Novel Irreversible Ligands Specific for "Peripheral" Type Benzodiazepine Receptors: (±)-, (+)-, and (−)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-(2-isothiocyanatoethyl)-3-isoquinolinecarboxamide and 1-(2-Isothiocyanatoethyl)-7-chloro-1,3-dihydro-5-(4-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one

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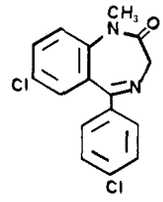
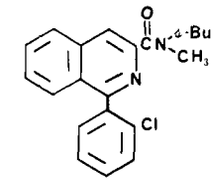
Novel ligands that bind irreversibly and selectively to "peripheral" type benzodiazepine receptors (PBR) have been prepared. These compounds inhibit radiolabeled binding to PBR in the nanomolar range. The 2-isothiocyanatoethyl analogue of Ro 5-4864 (1-methyl-7-chloro-1,3-dihydro-5-(4-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one) (**5**, AHN 086) was synthesized in three steps from desmethyl Ro 5-4864. The (±) (**11a**, AHN 070), *R*-(−) (**11b**), and *S*-(+) (**11c**) 2-isothiocyanatoethyl derivatives of PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide) were each prepared in three steps from PK 11209 (1-(2-chlorophenyl)-3-isoquinolinecarboxylic acid, **6**). All four compounds inhibited radioligand binding to the PBR in brain and kidney. The *R*-(−) stereoisomer **11b** was observed to be approximately 2.5-fold more potent than its enantiomer **11c**; this is the first report of stereoselectivity in the isoquinoline series of ligands selective for the PBR. Furthermore, pH dependency studies showed that, at lower pH, change in the affinities for the PBR ligands is a property of the receptor, substantiating the hypothesis that a histidine moiety on the PBR is the most likely site for covalent bond formation, whereas, at higher pH, the observed changes in affinities can be attributed to properties of the compounds. All four of these novel ligands are potentially useful tools in the investigation of the PBR.

Compounds such as Ro 5-4864[1] and PK 11195 have been shown to bind with high affinity and selectivity to "peripheral" type benzodiazepine receptors (PBR).[2–7]



Ro 5-4864



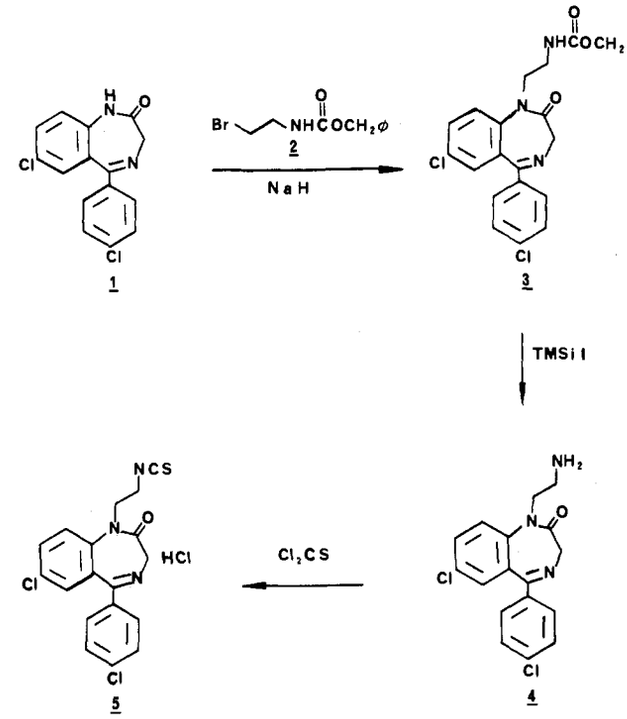
PK 11195

These specific ligands have been used to differentiate both the subcellular localization and structural requirements for high affinity drug binding to the PBR from those of "central" benzodiazepine receptors (CBR)[2–5,8,9] which are coupled to $GABA_A$ receptors and an associated chloride ion channel. A comparison of the affinities of a large series of benzodiazepines with their pharmacological effects suggests that the CBR mediate the anxiolytic, muscle relaxant, and anticonvulsant properties of this widely prescribed class of drugs.[10–13] In contrast, the physiological function of the PBR is still not known.[14]

