Synthesis and Evaluation of Imidazo[1,5-a][1,4]benzodiazepine Esters with High Affinities and Selectivities at "Diazepam-Insensitive" Benzodiazepine Receptors

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A series of imidazo[1,5-a][1,4]benzodiazepine esters have been synthesized with varying ester side chains and 8-position substituents. The affinities of these compounds were evaluated at both "diazepam-insensitive" (DI) and diazepam-sensitive (DS) subtypes of the benzodiazepine receptor (BZR). A profound steric effect of the 3-position ester side chain moiety was observed on ligand affinity at DI. In contrast, ester size had a less robust effect on ligand affinity at DS. The *tert*-butyl ester compound **8** displayed the highest affinity (K_i = 1.7 nM) for DI within a series of 8-chloro esters. Furthermore, halogens at the 8-position resulted in an enhancement of both ligand affinity and selectivity at DI among the series of *tert*-butyl esters examined. The 8-nitro derivative **23** and 8-isothiocyanato congener **25** had high affinities for both DI and DS but exhibited little subtype selectivity (10.8 and 2.7 nM at DI versus 14 and 3.7 nM at DS, respectively). The 8-azido *tert*-butyl ester **29** exhibited a significantly higher affinity (K_i = 0.43 nM) and selectivity (DI/DS ratio of 0.2) than the corresponding ethyl ester, the prototypic DI ligand **1** (Ro 15-4513). Among the compounds synthesized, **29** is the highest affinity ligand for DI described to date while its 8-bromo analog **18** is the most selective ligand (DI/DS ratio of 0.17) for this novel BZR subtype.

Introduction

The "diazepam-insensitive" (DI) subtype of the benzodiazepine receptor (BZR) has a pharmacological profile and neuroanatomical distribution which is distinct from other diazepam sensitive (DS) BZR subtypes.[1,2] Thus the DI BZR is characterized by low affinities (>1 μM) for prototypical 1,4-benzodiazepines (e.g. diazepam, flunitrazepam), triazolobenzodiazepines (e.g. triazolam), and triazolopyridazines that exhibit high affinities for DS BZR.[3-5] Moreover, DI are almost exclusively localized to the cerebellum of the mammalian species examined to date.[5] This unique BZR subtype has been linked to some of the pharmacological actions of ethanol since several high-affinity DI ligands including **1** (Ro 15-4513) and **2** (Ro 19-4603) (see tables for the structures of these compounds) antagonize biochemical and behavioral effects of ethanol.[6-10] Moreover, the alcohol nontolerant (ANT) rat line, which is far more sensitive to the motor-impairing effects of ethanol than the closely related alcohol tolerant line (AT), does not possess measurable levels of DI binding.[2] Molecular biology studies have revealed that a pharmacological profile similar to that of native cerebeller DI can be reconstituted in cell lines transfected with cDNA's encoding α6, β2, and γ2 subunits.[11] Nonetheless, the physiological and pharmacological role of DI remain controversial,[12] due in part to the paucity of ligands that exhibit selectivity for this subtype.

Recently, compounds from several other chemical classes including imidazobenzodiazepines,[3] pyrazoloquinolines,[13] quinolines,[4] and β-carbolines[4] have been shown to bind to DI with high affinities (<20 nM). Nonetheless, the structural requirements for ligand binding to DI appear far more restrictive than binding to DS BZR subtypes. The only reported BZR ligand with modest selectivity at DI is the prototypical **1** (Ro 15-4513) (DI/DS ratio of 0.6).[3,11] Previous structure–affinity relationship (SAR) studies have shown that both the ester side chain moiety and phenyl ring substitution may be critical for high-affinity binding.[3] In order to first define the structural requirements for high-affinity and selective DI binding, a series of novel 8-substituted imidazo[1,5-a][1,4]benzodiazepine esters were synthesized. These compounds exhibit a broad range of affinities and selectivities at DI. Several compounds were synthesized which, for the first time, have higher affinity and selectivity for DI than the prototypical DI ligand **1**.

Chemistry

Compounds **3a** and **3b** were readily obtained by heating commercially available isatoic anhydride or 5-chloroisatoic anhydride **4b** with sarcosine in dimethyl sulfoxide, respectively, using a modified literature method[14] (Scheme I). Deprotonation of **3a** or **3b** with sodium hydride in THF and DMF, followed by treatment with diethyl phosphorochloridate, formed the corresponding enol phosphate.[14,15] This was subsequently reacted with a solution of ethyl or *tert*-butyl isocyanoacetate and sodium hydride in DMF to afford compounds **5**, **6**, **7**, and **8**, respectively. The other ester analogs **9**–**16** were obtained by hydrolysis of compound **5** or **6**, followed by acid chloride formation and condensation with the corresponding alcohols.

Bromination of **3a** (Scheme II) with bromine in a mixture of acetic acid and concentrated sulfuric acid afforded the desired product **17** only. However, treatment of **3a** (Scheme II) with iodine monochloride under the same conditions afforded a mixture of mono- and diiodinated products **19** and **20** which were readily separated by flash chromatography. It is noted that the presence of concentrated sulfuric acid was necessary to achieve halogenation of **3a**. Compounds **17** and **20** were treated with *tert*-butyl isocyanoacetate as above to give target compounds **18** and **21**, respectively.

