in tumor declined slightly. These observations strongly suggest that the autoradiogram of the tumor at 120 min reflects specific binding of [^{125}I]-**6** to PBS sites.

The present work demonstrates that radioiodinated PBS ligands with good radiostability can be made in specific activities sufficient for in vivo mapping studies. Although carrier is present, the specific activity of these ligands are nonetheless 2–4 times higher than that of ^{3}H-labeled ligands normally employed for this purpose. More importantly, with use of this same methodology, the corresponding ^{123}I-labeled ligand could be synthesized for in vivo imaging of PBS-rich tissues by the technique of single photon emission computed tomography. More detailed autoradiography and pharmacology of ligand **6** will be reported in a subsequent paper.

Experimental Section

Melting points were obtained in open capillary tubes with a Thomas-Hoover or Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Varian EM360-A spectrometer with either $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (δ) are reported in parts per million (ppm) downfield from

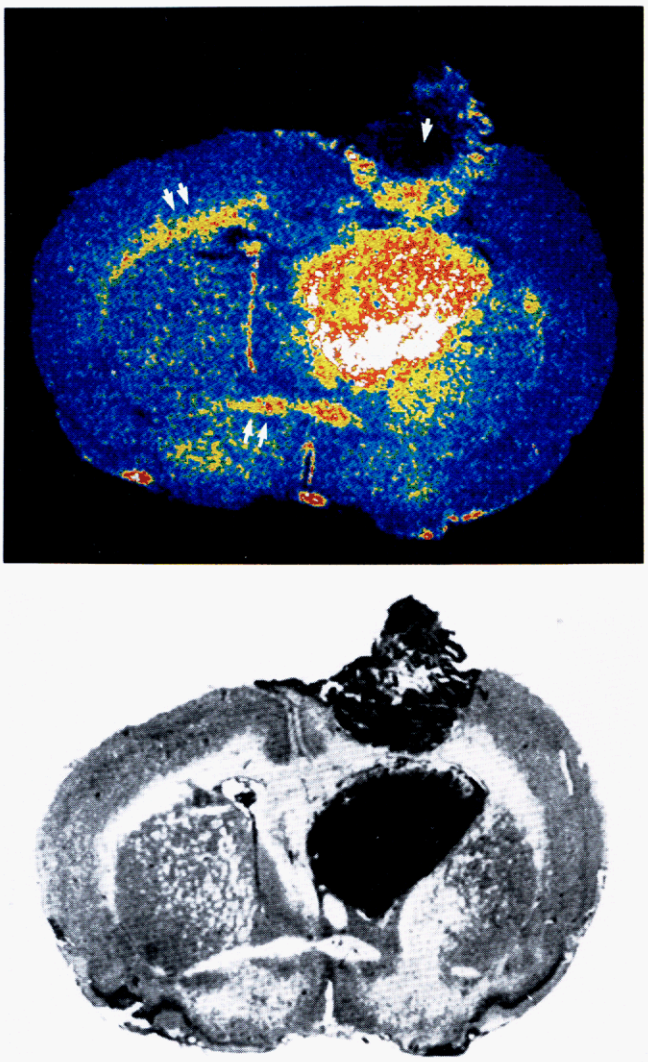

Figure 1. In vivo binding autoradiogram (panel A, top) in C_6 glioma rat 120 min after iv injection of [^{125}I]-**6** and corresponding cresyl violet stained section (panel B, bottom). [^{125}I]-**6** was concentrated in glioma tissue, approximately 2:1 over normal brain tissue, which clearly imaged the tumor dimensions (compare with cresyl violet stain of the identical tissue section). Binding within the tumor region is heterogeneous; low binding is likely due to necrotic tissue (arrow). The binding of radioligand to the glioma was blocked in animals predosed with 5 mg/kg PK 11195, a high-affinity ligand for the peripheral benzodiazepine binding site. Nonspecific binding in the tumor was similar to total, or nonspecific, binding in normal brain. [^{125}I]-**6** does concentrate in white matter (double arrows) which cannot be blocked by PK 11195. In panel A autoradiogram, radioactivity concentration increases in the following order: black < violet < blue < green < yellow < orange < red < white.

