Tetrahydroimidazo[1,5,4-*ef*][1,5]benzodiazepinones (isoTIBO's): Synthesis and Evaluation as HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors

Kathlyn A. Parker* and Apostolos Dermatakis

Department of Chemistry, Brown University, Providence, Rhode Island 02912

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Introduction

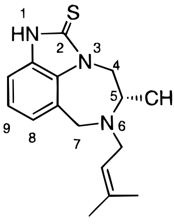
Recommended drug therapy for HIV-infected individuals has recently undergone a revolution. Combination therapy has, with exceptions, replaced monotherapy and early treatment is now suggested.[1] Despite continuing impressive progress in the clinic, however, the global nature of the AIDS epidemic requires that we search for improved approaches.

A particularly tantalizing target for drug development is the non-nucleoside binding site of HIV-1 reverse transcriptase. This hydrophobic pocket is adjacent to but distinct from the polymerase active site, the target of the nucleoside drugs such as zidovudine (AZT) and didanosine (ddI).

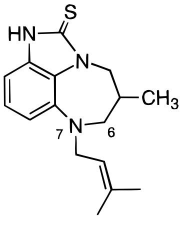
A number of small molecules, representing simple structural types, inhibit the enzyme by occupying the non-nucleoside binding site.[2] These compounds, the non-nucleoside reverse transcriptase inhibitors or NNRTI's, are highly active and remarkably nontoxic. Although they select for resistant mutants with exceptional speed, one of them, neviripine, is considered an effective component of a three-drug regimen in adults.[3] Also, trials of neviripine in combination therapy are underway in children.[4]

Strategy

The TIBO (4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-*j,k*][1,4]benzodiazepin-2(1*H*)-one) class of NNRTI's was among the first to be discovered, and a large number of analogs of the parent TIBO (R82150, **1**) have been studied.[5−8] The prospect of finding NNRTI's which would exhibit improved properties prompted us to prepare and test similar structures. IsoTIBO **2**, which differs from TIBO R82150 in that the nitrogen and carbon centers at positions 6 and 7 on the benzodiazepine skeleton are reversed,[9] seemed an attractive first candidate.

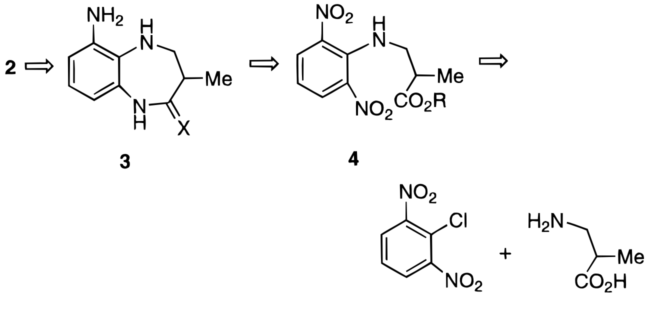


1, TIBO R82150



2, "isoTIBO"

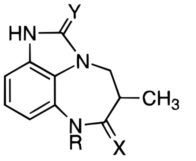
In the most likely retrosynthetic analysis, stripping the target of the thiocarbonyl "cap" and the prenyl side chain left the [1,5]benzodiazepine derivative **3**. Then, a strategy based on nucleophilic aromatic substitution, similar to that used for the TIBO's themselves,[10,11] was employed. Reduction and cyclization of a coupling product (**4**) of 2,6-dintrochlorobenzene (**5**) and β-aminoisobutyric acid (**6**) would afford the key intermediate **3**.



3 **4** **5** **6**

Synthesis of isoTIBO

This general strategy was parlayed into syntheses of the target isoTIBO **2** and four isoTIBO analogs (**7**−**10**). Cyclization substrate **11** was obtained as outlined (Scheme 1). Direct condensation of 2,6-dinitrochlorobenzene (**5**) with amino acid **6** afforded amino acid **4a**, and Fischer esterification followed by catalytic reduction provided the desired **11**.