Nitration of **3a** was carried out (Scheme III) according to a previously reported method[16,17] using potassium

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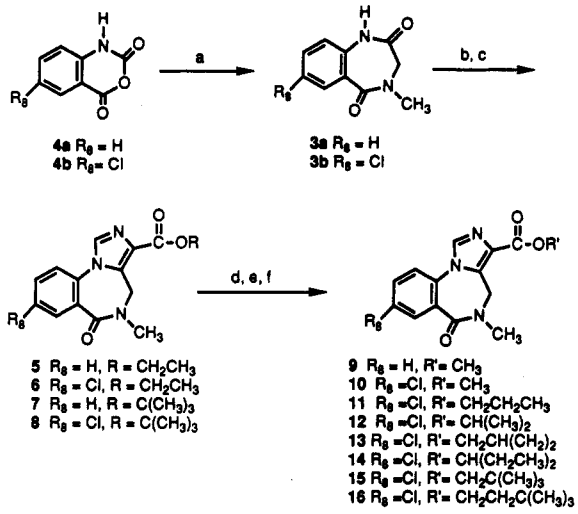
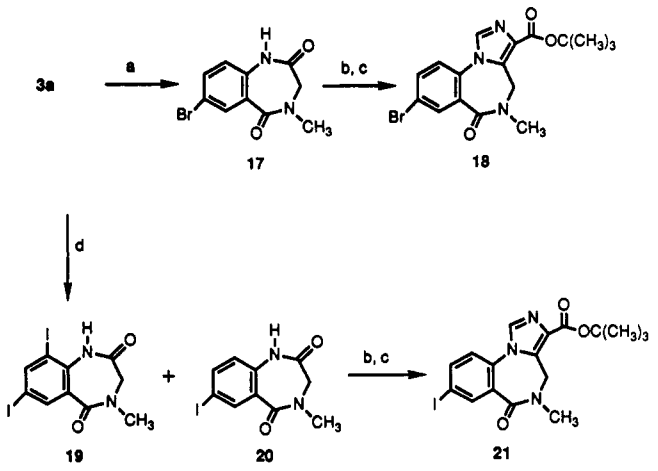
Scheme I [a]



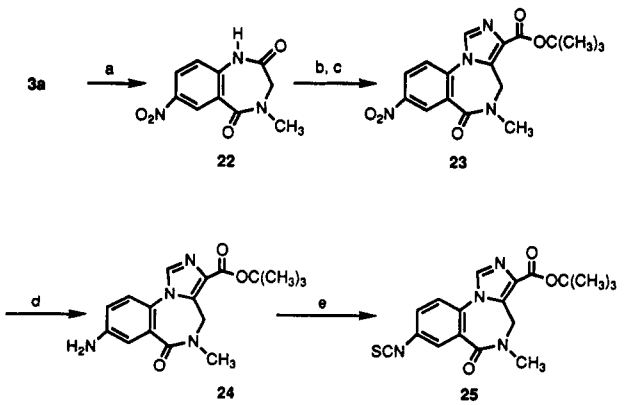
[a] $CH_3NHCH_2CO_2H$, DMSO, 140 °C; (b) NaH, THF, DMF, $(EtO)_2POCl$; (c) NaH, DMF, $CNCH_2CO_2R$; (d) 10% KOH, in CH_3OH, H_2O, HCl; (e) $SOCl_2$, toluene; (f) R'OH.

Scheme II [a]



[a] (a) Br_2, CH_3CO_2H, H_2SO_4; (b) NaH, THF, DMF, $(EtO)_2POCl$; (c) NaH, DMF, $CNCH_2CO_2C(CH_3)_3$; (d) ICl, CH_3CO_2H, H_2SO_4.

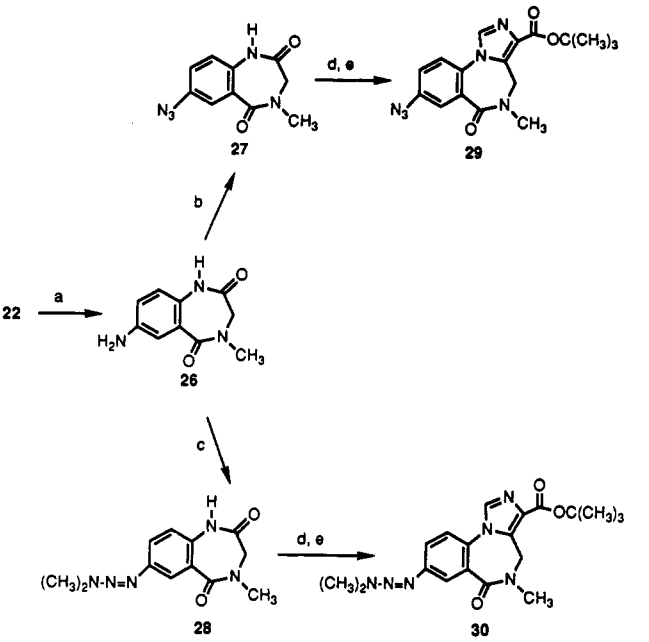
Scheme III [a]



[a] (a) KNO_3, H_2SO_4; (b) NaH, THF, DMF, $(EtO)_2POCl$; (c) NaH, DMF, $CNCH_2CO_2C(CH_3)_3$; (d) H_2, 10% Pd/C, EtOH, THF; (e) $SCCl_2$, $CHCl_3$, aqueous $NaHCO_3$.

nitrate and concentrated sulfuric acid to give the nitro intermediate **22** in 60% yield. The imidazo ester **23** was obtained in only 20% yield using *tert*-butyl isocyanoacetate as above. Catalytic reduction of the nitro group of **23** in the presence of 10% Pd/C afforded the corresponding amino compound **24** in quantitative yield. Treatment of **24** with thiophosgene under biphasic conditions[18] afforded

Scheme IV [a]



[a] (a) H_2, 10% Pd/C, EtOH, THF; (b) $NaNO_2$, NaN_3, 6 M HCl; (c) $NaNO_2$, 6 M HCl, $(CH_3)_2NH$, KOH; (d) NaH, THF, DMF, $(EtO)_2POCl$; (e) NaH, DMF, $CNCH_2CO_2C(CH_3)_3$.

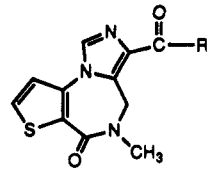
the 8-isothiocyanato derivative **25** in 57% overall yield from **23**.

Compound **22** was catalytically reduced in the presence of 10% Pd/C and hydrogen (Scheme IV) to afford primary amine **26**. This was transformed into either the azido[19] derivative **27** using HONO and NaN_3 or the triazene[19] derivative **28** using HONO followed by treatment with excess dimethylamine. **27** and **28** were readily transformed to the target compounds **29** and **30** as described above.