The development of irreversible ligands has been invaluable in the characterization, isolation, and purification of a number of receptor systems. The isothiocyanato function has proven to be extremely versatile as the electrophilic moiety in these high affinity ligands. This function is easily synthesized[15] from a primary amino group. Its high reactivity toward amino and sulfhydryl groups, along with low reactivity toward water and other hydroxyl functions,[16] plays a major role in its successful application to affinity ligand preparation and use. Thus, ligands containing an appropriate isothiocyanato function have been used to label CBR,[17] phencyclidine receptors,[18] and μ and δ opioid subpopulations[19,20] and in purifying a δ opioid receptor ligand complex to homogeneity.[21]

It was of interest to design potential irreversible ligands for the PBR, such as isothiocyanato derivatives of Ro 5-4864 and PK 11195 as selective receptor probes. Furthermore, the demonstrated utility of stereoisomers in elucidating receptor function in other systems and the

Scheme I



† Laboratory of Chemistry.
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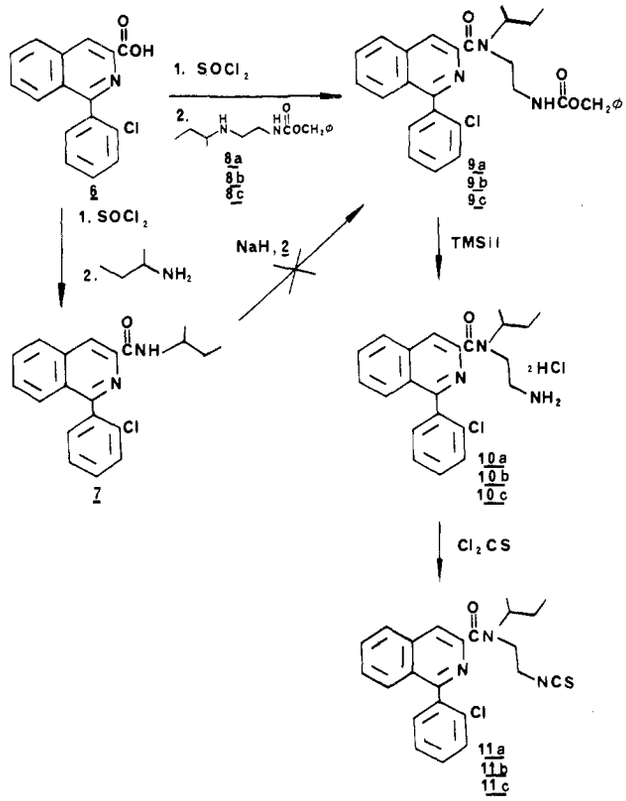
Scheme II



presence of an asymmetric center in PK 11195 suggested the synthesis of the stereoisomers of **11a**. The synthetic feasibility of replacement of the *N*-methyl substituent of both Ro 5-4864 and PK 11195 and the relatively minor effect, on the potency at the PBR, of small alkyl substituents at the nitrogen on Ro 5-4864[22] led to its replacement with the 2-isothiocyanatoethyl function in the high affinity parent ligands.

Table I. IC_{50} Values of **11a**[a]

ligand	tissue	IC_{50}[b]	Hill[c]	protein[d]
[^{3}H]Ro 5-4864	brain	3.0	1.0	255
[^{3}H]Ro 5-4864	kidney	2.0	0.97	62
[^{3}H]PK 11195	brain	15	1.0	263
[^{3}H]PK 11195	kidney	2.9	0.81	50

[a] The ligand concentration was 1 nM. Given are mean values of two experiments, each with six concentrations of the inhibitor in triplicate. [b] In nanomoles/liter. [c] Pseudo Hill coefficient. [d] In micrograms of protein/assay. For purposes of comparison, the IC_{50} values for **5** to inhibit [^{3}H]Ro 5-4864 binding in the brain and kidney (2.1 ± 0.2 nM and 1.2 ± 0.1 nM, respectively) were estimated by extrapolating the observed IC_{50} at different tissue concentrations to an infinite tissue dilution.[27] The IC_{50} of **5** to inhibit [^{3}H]PK 11195 binding was identical with the value obtained for [^{3}H]Ro 5-4864.[27]

Table II. IC_{50} Values of **11b** and **11c** at Two Protein Concentrations[a]

	11c		**11b**	
ligand	IC_{50}[b]	Hill[c]	IC_{50}[b]	Hill[c]
[^{3}H]Ro 5-4864	2.9[d]	0.98	1.2[d]	0.96
[^{3}H]Ro 5-4864	6.3[e]	1.0	2.9[e]	1.1
[^{3}H]PK 11195	3.9[d]	1.0	1.6[d]	1.0
[^{3}H]PK 11195	10[e]	1.2	3.5[e]	1.1

[a] The tissue source was rat kidney. The ligand concentration was 1 nM. Values are the means of two experiments, each with six concentrations of the inhibitor performed in triplicate. [b] In nanomoles/liter. [c] Pseudo Hill coefficient. [d] The protein concentration was 22 μg/assay. [e] The protein concentration was 110 μg/assay.