tetramethylsilane (TMS). IR spectra were obtained in thin KBr wafers and recorded on a Perkin-Elmer 283B spectrophotometer. All spectral results are in agreement with the assigned structures. Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI. All analyses (C, H, N) are within ±0.4% of the theoretical values. TLC was done with Whatman K6F silica gel coated glass plates with fluorescent indicator. Flash column chromatography[15] was done with E. Merck Kieselgel 60 (230–400 mesh). PK 11195 was a generous gift of Pharmuka Laboratories, Gennevilliers, France. 4-Iodobenzonitrile was obtained from Pfaltz and Bauer, Waterbury, CT. All other reagents were obtained from Aldrich Chemical Co., Milwaukee, WI. Sodium iodide-125 was obtained from Du Pont New England Nuclear, Boston, MA, as a no-carrier-added solution in 0.1 N NaOH (pH 10–12). Saturated KI solution for oral administration was obtained from Upsher-Smith Laboratories.

HPLC and Radio-HPLC Analyses. Analyses were performed with a Waters Associates (Milford, MA) HPLC system consisting of a Model 680 gradient controller, two Model 510 pumps, Model U6K injector, and Model 740 data module with a Kratos (Ramsey, NJ) Model SF773 variable-wavelength UV/vis detector and Radiomatic Instruments (Tampa, FL) Model DR, upgraded to Model IC, Flo-One radioactivity detector. For the separations, one of the following systems was used: system A, an Ultrasphere Si column (0.46 × 25 cm, 5-μm particle size, silica; Beckman Instruments, Berkeley, CA) with THF/hexane (1:9) as eluant, a flow rate of 2.0 mL min^{-1}, and detection at 254 nm; system B, and Ultrasphere ODS column (0.46 × 25 cm, 5-μm particle size, C-18; Beckman Instruments) with CH_3CN/H_2O (3:1) as eluant, a flow rate of 1.0 mL min^{-1}, and detection at either 235 or 206 nm, the λ max for **5** and **6**, respectively; or system C, conditions as in system B except that a μBondapak C-18 column (0.39 × 30 cm, 10-μm particle size, C-18; Waters Associates) was used. Retention times for **5** and **6** were 6.43 and 6.55 min (system A), 8.12 and 8.45 min (system B), and 10.77 and 11.69 min (system C), respectively, under these conditions. The retention time of Ro5-4864 was determined to be 7.04 min (system B).

Radio-TLC Analyses. Radio-TLC analyses of radioiodide exchanges were performed on 2.5 × 20 cm silica gel coated (250 μm) glass plates (Whatman K6F, 250 μm). Radio-TLC analysis of tissue metabolites were performed on E. Merck plastic-backed alumina plates (60F/254 neutral, 0.2 mm). The radioactive solutions were applied over a spot of the corresponding unlabeled compound. The plates were analyzed on a Berthold Model LB2832 TLC-Linear Analyzer equipped with a Model LB500 Data Acquisition System.

Chemical Syntheses. The chemical synthesis of each ligand was carried out by using essentially identical procedures. Therefore one representative example will be described in detail and only physical data for the remaining compounds will be given.

5-Iodo-2-amino-4′-chlorobenzophenone (1). A solution of 4-iodoaniline (8.50 g, 38.8 mmol) in dichloroethane (40 mL) was added dropwise to an ice-cold stirred solution of BCl_3 (1.0 M in CH_2Cl_2, 42.7 mL, 42.7 mmol) under an argon atmosphere. The resulting aniline–boron trichloride complex was treated successively with 4-chlorobenzonitrile (10.68 g, 77.6 mmol) and anhydrous $AlCl_3$ (5.70 g, 42.7 mmol) and stirred at room temperature for 30 min. The mixture was then slowly heated to 60 °C and most of the CH_2Cl_2 (approximately 43 mL) removed by distillation, after which the solution was refluxed at 78 °C for 6 h. The reaction was allowed to cool, treated with aqueous 2 N HCl (78 mL), and heated at 78 °C for 30 min to hydrolyze the ketimine to the corresponding benzophenone. Extraction of the mixture with CH_2Cl_2 (4 × 150 mL) and removal of the solvent afforded the benzophenone product and unreacted nitrile. A suspension of the crude mixture in 95% EtOH (40 mL) and aqueous 2 N NaOH (80 mL) was refluxed with stirring for 2 h to hydrolyze the unreacted nitrile. The EtOH was removed under reduced pressure, H_2O (200 mL) was added, the mixture was extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic layers were dried ($MgSO_4$). Removal of the CH_2Cl_2 in vacuo afforded 6.53 g of crude product, which on recrystallization from absolute EtOH gave 4.81 g (35%) of analytically pure **1**: mp 130–131 °C (EtOH); IR (KBr) 3470, 3345, 1618, 1603 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.78–7.15 (m, 6 H, Ar H), 6.45 (d, 1 H, Ar H), 6.02 (br s, 2 H, NH_2). Anal. ($C_{13}H_9ClINO$) C, H, N.