Radioligand Binding. Cerebella or cortices were obtained from adult, male (200–300 g) Sprague–Dawley rats (Taconic Farms, Germantown, NY) killed by decapitation. Tissues were dissected, weighed, and disrupted (Brinkmann Polytron, setting 6, 10 s) in 60 volumes of 50 mM Tris-citrate buffer (pH 7.8). Homogenates were centrifuged at 20000*g* for 20 min (4 °C), resuspended in 60 volumes of buffer, and recentrifuged. This "washing" procedure was repeated a total of five times.

DI and DS receptor binding were determined essentially as previously described.[3] Incubations were performed in a total volume of 0.5 mL consisting of 0.1 mL of tissue suspension (~100 mg protein), 0.05 mL [^{3}H]Ro 15-4513 ([^{3}H]-1) (sp. act. 24.3 Ci/mmol; final concentration ~2 nM), 0.05 mL of drug solution (final concentration 0.1 nM to 1 mM), 0.05 mL of 2 M NaCl, and Tris-citrate buffer (pH 7.8) to volume. Stock solutions of compounds (1–10 mM) in methanol or ethanol were diluted in buffer to yield the desired drug concentrations. In experiments examining the effects of GABA on ligand binding to DS benzodiazepine receptors, 0.05 mL of buffer was replaced with GABA (0.5 mM). Nonspecific binding was determined with **31** (Ro 15-1788) (10 μM) and typically represented ≤10% of total binding. [^{3}H]-1 binding displaced by **31** (10 μM) was defined as diazepam-sensitive (DS) + diazepam-insensitive (DI) binding; this typically represented 90–95% of [^{3}H]-1 binding. [^{3}H]-1 binding displaced by **31** (10 μM) but not by diazepam (10 μM) was defined as DI binding. This value typically represented 30–40% of specific [^{3}H]-1 binding. Subtraction of [^{3}H]-1 binding to DI as described above from DS + DI defined

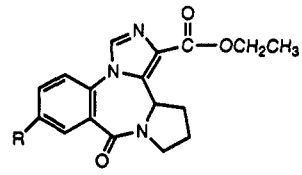
Table I. Affinities of Imidazo[1,4]thienodiazepinone Esters at DI and DS Benzodiazepine Receptors[a]



compd[b]	R	DI	DS	DI/DS ratio
2 (Ro 19-4603)	$OC(CH_3)_3$	2.6	0.2	13
32	$OCH_2CH_2CH_3$	38	1.4	27
33	OCH_3	52	0.9	57

[a] Values designated for DI and DS are K_i (nM), representing the average values of three experiments. [b] Compounds 32 and 33 were synthesized from 4 using the procedure described in method B. Their syntheses will be reported elsewhere. The Roche code definitions of previously reported compounds are shown in parentheses.

Table II. Affinities of Imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodiazepine Ester at DI and DS Benzodiazepine Receptors[a]



compd[b]	R	DI	DS	DI/DS ratio
34 (Ro 14-5974)	H	45	0.9	50
35 (Ro 14-5975)	Cl	53	72	0.73

[a] Values designated for DI and DS are K_i (nM), representing the average values of three experiments. [b] The Roche (Ro) code designations of previously published compounds are shown in parentheses.

ligand binding to DS. Incubations (0–4 °C) were initiated by the addition of tissue and terminated after 60 min by rapid filtration with two 5-mL washes of ice-cold Tris-citrate buffer through Whatman GF/B filters using a Brandel M-48R filtering manifold (Brandel Instruments, Gaithersburg MD, USA). Protein content was determined using the BCA Protein Assay Reagent (Pierce, Rockford, IL). Assays were in duplicate with at least six concentrations, and the values presented are the mean ± SEM of three experiments unless otherwise specified.

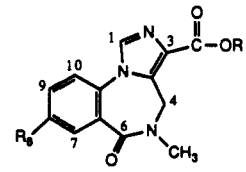
The GABA ratio was defined as the ratio of EC_{50} values in cortical membranes in the presence or absence of 50 μM GABA.

Results and Discussion

Ester analogs **32** and **33** of **2** (Ro 19-4603) (K_i = 2.6 nM at DI) were initially synthesized (see Table I legend) and evaluated for their affinities at DS and DI (Table I). This preliminary study suggested that the 3-position side chain may be important for high affinity binding to DI. Subsequent experiments with **34** (Ro 14-5974) (DI/DS ratio of 50) and its 8-chloro analog **35** (Ro 14-5975) (DI/DS ratio of 0.7) suggested that the 8-chloro substitution imparts DI selectivity (Table II). These results prompted the present investigation examining the effects of 3- and 8-position substitutions on ligand affinities at DI and DS in order to develop DI selective ligands.

The series of novel imidazo[1,5-a][1,4]benzodiazepine esters synthesized exhibit a broad range of affinities at DI (K_i = 0.43 nM to >1000 nM) and DS (K_i = 1.1 nM to >1000 nM) dependent upon both the ester side chain (Table III) and the 8-position substitution (Table IV). Chloro substitution at the 8-position (**6**, **8**, **10**) enhances affinity at DI (~12-fold) and decreases affinity at DS (~4-fold) in comparison to their corresponding nonsubstituted