We now describe the synthesis and biochemical evaluation of novel irreversible ligands of the PBR based on PK 11195 (**11a–c**) and the synthesis and further biochemical study of **5**, as well as the amino precursors **4** and **10a–c**.

Chemistry. The 2-isothiocyanatoethyl analogue **5** of Ro 5-4864 was prepared in three steps starting with **1**,[1] as shown in Scheme I. Via the procedure of Earley et al.,[23] formation of the anion of **1** with NaH, in dry DMF, followed by addition of freshly prepared 1-(carbobenzoxyamino)-2-bromoethane (**2**)[24] and extractive workup afforded crude **3** as an orange oil. Purification by column chromatography and by crystallization from anhydrous ether gave **3**, in 92% yield. Deprotection with iodotrimethylsilane using the method of Lott et al.[25] followed by extractive workup and crystallization from EtOAc gave **4**, in 90% yield. Via an established procedure,[17] treatment of **4** with freshly distilled thiophosgene in a biphasic $CHCl_3$/aqueous $NaHCO_3$ system gave the isothiocyanate **5** (AHN 086), which was isolated as the HCl salt in 87% yield.

Two approaches could be taken to prepare the 2-isothiocyanato derivative of PK 11195 from **6**, as shown in Scheme II. The first approach required the amidation of the carboxylic acid **6** with (±)-*sec*-butylamine, to **7**, followed by N-alkylation with **2** as described for **1** above. The (±)-*sec*-butyl amide **7** was prepared in 50% yield via the acid chloride formed from the carboxylic acid **6** by stirring in thionyl chloride at reflux and reaction with (±)-*sec*-butylamine, in dry THF. However, the conversion of **7** to **9a** did not proceed satisfactorily, necessitating utilization of the approach described below. Since formation of the acid chloride followed by amidation was found to be successful in the synthesis of **7**, it appeared

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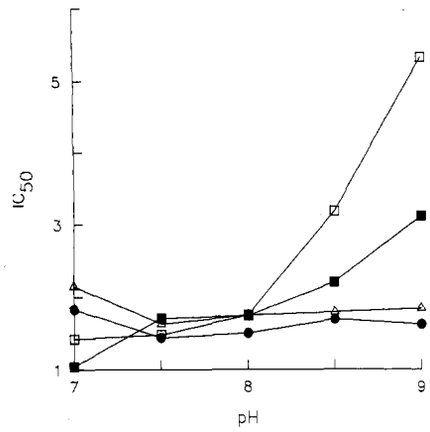

Figure 1. pH dependency of the IC_{50} of PBR ligands. The tissue was prepared as described. Each IC_{50} value was determined by using ten ligand concentrations with samples in triplicate. The assay buffer was 50 mM Tris-H_3PO_4 with the pH as indicated. The tissue concentration was in the range 33–35 μg of protein/assay. The radioligand ([^{3}H]Ro 5-4864) concentration was 1 nM. △, Ro 5-4864; ●, PK 11195; □, **11a**; ■, **5**.

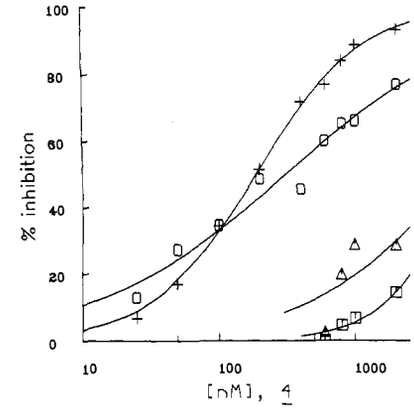

Figure 2. pH dependency of the IC_{50} of **4**. The assay was performed as described for Figure 1. The assay buffer was 50 mM potassium phosphate at all pH values, replacing the monobasic by the tribasic salt at pH 10.3. The tissue concentration was 28 and 25 μg of protein/assay at pH 7.0 and 10.3, respectively. No inhibition of [^{3}H]Ro 5-4864 and [^{3}H]PK 11195 was observed at pH 5.0. The radioligand concentrations were 1 nM. pH 7.0: △, [^{3}H]Ro 5-4864; □, [^{3}H]PK 11195. pH 10.3: +, [^{3}H]Ro 5-4864; ○, [^{3}H]PK 11195.