5-Chloro-2-amino-4′-iodobenzophenone (2). A procedure analogous to the above utilizing 4-chloroaniline (5.80 g, 45.5 mmol) and 4-iodobenzonitrile (15.0 g, 65.5 mmol) gave 3.61 g (22%) of analytically pure **2**: mp 149–151 °C (EtOH); IR (KBr) 3430, 3320, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.85–6.55 (m, 7 H, Ar H), 6.02 (br s, 2 H, NH_2). Anal. ($C_{13}H_9ClINO$) C, H, N.

7-Iodo-1,3-dihydro-5-(4-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one (3). A mixture of glycine ethyl ester hydrochloride (2.05 g, 14.70 mmol) and 1 (3.50 g, 9.80 mmol) in dry pyridine (22 mL) was refluxed with stirring for 15 h. The reaction flask was fitted with an addition funnel (containing 3A molecular sieves) to which a reflux condenser was attached for continuous removal of the H_2O formed during the reaction. Removal of the pyridine under vacuum distillation afforded a tarry residue which was

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partitioned between Et_2O (200 mL) and H_2O (200 mL). The Et_2O layer was removed, the aqueous layer made basic (pH 12) with 1 N NaOH, and the latter extracted further with Et_2O (2 × 200 mL). The combined Et_2O layers were dried (Na_2SO_4) and evaporated in vacuo to afford 4.03 g of crude product. Flash column chromatography on silica with EtOAc/CH_2Cl_2/hexanes (2:2:1) gave 2.95 g (76%) of pure **3**: mp 244–245 °C (toluene); IR (KBr) 3190, 3090, 2930, 1688 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.71 (s, 1 H, NH), 7.78–6.78 (m, 7 H, Ar H), 4.24 (s, 2 H, CH_2). Anal. ($C_{15}H_{10}ClIN_2O$) C, H, N.

7-Chloro-1,3-dihydro-5-(4-iodophenyl)-2*H*-1,4-benzodiazepin-2-one (4). In a procedure and workup analogous to the above treatment of **2** (2.40 g, 6.72 mmol) with glycine ethyl ester hydrochloride (1.41 g, 10.1 mmol) gave 1.84 g (69%) of analytically pure **4**: mp 260–261.5 °C dec (toluene); IR (KBr) 3200, 3110, 2945, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.45 (s, 1 H, NH), 7.70–7.02 (m, 7 H, Ar H), 4.00 (s, 2 H, CH_2). Anal. ($C_{15}H_{10}ClIN_2O$) C, H, N.