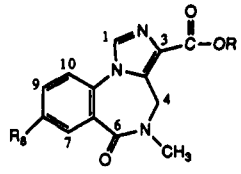
Table III. Affinities of Imidazo[1,4]benzodiazepine Esters at DS and DI Benzodiazepine Receptors and Their GABA Shift Ratio at DS[a]



compd[b]	R	R_8	DI	DS	DI/DS ratio	GABA shift[c]
5 (Ro 14-7437)	CH_2CH_3	H	214	1.3	164.6	0.98
6 (Ro 15-1310)	CH_2CH_3	Cl	16.9	5.4	3.1	0.96
7	$C(CH_3)_3$	H	21.2	1.1	19.2	1.05
8	$C(CH_3)_3$	Cl	1.7	4.0	0.4	1.16
9	CH_3	H	*>1000	6.3		0.99
10	CH_3	Cl	123.5	29.8	4.1	0.86
11	$CH_2CH_2CH_3$	Cl	33.3	24.8	1.3	1.28
12	$CH(CH_3)_2$	Cl	8.8	10.5	0.8	1.23
13	CPM	Cl	39.8	9.7	4.1	1.76
14	$CH(CH_2CH_3)_2$	Cl	122.6	26.9	4.6	1.58
15	$CH_2C(CH_3)_3$	Cl	299.6	499.3	0.67	1.77
16	$CH_2CH_2C(CH_3)_3$	Cl	*>1000	184		2.01

[a] Values represent the average values from three experiments; values designated for DI and DS are K_i (nM) and *IC_{50} (nM) as indicated. [b] The Roche code (Ro) designations of previously published compounds are shown in parentheses. [c] GABA shifts (values from two experiments) for diazepam (2.24) and **31** (Ro 15-1788) (1.04) were determined for internal comparison along with the new compounds.

Table IV. Affinities of Substituted *tert*-Butyl Imidazo[1,4]benzodiazepine Esters at DS and DI Benzodiazepine Receptors[a] and Their GABA Shift Ratio at DS



compd	R	R_8	DI	DS	DI/DS ratio	GABA shift[c]
8	$C(CH_3)_3$	Cl	1.7	4.0	0.42	1.16
18	$C(CH_3)_3$	Br	2.8	16.0	0.17	1.06
21	$C(CH_3)_3$	I	1.5	7.0	0.21	1.22
23	$C(CH_3)_3$	NO_2	10.8	14.0	0.77	1.46
25	$C(CH_3)_3$	NCS	2.7	3.7	0.73	1.35
29	$C(CH_3)_3$	N_3	0.43	2.1	0.20	1.76
30	$C(CH_3)_3$	$N_3(CH_3)_2$	*>1000	3489		1.12
1 (Ro 15-4513)	CH_2CH_3	N_3	3.1	5.3	0.6	0.77
31 (Ro 15-1788)	CH_2CH_3	F	58	0.8	73	1.04
diazepam			*>1000	6.6		2.24

[a] Values represent the average of three separate experiments; values designated for DI and DS are K_i (nM) and *IC_{50} (nM) as indicated. [b] The Roche code (Ro) designations of previously published compounds are shown in parentheses. [c] GABA shifts (values from two experiments) for diazepam (2.24) and **31** (1.04) were determined for internal comparisons along with the new compounds. GABA shift for **1** was previously determined.[22]

analogs (**5**, **7**, **9**). A substantial increase in affinity at DI (~72-fold) within the chloro-substituted compounds was demonstrated as the size of the ester side chain was increased from methyl, ethyl, isopropyl, to *tert*-butyl (**10**, **6**, **12**, **8**), while DS affinities increased moderately (~7-fold). Moreover, increasing the number of methyl groups on the ester carbon adjacent to the oxygen enhances the affinity at DI. Thus, compounds with three-carbon (linear except **13**) side chains, such as propyl, cyclopropylmethyl, isopentyl, and neopentyl (**11**, **13**, **14**, **15**), displayed lower affinities (15–176-fold decrease) at DI than the *tert*-butyl

ester analog (8), while their DI and DS affinities generally decreased with increased branching (14) or side chain bulk (15). Furthermore, the dimethyl butyl ester analog (16) with a four-carbon linear chain resulted in a complete loss of affinity at DI (>1000 nM), while maintaining modest affinity at DS (184 nM). These results demonstrate that the 3-position ester side chain plays a far more critical role in high-affinity binding to DI than DS. The DI binding site has a more restricted steric limitation for the ester side chain which may be an important factor to differentiate DI selectivity from DS along with other structural requirements.

Since 8-chloro-substituted compounds exhibited both a higher affinity and selectivity for DI than the corresponding unsubstituted compounds (compare 5 and 6; 7 with 8; 9 with 10), we extended 8-halogen substitution to include the bromo 18 and iodo 21 analogs. Both compounds 18 and 21 exhibited not only high affinity but also excellent selectivity at DI primarily mediated through a decreased affinity at DS. Furthermore, the 8-nitro- and 8-isothiocyanato-substituted *tert*-butyl ester derivatives 23 and 25 displayed high affinities for both DI and DS, but moderate selectivity. The 8-azido-substituted compound 29 showed a very high affinity for DI together with good selectivity for this subtype, whereas its triazene analog 30 had dramatically reduced affinities at both DI and DS. This demonstrates, for the first time, that a steric limitation may exist at the 8-position in this series of compounds. Thus, among 8-substituted *tert*-butyl esters, the rank order of affinity for DI was $N_3 > I > Cl > Br > NCS > NO_2$, while the rank order of selectivity was $Br > N_3 > I > Cl > SCN > NO_2$. These results do not imply any correlation between the electronic properties of 8-position substituents and affinity at DI, but rather the enhanced affinity and selectivity of the 8-substituted compounds (compared with 8-unsubstituted analogs) suggest that this substitution, and in particular with halogens and azido, has a more favorable influence on ligand affinity at DI than DS. This effect may be due to either a direct interaction with a receptor site or an indirect interaction altering the lipophilicity of the aryl ring or entire molecule.

The ability of GABA to increase the apparent affinity[20] (GABA shift ratio) of many of the newly synthesized derivatives was substantially lower than diazepam (Table III and IV). On the basis of a wide variety of chemically disparate compounds, a positive GABA shift ratio lower than that observed for a "classical" 1,4-benzodiazepine such as diazepam often predicts compounds that are anxiolytic/anticonvulsant but lacking prominent muscle relaxant or sedative actions.[21] Previous studies[3] have shown that certain compounds exhibiting a range of pharmacological actions and GABA shift ratios are GABA neutral (GABA shift ratio ~1) at DI. Nonetheless, the synthesis of novel compounds with greater affinity and selectivity at DI than 1 (Ro 15-4513) and different pharmacological profiles at DS may prove useful in evaluating the functional role of DI BZR.