that employing this method and using the preformed side chains **8a–c** would be a viable route for the preparation of the carbobenzoxy-protected intermediates **9a–c**. The required side chain **8a** was synthesized in the racemic series by treating freshly prepared 1-(carbobenzoxyamino)-2-bromoethane (**2**) with (±)-*sec*-butylamine in dry DMF and anhydrous K_2CO_3 followed by formation of the hydrochloride salt, giving **8a** in 56% yield. The acid chloride of **6** was prepared by stirring in thionyl chloride at reflux, as described above, and reacted with **8a** in a two-phase $CHCl_3$/aqueous $NaHCO_3$ system. Extractive workup followed by crystallization gave **9a** in 70% yield. Deprotection of **9a** with iodotrimethylsilane and formation of the dihydrochloride salt afforded **10a** in 80% yield. Preparation of the isothiocyanate **11a** (AHN 070) proceeded as described for **5**, in 86% yield.

Synthesis of the (–) and (+) stereoisomers **8b,c–11b,c** proceeded by the method described for (±)-**8a–11a**. Since the absolute configuration[25] of chiral *sec*-butylamine has been established as R-(–) and *S*-(+), the absolute configurations of **8b,c–11b,c** follow. All spectral data (IR, NMR, MS), and TLC R_f values were identical with those of the racemic mixtures. Optical rotations are reported in the Experimental Section.

Results

Both **11a**, the 2-isothiocyanatoethyl analogue of PK 11195 (Table I), and **5**,[27] the 2-isothiocyanatoethyl derivative of Ro 5-4864, inhibited radioligand binding to the PBR in both kidney and brain with IC_{50} values from 2 to 15 nM (Table I). The pseudo Hill coefficients were not significantly different from unity under all conditions tested. The IC_{50} of **11b** and **11c** for both [^{3}H]Ro 5-4864 and [^{3}H]PK 11195 increased with increasing tissue concentration, indicative of irreversible binding.[17,27]

The (–) stereoisomer (**11b**) and the (+) stereoisomer (**11c**) of **11a** exhibited differential effects on the inhibition of the binding of both radioligands tested, with the (–) stereoisomer being ~2.5-fold more potent than (+) stereoisomer (Table II).

We evaluated the pH dependency of **5** and **11a** in comparison to their parent compounds (Figure 1). The IC_{50} of Ro 5-4864 and PK 11195, measured against [^{3}H]Ro 5-4864, does not vary with the pH,[27] but the IC_{50} of **5** is increased by ~3-fold in the pH range of 8.5–9, as compared to pH 7–8.5. The IC_{50} of **11a** increased by ~4-fold in the same pH range. The pseudo Hill coefficient is near unity for all conditions.

These results prompted us to study the affinity of the aminoethyl derivative of Ro 5-4864 (**4**), the direct precursor of **5**. As shown in Figure 2, **4** displaces both [^{3}H]Ro 5-4864 and [^{3}H]PK 11195. The IC_{50} values at pH 7 are >1 μM, but at pH 10.3 (the highest pH at which it is feasible to do radiolabeled binding studies in this system), the IC_{50} decreased to the nanomolar range.

Discussion

In this paper we describe the synthesis of novel, irreversible, high affinity ligands selective for the PBR. We have previously reported[27] that one of these compounds, **5**, the 2-isothiocyanatoethyl analogue of Ro 5-4864, binds irreversibly to the PBR, reducing the B_{max} for [^{3}H]Ro 5-4864 without affecting the B_{max} of [^{3}H]PK 11195, but decreasing the affinity for both ligands. We now report that **11a**, the 2-isothiocyanatoethyl derivative of PK 11195, as well as the (–) and (+) stereoisomers of this compound (**11b** and **11c**, respectively), inhibits both [^{3}H]Ro 5-4864 and [^{3}H]PK 11195 binding. Like **5**, **11a** decreases the affinity of [^{3}H]Ro 5-4864 and [^{3}H]PK 11195 for the PBR, but does not affect the B_{max}. The dependence of the IC_{50} of the two stereoisomers on tissue concentration is a strong indication that **11b** and **11c** bind irreversibly to the PBR, as do **5**[27] and **11a** (Table II). Furthermore, extensive washing of tissues preincubated with **5**[27] or **11a** (results not shown) failed to remove the inhibition of radiolabeled ligand seen with these compounds. The difference in potencies between the two stereoisomers is only slight, but preliminary studies with the amino precursors of these compounds, **10b** and **10c**, indicate that the stereoselectivity is not restricted to the isothiocyanato derivatives but is inherent in the structure of the whole side chain. The *R*-(–) enantiomer **10b**, at pH 7.0, has an IC_{50} of ~2 μM. The *S*-(+) enantiomer **10c** has an IC_{50} of ~10 μM. Increasing the pH to 8.5 decreases the IC_{50} values to ~0.5 and ~2 μM, respectively. IC_{50} values were measured against [^{3}H]Ro 5-4864. The pseudo Hill coefficient is in the range 0.5–0.8 for these compounds. Recently, a higher degree