7-Iodo-1,3-dihydro-1-methyl-5-(4-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one (5). Sodium hydride (60% dispersion in mineral oil; 112 mg, 2.8 mmol) was added in two portions to a stirred, cooled (0 to 5 °C) solution of **3** (1.0 g, 2.5 mmol) in dry DMF (10 mL), under an argon atmosphere. The slurry was stirred for a further 30 min at room temperature, treated with CH_3I (0.43 g, 3.03 mmol), and stirred overnight at ambient temperature. Removal of the solvent under reduced pressure afforded a brown gum which was partitioned between CH_2Cl_2 (50 mL) and saturated brine (100 mL). The organic layer was removed and the aqueous layer further extracted with CH_2Cl_2 (2 × 50 mL), and the combined organic layers were washed with H_2O (1 × 50 mL) and dried ($MgSO_4$). Evaporation under reduced pressure afforded an oil which crystallized to a yellow solid on trituration with absolute EtOH. Flash chromatography on silica with EtOAc/CH_2Cl_2 hexanes (2:2:1) gave **5** (0.98 g, 95%): mp 157–158.5 °C (Et_2O); IR (KBr) 1675–1610 cm^{-1}; ^1H NMR δ 7.86–6.93 (m, 7 H, Ar H), 4.78 (d, 1 H, J_{AB} = 11 Hz, H_A of CH_2), 3.73 (d, 1 H, J_{AB} = 11 Hz, H_B of CH_2), 3.37 (s, 3 H, CH_3). Anal. ($C_{16}H_{12}ClIN_2O$) C, H, N.

7-Chloro-1,3-dihydro-1-methyl-5-(4-iodophenyl)-2*H*-1,4-benzodiazepin-2-one (6). A procedure analogous to the above utilizing **4** (1.0 g, 2.5 mmol) gave analytically pure **6** (0.99 g, 96%): mp 185–186 °C (CH_2Cl_2/hexanes, 1:1); IR (KBr) 1675, 1608 cm^{-1}; ^1H NMR δ 7.77–7.19 (m, 7 H, Ar H), 4.80 (d, 1 H, J_{AB} = 11 Hz, H_A of CH_2), 3.69 (d, 1 H, J_{AB} = 11 Hz, H_B of CH_2), 3.37 (s, 3 H, CH_3). Anal. ($C_{16}H_{12}ClIN_2O$) C, H, N.

Radiochemistry. In a typical procedure, a solution of $(NH_4)_2SO_4$ (5.0 mg in 15 μL of deionized H_2O), **5** (15 μg in 15 μL of acetone), and 7.0–8.0 mCi of $Na^{125}I$ were combined, and the mixture was heated to dryness at 140 °C in an oil bath. Air was then slowly passed through the reaction vial for 1 min and the dry reaction mixture maintained at 135 to 140 °C for 30 min. After cooling, the reaction mixture was dissolved in acetone (200 μL) and subjected to radio-TLC analysis (silica; EtOAc/CH_2Cl_2/hexanes 2:2:1; radiochemical yield 76%). Following removal of the acetone by means of a gentle stream of argon, the reaction mixture was dissolved in CH_2Cl_2 (2 × 0.5 mL) and transferred to a silica Sep-Pak (Waters). Elution with 4.5 mL of CH_2Cl_2 followed by 6.0 mL of EtOAc/hexanes (1:9) removed the less polar impurities. Further elution with EtOAc/hexanes (3:7) afforded 3.16 mCi of radioiodinated **5** in >98% radiochemical purity as determined by radio-TLC. The radiochemical yield was 26%.

An aliquot of the ^{125}I-ligand in CH_3CN/H_2O (1:1) was assayed for total radioactivity in a Capintec dose calibrator. A small portion of this aliquot was subjected to HPLC analysis (system C), and the area of the UV-absorbance peak was determined by means of an automated integrating recorder. The specific activity was calculated from a standard curve relating mass to UV absorbance peak area.

The radioiodinated ligands were formulated in ethyl alcohol/phosphate-buffered saline, pH 6.0 (15:85), prior to intravenous administration to rats. Radio-TLC analysis 4 days after formulation (stored at 4 °C) indicated less than 5% radiolytic decomposition of these ligands; ligand **5** 45 days in formulation was 86% radiochemically pure.