Conclusions

Our studies demonstrate a steric tolerance associated with the 3-position ester side chain of imidazo[1,5-*a*][1,4]-benzodiazepine compounds which is far more pronounced at DI than DS. This together with other structural requirements may be an important factor in differentiating DI selectivity. Although 8-position halogen and azido substitution enhances ligand affinity at DI more than DS, a limitation associated with this position was also observed. These studies yielded ligands with the highest affinities and selectivities for DI described to date. Moreover, these compounds demonstrated different GABA shift ratios at DS than the prototypical DI ligand 1. These compounds should be valuable in studies to determine the pharmacological functions of BZR subtypes such as DI.

Experimental Section

Melting points were determined on a Mel-Temp II capillary apparatus and are uncorrected. Elemental analyses were performed at Atlantic Microlabs, Norcross, GA. Chemical ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. Electron ionization mass spectra (EIMS) and high-resolution mass measurements (HRMS) were obtained using a V. G. Micro Mass 7070F mass spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using Varian XL-300 Fourier transform spectrometers in $CDCl_3$ or DMSO-d_6. Chemical shifts are expressed in parts per million (ppm) on the δ scale relative to a TMS internal standard. Thin-layer chromatography (TLC) was performed on 250 μm Analtech GHLH silica gel plates. TLC system A corresponds to CH_2Cl_2–EtOAc–MeOH (3:3:0.5), and TLC system B corresponds to CH_2Cl_2–EtOAc–MeOH (9:3:0.5). No attempt was made to optimize the yield of the intermediates and the final products.

Method A. A solution of **3a** or **3b** in DMF (1 mmol/2 mL) and THF (1 mmol/3 mL) was cooled in ice–water, and sodium hydride (1.2 equiv) was added in one portion. After 20 min, diethyl phosphorochloridate (1.5 equiv) was added dropwise, and the solution was continuously stirred for 30 min with cooling from an ice bath. A solution of *tert*-butyl isocyanoacetate (1.2 equiv) and sodium hydride (1.4 equiv) in DMF (1 mmol/mL), which had been stirred for 15 min with ice-bath cooling, was added slowly. After stirring for another 30 min with cooling, the reaction mixture was allowed to stir at room temperature overnight. Acetic acid was added to quench the reaction, and it was then poured into ice water and extracted with ethyl acetate. The combined extracts were washed with water and brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was chromatographed on a silica gel column and crystallized from an appropriate solvent.

Method B. Compound **5** or **6** was stirred in a 10% KOH methanol solution (0.3 mmol/10 mL) for 2 h at room temperature. The solvent was evaporated under reduced pressure, the residue was dissolved in water, and the solution was acidified by addition of concentrated HCl to pH 2 followed by cooling in the refrigerator. The resulting precipitate was collected by filtration, washed with ether, dried, and then refluxed in thionyl chloride (0.3 mmol/5 mL) and toluene (0.3 mmol/5 mL) for 1 h. The solvent was evaporated, and then a further 10 mL of toluene was added and evaporated again. The residue was heated under reflux in an appropriate alcohol for 2 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in methylene chloride, washed with water and brine, and then dried over anhydrous sodium sulfate. After the solvent was evaporated under reduced pressure, the residue was crystallized from an appropriate solvent. In some cases, silica gel chromatography was required for further purification.

***tert*-Butyl 5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*]-[1,4]benzodiazepine-3-carboxylate (7)** was prepared from **3a** using the procedure described in method A. Crystallization from ethyl acetate afforded **7** in 34% yield: mp 202–204 °C; ^{1}H NMR ($CDCl_3$) δ 8.07 (dd, 1 H, J = 1.5, 7.8 Hz), 7.88 (s, 1 H), 7.64 (dt, 1 H, J = 1.6, 7.6 Hz), 7.55 (t, 1 H, J = 7.3 Hz), 7.41 (d, 1 H, J = 7.0 Hz), 5.18 (br s, 1 H), 4.35 (br s, 1 H), 3.25 (s, 3 H), 1.65 (s, 9 H); MS (CI) m/z 314 (M + H), 258, 182. Anal. ($C_{17}H_{19}N_3O_3$) C, H, N.

***tert*-Butyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (8)** was prepared from **3b** using the procedure described in method A. Crystallization from ethyl acetate afforded **8** in 22% yield: mp 207–209 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, 1 H, J = 2.2 Hz), 7.65 (s, 1 H), 7.60 (dd, 1 H, J = 2.3, 8.5 Hz), 7.35 (d, 1 H, J = 8.5 Hz), 5.17 (br s, 1 H), 4.36 (br s, 1 H), 3.25 (s, 3 H), 1.64 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 164.9, 161.6, 134.4, 134.2, 132.4, 132.3, 132.2, 130.4, 130.2, 129.9, 123.0, 81.8, 42.3, 35.7, 28.4, 28.3, 28.2; MS (CI) m/z 350 (M + H), 348 (M + H), 314, 292. Anal. ($C_{17}H_{18}ClN_3O_3$) C, H, N.

Methyl 5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (9) was prepared from **5** using the procedure described in method B. Crystallization from ethyl acetate afforded **9** in 75% yield; mp 211–212 °C; ^{1}H NMR ($CDCl_3$) δ 8.08 (dd, 1 H, J = 1.4, 7.7 Hz), 7.90 (s, 1 H), 7.65 (dt, 1 H, J = 1.5, 7.6 Hz), 7.55 (t, 1 H, J = 7.6 Hz), 7.43 (d, 1 H, J = 7.9 Hz), 5.22 (br s, 1 H), 4.40 (br s, 1 H), 3.90 (s, 3 H), 3.20 (s, 3 H); HR-MS (M^+) calcd 271.0956, found 271.0949. Anal. ($C_{14}H_{13}N_3O_3 \cdot 0.25H_2O$) C, H, N.

Methyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (10) was prepared from **6** using the procedure described in method B. The crude product was crystallized from ethyl acetate to give **10** in 50% yield: mp 205–206 °C; ^{1}H NMR ($CDCl_3$) δ 8.07 (d, 1 H, J = 2.4 Hz), 7.87 (s, 1 H), 7.61 (dd, 1 H, J = 2.4, 8.5 Hz), 7.39 (d, 1 H, J = 8.5 Hz), 5.20 (br s, 1 H), 4.37 (br s, 1 H), 3.97 (s, 3 H), 3.25 (s, 3 H); MS (CI) m/z 308 (M + H), 306 (M + H), 273, 245. Anal. ($C_{14}H_{12}ClN_3O_3$) C, H, N.

Propyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (11) was prepared from **6** using the procedure described in method B. The crude product was crystallized from ethyl acetate and methanol to give **11** in 85% yield: mp 133–135 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 1 H, J = 2.4 Hz), 7.88 (s, 1 H), 7.60 (dd, 1 H, J = 2.4, 8.5 Hz), 7.38 (d, 1 H, J = 8.6 Hz), 5.20 (br s, 1 H), 4.35 (br s, 1 H), 4.32 (t, 2 H, J = 6.7 Hz), 3.24 (s, 3 H), 1.85 (m, 2 H), 1.04 (t, 3 H, J = 7.4 Hz); HR-MS (M^+) calcd 333.0880, found 333.0889. Anal. ($C_{16}H_{16}ClN_3O_3 \cdot 0.5CH_3OH$) C, H, N.

Isopropyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (12) was prepared from **6** using the procedure described in method B. The crude product was crystallized from ethyl acetate in 67% yield: mp 190–192 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 1 H, J = 2.4 Hz), 7.87 (s, 1 H), 7.60 (dd, 1 H, J = 2.4, 8.5 Hz), 7.38 (d, 1 H, J = 8.5 Hz), 5.35 (m, 1 H), 5.17 (br s, 1 H), 4.36 (br s, 1 H), 3.25 (s, 3 H), 1.43 (d, 6 H, J = 6.3 Hz). MS (CI) m/z 336 (M + H), 334 (M + H), 300. Anal. ($C_{16}H_{16}ClN_3O_3$) C, H, N.

Cyclopropylmethyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (13) was prepared from **6** using the procedure described in method B. Crystallization from ethyl acetate afforded **13** in 45% yield: mp 163–164 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 1 H, J = 2.4 Hz), 7.88 (s, 1 H), 7.60 (dd, 1 H, J = 2.4, 8.6 Hz), 7.38 (d, 1 H, J = 8.6 Hz), 5.21 (br s, 1 H), 4.37 (br s, 1 H), 4.20 (t, 2 H, J = 4.5 Hz), 3.25 (s, 3 H), 1.32 (m, 1 H), 1.04 (m, 2 H), 0.40 (m, 2 H); MS (CI) m/z 346 (M + H), 334, 312, 286; HR-MS (M^+) calcd 345.0880, found 345.0893. Anal. ($C_{17}H_{16}ClN_3O_3$) C, H, N.

3-Pentyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (14) was prepared from **6** using the procedure described in method B. Crystallization from ethyl acetate afforded **14** in 33% yield: mp 148–150 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 1 H, J = 2.4 Hz), 7.88 (s, 1 H), 7.62 (dd, 1 H, J = 2.3, 8.5 Hz), 7.38 (d, 1 H, J = 8.5 Hz), 5.20 (br s, 1 H), 5.07 (m, 1 H), 4.35 (br s, 1 H), 3.25 (s, 3 H), 1.75 (m, 4 H), 0.97 (t, 6 H, J = 8.5 Hz); MS (EI) m/z 363 (M^+), 361 (M^+), 291, 274, 245, 217, 157, 125, 70, 55. Anal. ($C_{18}H_{20}ClN_3O_3$) C, H, N.

Neopentyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (15) was prepared from **6** using the procedures described in method B. Crystallization from ethyl acetate afforded **15** in 37% yield; mp 192–194 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 1 H, J = 2.4 Hz), 7.89 (s, 1 H), 7.60 (dd, 1 H, J = 2.4, 8.5 Hz), 7.37 (d, 1 H, J = 8.5 Hz), 5.20 (br s, 1 H), 4.40 (br s, 1 H), 4.10 (s, 2 H), 3.25 (s, 3 H), 1.05 (s, 9 H); MS (CI) m/z 364 (M + H), 362 (M + H), 328, 292, 273, 245, 219. Anal. ($C_{18}H_{20}ClN_3O_3$) C, H, N.

3,3-Dimethyl-1-butyl 8-chloro-5,6-dihydro-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (16) was prepared from **6** using the procedure described in method B. The product was crystallized from ethyl acetate to give **16** in 47% yield: mp 182–183 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 1 H, J = 2.4 Hz), 7.87 (s, 1 H), 7.60 (dd, 1 H, J = 2.2, 8.5 Hz), 7.38 (d, 1 H, J = 8.5 Hz), 5.20 (br s, 1 H), 4.43 (t, 2 H, J = 7.7 Hz), 4.26 (br s, 1 H), 3.25 (s, 3 H), 1.77 (d, 2 H, J = 7.8 Hz), 0.98 (s, 9 H); MS (CI) m/z 378 (M + H), 376 (M + H), 342. Anal. ($C_{19}H_{22}ClN_3O_3$) C, H, N.