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of stereospecificity to the PBR has been reported for quinoline derivatives.[28] These stereoisomers are structurally quite different from the compounds described herein, and the asymmetric center is located in a different position on the molecule, implying that the stereochemistry at the *sec*-butyl side chain of the isoquinoline type compounds is less important to stereoselective binding to the PBR than the center of asymmetry on the quinoline molecule. The difficulty in studying compounds 4 and **10a–c** is their high pH dependency, which makes it necessary to perform the assays at pH values that are no longer physiologically relevant. Though the pseudo Hill coefficients are near unity for all the above ligands, it is still possible that the (+) stereoisomer binds to a subpopulation of the PBR with a slightly lower affinity. This problem is currently under investigation.

The affinity of Ro 5-4864 for the PBR has been reported previously to remain unaffected at pH 5;[27] the affinity of PK 11195 for the PBR was reported to be only slightly reduced at this lower pH.[27] It has been reported that the rate of reaction of **5** with the PBR decreased at pH 5, which led to the hypothesis that the nucleophile on the PBR which forms a covalent bond with the isothiocyanato moiety of the irreversible ligands is an amino group, most probably from histidine.[27] This putative histidine moiety would be protonated, at pH 5, to a degree that would decrease its nucleophilicity and consequently its ability to form a covalent bond with the isothiocyanato moiety of **5** and **11a**.

The study of the pH dependency (Figure 1) establishes further that the PBR are stable through a pH range of 7–9. The IC_{50} values for Ro 5-4864 and PK 11195 remain unchanged throughout this pH range (Figure 1), and only at pH 8.5–9 do the IC_{50} values of **5** and **11a** begin to increase modestly. The modest increase in IC_{50} values may be due to adverse effects of the higher pH on the ligands themselves and is currently under investigation. Furthermore, the potency of the amino precursor (4) of **5** increases ~ 10-fold from pH 7 to 10.3 (Figure 2), which appears to correspond to the decreased degree of protonation of this compound at the elevated pH. Conversely, at lower pH, e.g., less than 9, the primary amino function of 4 is protonated and, presumably due to the size and electrical charge of the protonated amino group, affinity of this compound for the PBR is adversely affected. Increased potencies of the amino precursors (**10b** and **10c**) of the stereoisomers **11b** and **11c** at elevated pH were also observed. Taken together, these observations demonstrate that, at lower pH, a change in affinity for the PBR ligands is a property of the receptor, whereas, at higher pH, the change in affinities of these ligands can be attributed to the properties of the compounds.

All four compounds promise to become valuable tools in the further investigation of the PBR. The availability of radiolabeled forms of these compounds will undoubtedly aid in studies on the purification, turnover rate, and functional significance of the PBR.

Experimental Section

Biochemical Materials and Methods. Male Sprague–Dawley rats (150–200 g) (Taconic Farms, Germantown, NY) were killed by decapitation. The kidneys were decapsulated, minced with scissors, and homogenized in 20 volumes of ice cold 50 mM potassium phosphate buffer (pH 7.0) in a Brinkman Polytron (setting 6–7, 15 s). This tissue homogenate was centrifuged at 23000*g* for 20 min (4 °C) and the resulting pellet resuspended in 500–2000 volumes of potassium phosphate buffer. The binding assays for [^{3}H]Ro 5-4864 and [^{3}H]PK 11195 have been described elsewhere.[27] The 1,4-benzodiazepines utilized in these assays were obtained from Hoffmann-La Roche, Nutley, NJ. PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide) and PK 11209 (6, 1-(2-chlorophenyl)-3-isoquinolinecarboxylic acid) were the generous gifts of Dr. Gerard LeFur, Pharmuka Industries, Gennevilliers, France. The desmethyl Ro 5-4864 (1*H*-7-chloro-1,3-dihydro-5-(4-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one, 1) was a gift from Dr. Karl Weber, Boehringer-Ingelheim, W. Germany.