7 X = O, Y = S, R = H
8 X = O, Y = O, R = H
9 X = H$_2$, Y = S, R = H
10 X = H$_2$, Y = S, R = COCH=C(CH$_3$)$_2$

This fully reduced compound was very air sensitive and its cyclization to aminodiazepine **3** (X = O, R = H) was problematic. Heating at 1 atm or distillative conditions resulted only in the retrieval of the starting material. The

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[1] Lipsky, J. J. *Lancet* **1996**, *348*, 800−803.

[2] De Clercq, E. *Med. Res. Rev.* **1996**, *16*, 125−157.

[3] D'Aquila, R. T.; Hughes, M. D.; Johnson, V. A.; Fischl, M. A.; Sommadossi, J.-P.; Liou, S-h.; Timpone, J.; Myers, M. Basgoz, N.; Niu, M.; Hirsch, M. S.; et al. *Ann. Intern. Med.* **1996**, *124*, 1019−1030.

[4] Luzuriaga, K.; Bryson, Y.; McSherry, G.; Robinson, J.; Stechenberg, G. Scott, G.; Lamson, M.; Cort, S.; Sullivan, J. L. *J. Infect. Dis.* **1996**, *174*, 713−721.

[5] For a lead reference to the many TIBO compounds which have been prepared and tested, see: Ho, W.; Kukla, M. J.; Breslin, H. J.; Ludovici, D. W.; Grous, P. P.; Diamond, C. J.; Miranda, M.; Rodgers, J. D.; Ho, C. Y.; De Clercq, E.; Pauwels, R.; Andries, K.; Janssen, M. A. C.; Janssen, P. A. J. *J. Med. Chem.* **1995**, *38*, 794−802.

[6] For synthesis and evaluation of tetracyclic TIBO's, designed as conformationally restricted analogs, see: Breslin, H. J.; Kukla, M. J.; Ludovici, D. W.; Mohrbacher, R.; Ho, W.; Miranda, M.; Rodgers, J. D.; Hitchens, T. K.; Leo, G.; gauther, D. A.; Ho, C. Y.; Scott, M. K.; De Clercq, E.; Pauwels, R.; Andries, K.; Janssen, M. A. C.; Janssen, P. A. J. *J. Med. Chem.* **1995**, *38*, 771−793.

[7] For the synthesis and examination of bicyclic TIBO analogs, see: (a) Gardiner, J. M.; Loyns, C. R. *Tetrahedron* **1995**, *51*, 11515−11530, and (b) Gardiner, J. M.; Loyns, C. R.; Burke, A.; Khan, A.; Mahmood, N. *Bioorg. Med. Chem. Lett.* **1995**, *51*, 1251−1254.

[8] The synthesis of "carba" TIBO analogs, in which N-6 has been replaced with a carbon, was recently reported; see: Salaski, E. J. *Tetrahedron Lett.* **1995**, *36*, 1387−90.

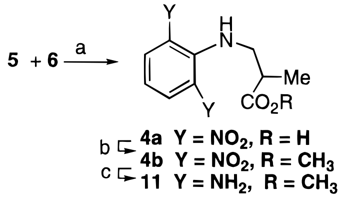
[9] Examples of tetrahydroimidazo[1,5,4-*ef*][1,5]benzodiazepinones were produced as the minor products of the Schmidt reaction of imidazo[4,5,1-*ij*]quinolin-6-ones; see: Geneste, P. Kamenka, J. M.; Vidal, Y.; Fauquet, J. P.; Morel, E.; Muller, P. Warolin, C. *Eur. J. Med. Chem.*−*Chem. Thir.* **1978**, *13*, 53−59.

[10] Parker, K. A.; Coburn, C. A. *J. Org. Chem.* **1991**, *56*, 4600−4601.

[11] Parker, K. A.; Coburn, C. A. *J. Org. Chem.* **1992**, *57*, 97−100.