Biological Methods. In Vitro Benzodiazepine Binding Site Assays. Male Sprague–Dawley rats were decapitated, and the brains and kidneys were removed and frozen on dry ice. Thin sections (20 μm) were cut from the tissue in a cryostat–microtome at −20 °C. The sections were thaw mounted onto gelatin coated microscope slides and stored at −20 °C until the time of the assay. The sections were prewashed three times for 5 min in 50 mM Tris buffer, pH 7.4 at 0 °C, and then immediately incubated with 5 nM [^3H]flunitrazepam (Amersham, Chicago, IL; 85 Ci/mmol) in the same buffer for 90 min in the presence of various concentrations of **5** and **6**. Nonspecific binding was determined in the presence of 1 μM PK 11195. The sections were washed for 5 min in fresh buffer and blown dry under a stream of cool air. LKB Ultrofilm-^3H was apposed to the radiolabeled sections along with ^{14}C standards (American Radiolabeled Chemicals, Inc., St. Louis, MO) for 10 days. The film was developed in D-19 developer for 3 min, and the resulting autoradiograms were analyzed for tritium binding by microspot densitometry. Inhibition constants (K_i values) were determined by the equation $K_i = IC_{50}/(1 + [t]/K_d)$, where IC_{50} = the concentration of competitor needed to inhibit 50% of the control binding, [t] = the radioligand concentration, and K_d = the radioligand's dissociation constant (40 nM, determined in separate experiments). K_i values for the central receptor were determined from the binding data in brain tissue while K_i values for the peripheral site were determined from the data in kidney tissue.

Biodistribution Studies. Male Sprague–Dawley rats weighing approximately 200 g were given water and standard chow ad libitum. Under light ether anesthesia the femoral vein was exposed and 16–19 μCi of ^{125}I-labeled ligand in 0.20–0.25 mL of 15:85 EtOH/phosphate-buffered saline (pH 6.0) was injected. At various time intervals after injection, the animals were killed by decapitation, selected tissues were excised and weighed, and the ^{125}I radioactivity was determined in an autogamma counter. Radioactivity concentrations were expressed as percent injected dose/gram of wet tissue. Three additional rats were given oral KI 2 days prior to iv injection of [^{125}I]-**6** to block thyroid sequestration of metabolic radioiodide. Ten drops of KI (1 g/mL) were dissolved in 250 mL of drinking water. Animals were sacrificed by decapitation 30 min after tracer injection; samples of 10 tissues were excised and counted as described above. In blocking studies, either vehicle (0.1 mL/kg, 1:1 dimethyl sulfoxide/ethanol) or 10 mg/kg PK 11195 was injected iv 30 min prior to administration of 18.10–19.2 μCi of [^{125}I]-**6** into the contralateral femoral vein. Animals were killed by decapitation 30 min after tracer administration. Five rats were used for both the control and experimental groups.

In Vivo Binding Autoradiography. Male Wistar rats (200–300 g) were innoculated with a C_6 glioma cell suspension as described previously.[10,16] Two to three weeks after innoculation, the animals were catheterized via the femoral blood vessel under ether anesthesia.[17] Thirty minutes prior to injection of [^{125}I]-**6**, the rats were pretreated iv with either vehicle (0.1 mL/kg, 1:1 dimethyl sulfoxide/ethanol) or 5 mg/kg PK 11195. The rats were injected with a bolus of approximately 400 μCi of [^{125}I]-**6**. Ten minutes, 30 min, or 120 min later, the animals were sacrificed by injection of 1 mL of saturated aqueous KCl. The brains, with intact gliomas, were rapidly removed by blunt dissection and frozen on dry ice for future ultracryotomy. Thin sections (20 μm) through the tumor tissue were thaw mounted onto gelatin coated glass microscope slides and apposed to SB-5 X-ray film (Eastman Kodak Co., Rochester, NY). After 3–7 days the film was developed in GBX developer to produce the autoradiogram shown in Figure 1.

Metabolism Studies. Three female Sprague–Dawley rats (210–250 g) were sedated with pentobarbital and injected via the femoral vein with 0.4 mCi [^{125}I]-**6**. Animals were sacrificed 30 min later. The hearts were frozen on dry ice, and the blood was chilled until homogenized in a 3-fold volume of acetonitrile with a Brinkman homogenizer. The supernatants were centrifuged for 15 min at 12000*g* at room temperature. The resulting supernatants and pellets were counted to determine the extraction efficiency of radioactivity into acetonitrile.