Synthesis. Melting points were determined on a Thomas-Hoover melting point apparatus and are corrected. Thin-layer chromatography (silica gel GF, Analtech, Newark, DE; EtOAc/HOAc (99:1) or $CHCl_3$/MeOH/NH_4OH (90:10:1)) was used to detect product homogeneity. Elemental analyses were performed by Atlanta Microlabs, Atlanta, GA. IR spectra were determined on a Beckman IR 4230 spectrophotometer, mass spectra (MS) were obtained on a Finnigan 1015 mass spectrometer (chemical ionization–NH_3), and ^{1}H NMR spectra were obtained on a Varian XL 300 or a Varian 220 spectrometer. Optical rotations were determined on a Perkin-Elmer 241MC polarimeter. All IR, NMR, and mass spectra supported the structure assigned, and all data for the stereoisomers were identical except for opposite optical rotations. The enantiomers of *sec*-butylamine were obtained from Norse Laboratories, Inc., Newbury Park, CA.

1-[2-(Carbobenzoxyamino)ethyl]-7-chloro-1,3-dihydro-5-(4-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one (3). In a modification of the procedure of Earley et al.,[23] 1.53 g (5.0 mmol) of 7-chloro-1,3-dihydro-5-(4-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one (**1**)[1] was dissolved in 10 mL of dry DMF. To the orange reaction mixture was added 0.40 g (10.0 mmol) of NaH (60% in oil dispersion washed with 3 × 2 mL of dry toluene and taken up in 2 mL of dry DMF for transfer). The reaction mixture was stirred at room temperature for 30 min, at which time 2.44 g (10.0 mmol) of freshly prepared 1-(carbobenzoxyamino)-2-bromoethane (**2**)[24] was added. An evolution of hydrogen gas was observed for 1 h; the reaction mixture was stirred overnight (after 4 h, reaction is not complete). The reaction mixture was quenched with 60 mL of H_2O and extracted with 3 × 50 mL of ether. The combined ether fractions were washed with 2 × 50 mL of brine followed by 2 × 50 mL of H_2O, dried (Na_2SO_4), and evaporated to an orange oil. Purification by column chromatography (silica gel, $CHCl_3$/MeOH/NH_4OH (95:5:1)) followed by recrystallization in anhydrous ether afforded 2.20 g (92%) of colorless prisms, mp 114–115 °C. Anal. ($C_{25}H_{21}N_3O_3Cl_2$) C, H, N.

1-(2-Ethylamino)-7-chloro-1,3-dihydro-5-(4-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one (4). To a solution of 0.48 g (1.0 mmol) of **3** in 9 mL of acetonitrile was added 0.6 mL of iodotrimethylsilane (3.6 mmol).[25] This reaction mixture was stirred for 10 min at room temperature, then quenched with 5 mL of MeOH, and stirred for an additional 10 min. Volatiles were removed in vacuo; the residue was dissolved in 10 mL of 10% HCl and extracted with 3 × 10 mL of ether. The combined ether portion was washed with 10 mL of 10% HCl, and the aqueous fractions were combined and neutralized to pH 9 with aqueous NH_4OH. Extraction with 3 × 10 mL of $CHCl_3$ and drying (Na_2SO_4) followed by evaporation yielded a white foam, which crystallized from EtOAc to give 0.31 g (90%) of **4**, mp 172–174 °C. Anal. ($C_{17}H_{15}N_3OCl_2 \cdot H_2O$) C, H, N.

1-(2-Isothiocyanatoethyl)-7-chloro-1,3-dihydro-5-(4-chlorophenyl)-2*H*-1,4-benzodiazepin-2-one Hydrochloride (5, AHN 086). In an established procedure,[17] to a solution of **4** (0.17 g, 0.50 mmol) in 25 mL of $CHCl_3$ were added 10 mL of H_2O and 0.17 g of $NaHCO_3$ (2.0 mmol), and the reaction mixture was stirred vigorously at room temperature for 10 min. To the biphasic reaction mixture was added 50 μL (0.64 mmol) of freshly distilled thiophosgene, and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was poured into a separatory funnel, and the organic layer was removed. The aqueous layer was washed with 3 × 5 mL of $CHCl_3$, and the combined $CHCl_3$ portion was washed with 5 mL of H_2O, dried (Na_2SO_4), and evaporated to a cream-colored foam, which was dissolved in 2 mL of absolute EtOH and treated with a saturated solution of HCl/EtOH to pH 2 (moist hydrion paper). Crystallization occurred upon addition of anhydrous ether to yield 0.19 g of **5** (87%) as pale yellow crystals: mp 173–175 °C; IR 2250

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cm^{-1} (NCS). Anal. ($C_{18}H_{13}N_3SOCl_2 \cdot HCl$) C, H, N.