7-Bromo-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5-(1*H*)-dione (17). To solution of **3a** (3.8 g, 20 mmol) in acetic acid (30 mL) and concentrated sulfuric acid (3 mL) was added a mixture of bromine (2 mL) and acetic acid (10 mL). After stirring at room temperature for 4 h, the reaction mixture was poured into ice water (400 mL). It was neutralized with concentrated ammonium hydroxide. The precipitated product was collected by filtration, washed with water, and dried (vacuum oven, 90 °C, overnight) to afford **17** as an off-white powder (3.22 g, 60%): mp 240–242 °C; ^{1}H NMR (DMSO-d_6) δ 10.54 (br s, 1 H), 7.81 (d, 1 H, J = 2.2 Hz), 7.67 (dd, 1 H, J = 2.4, 8.6 Hz), 7.04 (d, 1 H, J = 8.7 Hz), 3.87 (s, 2 H), 3.09 (s, 3 H); MS (CI) m/z 269 (M + H), 271 (M + H), 208, 191. Anal. ($C_{10}H_9BrN_2O_2$) C, H, N.

***tert*-Butyl 8-bromo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (18)** was prepared from **17** using the procedure described in method A. The crude product was crystallized from ethyl acetate to afford **18** in 31% yield: mp 206–208 °C; ^{1}H NMR ($CDCl_3$) δ 8.25 (d, 1 H, J = 2.2 Hz), 7.85 (s, 1 H), 7.40 (dd, 1 H, J = 2.3, 8.5 Hz), 7.29 (d, 1 H, J = 8.5 Hz), 5.17 (br s, 1 H), 4.34 (br s, 1 H), 3.25 (s, 3 H), 1.64 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 164.9, 161.8, 135.5, 135.4, 135.3, 134.4, 130.9, 130.4, 130.1, 123.2, 122.1, 81.9, 42.4, 35.8, 28.5, 28.4, 28.3; MS (CI) m/z 394 (M + H), 392 (M + H), 336, 364, 314, 258. Anal. ($C_{17}H_{18}BrN_3O_3$) C, H, N.

7,9-Diiodo-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5(1*H*)-dione (19) and 7-Iodo-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5(1*H*)-dione (20). To a solution of **3a** (4.6 g, 24 mmol) in acetic acid (35 mL) and concentrated sulfuric acid (2 mL) was added iodine monochloride (6.0 g, 37 mmol). After being stirred at room temperature overnight, the reaction mixture was poured into ice water (400 mL) and neutralized with concentrated aqueous ammonia. The precipitate (3.0 g) was collected by filtration. Part of the crude product (1.2 g) was used for further purification by column chromatography, eluting with solvent system B. The first fraction (R_f 0.72) was collected, evaporated, and crystallized from ethyl acetate to afford **19** (0.25 g): mp 252–253 °C; ^{1}H NMR ($CDCl_3$) δ 8.27 (d, 1 H, J = 2.0 Hz), 8.20 (d, 1 H, J = 2.0 Hz), 7.50 (br s, 1 H), 3.86 (s, 2 H), 3.27 (s, 3 H); MS (CI) m/z 443 (M + H), 338, 334. Anal. ($C_{10}H_8I_2N_2O_2$) C, H, N.

Later fractions furnished the second product (R_f 0.51), which was evaporated and crystallized from ethyl acetate to afford **20** (0.45 g): mp 249–250 °C; ^{1}H NMR ($CDCl_3$) δ 8.27 (d, 1 H, J = 2.2 Hz), 8.26 (br s, 1 H), 7.50 (dd, 1 H, J = 2.2, 8.5 Hz), 6.74 (d, 1 H, J = 8.5 Hz), 3.88 (s, 2 H), 3.28 (s, 3 H); MS (CI) m/z 317 (M + H), 208, 291. Anal. ($C_{10}H_9IN_2O_2$) C, H, N.

***tert*-Butyl 8-iodo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (21)** was prepared from **20** using the procedure described in method A. Crystallization from ethyl acetate gave **21** in 35% yield: mp 212–214 °C; ^{1}H NMR ($CDCl_3$) δ 8.38 (d, 1 H, J = 2.0 Hz), 7.93 (dd, 1 H, J = 2.0, 8.5 Hz), 7.84 (s, 1 H), 7.14 (d, 1 H, J = 8.7 Hz), 5.18 (br s, 1 H), 4.33 (br s, 1 H), 3.24 (s, 3 H), 1.64 (s, 9 H); MS (CI) m/z 440 (M + H), 314. Anal. ($C_{17}H_{18}IN_3O_3$) C, H, N.

7-Nitro-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5-(1*H*)-dione (22). To a stirred and cooled (0 °C) solution of **3a** (3.8 g, 20 mmol) in concentrated sulfuric acid (10 mL) was added a solution of potassium nitrate (3.1 g, 30 mmol) in concentrated sulfuric acid (8 mL). After 15 min, the mixture was allowed to stir at room temperature for 4 h and then poured into ice water (400 mL). The orange precipitate was collected by filtration, washed thoroughly with water, and dried in vacuo at 90 °C overnight to give **22** (1.8 g, 38%): mp 238–240 °C; ^{1}H NMR (DMSO-d_6) δ 8.53 (d, 1 H, J = 2.6 Hz), 8.36 (dd, 1 H, J = 2.6, 9.0 Hz), 7.3 (d, 1 H, J = 9.0 Hz), 3.98 (s, 2 H), 3.14 (s, 3 H); MS (CI) m/z 236 (M + H), 222, 206. This material was used for the next step without further purification.

***tert*-Butyl 8-nitro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5*a*][1,4]benzodiazepine-3-carboxylate (23)** was prepared from **22** using the procedure described in method A. Crystallization of the crude product from ethanol gave **23** in 20% yield: mp 210–212 °C; ^{1}H NMR ($CDCl_3$) δ 8.95 (d, 1 H, J = 2.5 Hz), 8.47 (dd, 1 H, J = 2.5, 8.8 Hz), 7.90 (s, 1 H), 7.60 (d, 1 H, J = 8.7 Hz), 5.25 (br s, 1 H), 4.38 (br s, 1 H), 3.29 (s, 3 H), 1.63 (s, 9 H); MS (CI) m/z 359 (M + H), 329, 208. Anal. ($C_{17}H_{18}N_4O_5$) C, H, N.