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A control rat was injected with saline and sacrificed 30 min later. Tissues were prepared as above with the addition of authentic [^{125}I]-**6** (10 μCi) to the supernatant prior to centrifugation. The supernatants were concentrated by evaporation of acetonitrile to a volume of approximately 100 μL (to 10% of the starting volume). The samples were applied to alumina TLC plates and eluted with $CHCl_3$. The R_f values of **6** and desmethyl **6** were 0.58 and 0.08, respectively. The extraction efficiency of radioactivity from the supernatant was 93% for heart tissue and 83% for blood for both control and experimental samples. The results with blood show 22% of the radioactivity co-migrates with desmethyl **6**. The remaining radioactivity (78%) is present as [^{125}I]-**6**. There was less metabolite in heart with 5% as ^{125}I-labeled desmethyl **6** and the remaining 95% as [^{125}I]-**6**. The controls showed 97% and 99% as [^{125}I]-**6** in blood and heart, respectively.

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Synthesis and Antiviral Activity of Certain 4- and 4,5-Disubstituted 7-[(2-Hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidines

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In vitro evaluation of a series of previously prepared tubercidin analogues revealed that certain 5-halogen-substituted analogues were active against human cytomegalovirus (HCMV) at concentrations lower than those that produced comparable cytotoxicity in uninfected cells. In contrast, tubercidin was cytotoxic at all antiviral concentrations. Even though the antiviral selectivity of the 5-substituted compounds was slight, this observation led us to prepare a series of acyclic analogues. Treatment of the sodium salt of 4-chloropyrrolo[2,3-*d*]pyrimidine (**2**) with (2-acetoxyethoxy)methyl bromide (**2a**) provided the acyclic nucleoside 4-chloro-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**3**). A nucleophilic displacement of the 4-chloro group with methoxide, methylamine, and dimethylamine yielded the corresponding 4-substituted compounds **4**, **5**, and **6**, respectively, in good yield. Electrophilic substitution (chlorination, bromination, and iodination) was effected at the C-5 position of compound **3** with *N*-chlorosuccinimide, *N*-bromosuccinimide, and iodine monochloride, respectively, in methylene chloride. Removal of the acetyl group from these intermediates (**7a–9a**) with methanolic ammonia at room temperature afforded the 5-chloro (**7b**), 5-bromo (**8b**), and 5-iodo (**9b**) derivatives of 4-chloro-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine. Treatment of compounds **7b–9b** with methanolic ammonia at an elevated temperature produced the corresponding 5-halotubercidin analogues **10**, **11**, and **12**, respectively. An alternate procedure for the preparation of these 4,5-disubstituted 7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidines involved an electrophilic substitution prior to the condensation of the heterocycle with **2a**. Treatment of **2** with *N*-chlorosuccinimide and *N*-bromosuccinimide gave compounds **13a** and **13b**, respectively. The condensation of **13a** and **13b** with **2a** and subsequent treatment with methylamine and ethylamine furnished the corresponding 5-halo-4-substituted-pyrrolo[2,3-*d*]pyrimidines **14a**, **14b**, **14c**, and **14d**, respectively. Evaluation of the target compounds (**4–6**, **7b–9b**, **10–12**, and **14a–14d**) for cytotoxicity and activity against HCMV and herpes simplex virus type 1 (HSV-1) revealed that all compounds except the 5-halogen-substituted compounds **10**, **11**, and **12** were inactive. Compounds **10**, **11**, and **12** were active against both viruses at noncytotoxic concentrations. The activity of compound **11** was particularly noteworthy, being at least 10-fold more potent than acyclovir.

The most important antiviral drug discovered during the past several years is the acyclic analogue of guanosine, 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir). This compound potently and selectively inhibits the in vitro and in vivo replication of herpes simplex viruses[1,2] and is clinically efficacious in the treatment of certain herpesvirus infections.[3-6] The biochemical basis for the antiviral activity of acyclovir involves its specific phosphorylation to the corresponding monophosphate by a herpesvirus-encoded pyrimidine deoxynucleoside kinase.[1,7] The monophosphate is phosphorylated further by cellular kinases[8,9] to acyclovir triphosphate, a potent and selective inhibitor of the virus-encoded DNA polymerase.[1,10-12] The combination of specificity and selectivity for virus-encoded enzymes leads to a paucity of cytotoxic effects by the drug in uninfected cells and assures the usefulness of acyclovir as an antiviral agent.

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