(±)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide (7). A solution of 0.57 g (2.0 mmol) of 1-(2-chlorophenyl)-3-isoquinolinecarboxylic acid (**6**) in 0.6 mL of thionyl chloride (8.0 mmol) was stirred at reflux for 3 h. Excess thionyl chloride was removed by distillation followed by addition and distillation of 3 × 2 mL of dry toluene. The residue was taken up in 2 mL of dry THF and added dropwise to a solution of 0.37 g (5.0 mmol) of (±)-*sec*-butylamine in 2 mL of THF at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. (±)-*sec*-Butylamine hydrochloride (0.12 g, 1.1 mmol) was removed by suction filtration as white needles. Volatiles were removed in vacuo, and 0.34 g of **7** (50%) crystallized from 2-propanol, mp 124.5–125.5 °C. Anal. ($C_{20}H_{19}N_2OCl$) C, H, N.

(±)-*N*-Carbobenzoxy-2-[(1-methylpropyl)amino]ethylamine Hydrochloride (8a). To a solution of 0.75 g of (±)-*sec*-butylamine (10.0 mmol) in 10 mL of dry DMF were added 1.03 g of **2**[24] (4.0 mmol) and 0.70 g of anhydrous K_2CO_3 (4.8 mmol); the heterogeneous reaction mixture was stirred at 60–70 °C for 1 h. Inorganic material was removed and washed with 50 mL of anhydrous ether, and the filtrate was washed with 2 × 25 mL of H_2O and 2 × 25 mL of brine, dried (Na_2SO_4), and evaporated to a clear oil. The product was dissolved in 2 mL of MeOH, 10 mL of anhydrous ether was added, and the solution was acidified with a saturated solution of HCl in 2-propanol, to pH 2. Crystallization resulted in 0.64 g (56%) of **8a** as white mirrors, mp 108–109 °C. Anal. ($C_{14}H_{22}N_2O_2 \cdot HCl$) C, H, N.

(*R*)-(+)-*N*-Carbobenzoxy-2-[(1-methylpropyl)amino]ethylamine Hydrochloride (8b). Following the procedure described for **8a** and using (−)-*sec*-butylamine, we prepared 0.69 g of **8b** (60%) as a white feathery hydrochloride salt: mp 126.5–127.5 °C; $[\alpha]^{22}_D$ +3.0° (*c* 1.0, $CHCl_3$). Anal. ($C_{14}H_{22}N_2O_2 \cdot HCl$) C, H, N.

(*S*)-(−)-*N*-Carbobenzoxy-2-[(1-methylpropyl)amino]ethylamine Hydrochloride (8c). Following the procedure described for **8a** and using (+)-*sec*-butylamine, we prepared 0.56 g of **8c** (50%) as a white feathery hydrochloride salt: mp 126.5–127.5 °C; $[\alpha]^{22}_D$ −2.7° (*c* 1.0, $CHCl_3$). Anal. ($C_{14}H_{22}N_2O_2 \cdot HCl$) C, H, N.

(±)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-[2-(carbobenzoxyamino)ethyl]-3-isoquinolinecarboxamide (9a). A solution of 0.28 g of **6** (1.0 mmol) in 1.0 mL of thionyl chloride was stirred at reflux for 3 h. Excess thionyl chloride was removed by distillation followed by addition and distillation of 3 × 2 mL of dry toluene. The pale yellow acid chloride was taken up in 1 mL of pentene-stabilized $CHCl_3$ and added dropwise to the two-phase reaction mixture of 0.29 g of **8a** (1.0 mmol), 5 mL of H_2O, 16 mL of $CHCl_3$, and 0.50 g of $NaHCO_3$ (6.0 mmol) at pH 8–9, at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The $CHCl_3$ layer was removed, and the aqueous portion was washed with 3 × 3 mL of $CHCl_3$. The combined organic portions were washed with 5 mL of H_2O, dried (Na_2SO_4), and evaporated to 0.54 g of white foam (100% crude). Crystallization in anhydrous ether gave 0.38 g (70%) of white crystalline **9a** mp 107–108 °C. Anal. ($C_{30}H_{30}N_3O_3Cl$) C, H, N.