***tert*-Butyl 8-Amino-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (24).** A solution of **23** (0.25 g, 0.7 mmol) in THF (10 mL) and ethanol (10 mL) was hydrogenated overnight in a Parr apparatus, in the presence

of 10% Pd/C (0.2 g). The catalyst was removed by filtration through a pad of Celite, and the filtrate was concentrated under reduced pressure to afford a yellowish oil (~0.24 g): HRMS (EI) calcd 328.1535, found 328.1520. The oil was used for the next step without further purification.

***tert*-Butyl 8-Isothiocyanato-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (25).** To a rapidly stirred solution of **24** (0.24 g, 0.75 mmol) in hydrocarbon-stabilized chloroform (10 mL) and saturated sodium bicarbonate solution (10 mL) was added freshly redistilled thiophosgene (0.12 mL, 1.5 mmol). The mixture was allowed to stir for 1 h at room temperature. The organic layer was then separated, and the aqueous layer was extracted with chloroform. The combined organic layer was washed with saturated sodium bicarbonate solution, water, and brine and dried (Na_2SO_4), and solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (solvent system A) and then crystallized from ethyl acetate to afford **25** (145 mg) in an overall yield of 57% from **23**: mp 211–213 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (d, 1 H, J = 2.0 Hz), 7.86 (s, 1 H), 7.41 (m, 2 H), 5.18 (br s, 1 H), 4.33 (br s, 1 H), 3.25 (s, 3 H), 1.63 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 164.9, 161.7, 139.1, 134.5, 134.3, 132.1, 130.4, 130.2, 130.1, 129.8, 129.1, 123.2, 82.1, 42.5, 35.9, 28.6, 28.5, 28.4; MS (CI) m/z 317 (M + H), 314, 268. Anal. ($C_{18}H_{18}N_4O_3S$) C, H, N, S.

7-Amino-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5-(1*H*)-dione (26). A solution of **22** (2.3 g, 10 mmol) in THF (50 mL) and ethanol (50 mL) was hydrogenated overnight in a Parr hydrogenator, in the presence of 10% Pd/C (0.8 g). The catalyst was removed by filtration through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was crystallized from ethanol to afford **26** (1.6 g, 78%): mp 246–248 °C dec; MS (CI) m/z 206 (M + H). This material was used for the next step without further characterization.

7-Azido-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5-(1*H*)-dione (27). To a cooled (0 °C) solution of **26** (0.45 g, 2.2 mmol) in 6 N HCl was added a previously cooled solution of sodium nitrite (0.24 g, 3.4 mmol) in water (3 mL). After the mixture was stirred for 30 min at 0 °C, a solution of sodium azide (0.31 g, 4.8 mmol) in water (5 mL) was added dropwise. The reaction mixture was stirred for 20 min at 0 °C and then for 10 min at room temperature. The precipitate was collected by filtration and washed with water. It was dried in vacuo at 90 °C overnight to afford **27** (0.48 g, 95%): mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 8.12 (br, s, 1 H), 7.65 (d, 1 H, J = 2.6 Hz), 7.11 (dd, 1 H, J = 2.6, 8.5 Hz), 3.90 (s, 2 H), 3.30 (s, 3 H); MS (CI) m/z 232 (M + H), 223, 206. Anal. ($C_{10}H_9N_5O_2$) C, H, N.

***tert*-Butyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (29)** was prepared from **27** using the procedure described in method A. Crystallization from ethyl acetate gave **29** in 22% yield: mp 155–157 °C; ^{1}H NMR ($CDCl_3$) δ 7.86 (s, 1 H), 7.73 (d, 1 H, J = 2.6 Hz), 7.41 (d, 1 H, J = 8.5 Hz), 7.24 (dd, 1 H, J = 8.5, 2.6 Hz), 5.18 (br s, 1 H), 4.36 (br s, 1 H), 3.25 (s, 3 H), 1.64 (s, 9 H); MS (CI) m/z 355 (M + H), 329, 168; HR-MS (M^+) calcd 354.1440, found 354.1419.

7-(Dimethylazido)-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5(1*H*)-dione (28). To an ice-cooled solution of **26**, (0.50 g, 2.4 mmol) in 6 N HCl (8 mL) was added a previously cooled solution of sodium nitrite (0.20 g, 3.0 mmol) in water (3 mL). After 15 min, an ice-cooled solution of dimethylamine (0.50 mL, 4.0 mmol) and potassium hydroxide (0.84 g, 15 mmol) in water (5 mL) was added. The reaction mixture was allowed to stir for 20 min with ice-bath cooling, and then a solution of concentrated aqueous ammonia–water (1:1, 10 mL) was added. The precipitate was collected by filtration and dried in vacuo overnight at 85 °C to give **28** (0.23 g, 37%): mp 194–196 °C; ^{1}H NMR ($CDCl_3$) δ 8.45 (br s, 1 H), 7.98 (d, 1 H, J = 2.3 Hz), 7.53 (dd, 1 H, J = 2.4, 8.5 Hz), 6.95 (d, 1 H, J = 8.5 Hz), 3.89 (s, 2 H), 3.33 (br s, 6 H), 3.30 (s, 3 H); MS (CI) m/z 262 (M + H), 223, 208, 191. Anal. ($C_{12}H_{15}N_5O_2$) C, H, N.

***tert*-Butyl 8-(dimethylazido)-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (30)** was prepared from **28** using the procedure described in method A. Crystallization of the crude product from ethyl acetate afforded **30** in 34% yield: mp 169–171 °C; ^{1}H NMR ($CDCl_3$) δ 8.08 (d, 1 H, J = 2.3 Hz), 7.86 (s, 1 H), 7.64 (dd, 1 H, J = 2.3, 8.5 Hz), 7.34 (d, 1 H, J = 8.5 Hz), 5.18 (br s, 1 H), 4.35 (br s, 1 H), 3.54 (br s, 3 H), 3.25 (br s, 6 H), 1.64 (s, 9 H); MS (CI) m/z 385 (M + H), 329, 314, 257, 214, 136. Anal. ($C_{19}H_{24}N_6O_3$) C, H, N.

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