(*R*)-(−)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-[2-(carbobenzoxyamino)ethyl]-3-isoquinolinecarboxamide (9b). Following the procedure described for **9a** and using **8b** afforded 0.38 g (73%) of **9b** as white prisms: mp 106–108 °C; $[\alpha]^{22}_D$ −28.3° (*c* 0.7, $CHCl_3$). Anal. ($C_{30}H_{30}N_3O_3Cl$) C, H, N.

(*S*)-(+)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-[2-(carbobenzoxyamino)ethyl]-3-isoquinolinecarboxamide (9c). Following the procedure described for **9a** and using **8c** afforded 0.39 of **9c** (75%) as white prisms: mp 106–108 °C; $[\alpha]^{22}_D$ +31.7° (*c* 0.5, $CHCl_3$). Anal. ($C_{30}H_{30}N_3O_3Cl$) C, H, N.

(±)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-(2-aminoethyl)-3-isoquinolinecarboxamide Dihydrochloride (10a). Deprotection of **9a** was performed in a similar manner to that which is described for the deprotection of **3**, by using 0.52 g (1.0 mmol) of **9a** in 12 mL of MeCN and 0.51 mL (3.6 mmol) of iodotrimethylsilane, quenching with MeOH, and working up, afforded 0.38 g of white foam (100% crude). Formation of the hydrochloride salt in MeOH/anhydrous ether gave 0.36 g of **10a** (80%) as white crystals, mp 200–203 °C. Anal. ($C_{22}H_{24}N_3OCl \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N.

(*R*)-(−)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-(2-aminoethyl)-3-isoquinolinecarboxamide Dihydrochloride (10b). Following the procedure described for **10a** and using **9b** afforded 0.37 g (81%) of **10b** as white crystals: mp 204–206 °C; $[\alpha]^{22}_D$ −54.2° (*c* 0.5, $CHCl_3$). Anal. ($C_{22}H_{24}N_3OCl \cdot 2HCl$) C, H, N.

(*S*)-(+)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-(2-aminoethyl)-3-isoquinolinecarboxamide Dihydrochloride (10c). Following the procedure described for **10a** and using **9c** afforded 0.35 g (78%) of **10c** as white crystals; mp 204–206 °C; $[\alpha]^{22}_D$ +51.5° (*c* 0.5, $CHCl_3$). Anal. ($C_{22}H_{24}N_3OCl \cdot 2HCl$) C, H, N.

(±)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-(2-isothiocyanatoethyl)-3-isoquinolinecarboxamide (11a) (AHN 070). Via the procedure described for the preparation of **5**, 0.23 g of **10a** (0.5 mmol) in a two-phase system of 10 mL of H_2O, 25 mL of $CHCl_3$, and 0.25 g of $NaHCO_3$ (3.0 mmol) was stirred at 0 °C. Addition of 55 μL of freshly distilled thiophosgene and stirring at room temperature for 45 min followed by workup and crystallization from absolute EtOH afforded 0.18 g of **11a** (86%) as cream-colored prisms: mp 126–128 °C; IR 2250 cm^{-1} (NCS). Anal. ($C_{23}H_{22}N_3OSCl$) C, H, N.

(*R*)-(−)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-(2-isothiocyanatoethyl)-3-isoquinolinecarboxamide (11b). Following the procedure described for **11a** and using **10b** afforded 0.21 g of **11b** (95%) as cream-colored crystals: mp 124–125.5 °C; $[\alpha]^{22}_D$ −42.9° (*c* 1.0, $CHCl_3$). Anal. ($C_{23}H_{22}N_3OSCl$) C, H, N.

(*S*)-(+)-1-(2-Chlorophenyl)-*N*-(1-methylpropyl)-*N*-(2-isothiocyanatoethyl)-3-isoquinolinecarboxamide (11c). Following the procedure described for **11a** and using **10c** afforded 0.20 g of **11c** (90%) as cream-colored prisms: mp 124–125.5 °C; $[\alpha]^{22}_D$ +43.8° (*c* 1.3, $CHCl_3$). Anal. ($C_{23}H_{22}N_3OSCl$) C, H, N.

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