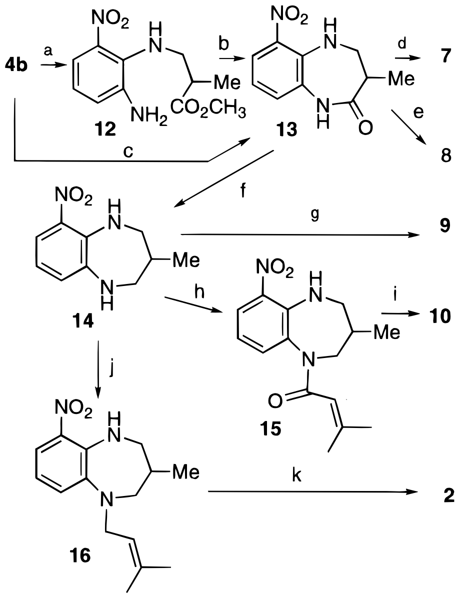
S0022-3263(96)02050-6 CCC: $14.00

Scheme 1. Coupling and Reduction[a]



[a] (a) EtOH, K_2CO_3, reflux, 98%; (b) MeOH, cat. H_2SO_4, reflux, 98%; (c) H_2, 10% Pd/C, EtOH.

Scheme 2. Synthesis of IsoTIBO and Analogs[a]



[a] Reagents and conditions: (a) 3.8 equiv of HCO_2H, 4.0 equiv of Et_3N, 10% Pd/C catalyst, CH_3CN, 25 °C, 15 min, 82%; (b) CH_3CN, H_2SO_4, 30 min, reflux, 82%; (c) 8 equiv of Fe dust, glacial HOAc, 40 min, reflux, 38%; (d) H_2, 10% Pd/C, EtOH, 1 h, then filtered and $NaHCO_3$ and CS_2 added, 37 °C, 16 h, 37%; (e) H_2, 10% Pd/C, EtOH, rt, 12 h, then filtered and added 4 equiv of $(CCl_3O)_2CO$, rt, 12 h, 26%; (f) 3.6 equiv of BH_3, THF, 2.5 h, reflux, 86%; (g) procedure (d), 45%; (h) 3,3-dimethylacryloyl chloride, $NaHCO_3$, DMAP, CH_2Cl_2; 3 h, rt, 98%; (i) procedure d, 52%; (j) 3-methyl-2-butenal, MeOH, $NaCNBH_3$ 72 h, 35% or prenyl bromide, K_2CO_3, DMF, rt, 93%; (k) H_2, PtO_2, 6 equiv of K_2CO_3, EtOH, then filtered and $NaHCO_3$ and CS_2 added, 53%.

inherent instability of **11** to oxidation suggested that a partially reduced cyclization substrate might be more manageable.

Treatment of compound **4b** with HCO_2H and Et_3N in the presence of 10% Pd on carbon[12] afforded the air-stable mononitro ester **12**. This compound, like the fully reduced substrate **11**, failed to cyclize on heating in acetonitrile. However, the addition of concd H_2SO_4 to the reaction medium effected cyclization to nitrobenzodiazepine **13**. More conveniently, dinitrobenzene **4b** was converted to diazepine **13** directly by reduction with Fe in refluxing glacial AcOH.[13] With this key intermediate in hand, we were able to complete the synthesis of isoTIBO analogs **2** and **7**–**10** according to the original strategy (see Scheme 2).

Reduction of nitrobenzodiazepine **13** and "capping" of the intermediate aminobenzodiazepinone with CS_2[14] afforded compound **7** in a "one-pot" procedure. Similarly, the oxo derivative **8** was obtained from diazepinone **13** by reduction followed by "capping" with triphosgene. Thione **9** was prepared by borane reduction of the common intermediate **13** to give nitrodiazepine **14** and then the one-pot reduction–"capping"' procedure with CS_2.

Treatment of diazepine **14** with 3,3-dimethylacryloyl chloride resulted in the acylation of the more nucleophilic nitrogen, N-7, and the exclusive formation of derivative **15**. The one-pot reduction–"capping" procedure with CS_2, as previously described, led to the formation of benzodiazepinethione **10** in 52% yield.

Compound **16**, the key intermediate to thione **2**, was accessible by the reductive alkylation of **14** with 3-methyl-2-butenal. However, a better yield was obtained by the direct alkylation of **14** with prenyl bromide

An attempt to apply the reduction–"capping" (with CS_2) procedure to compound **16** resulted in the unexpected loss of the N-7 substituent and the isolation of benzodiazepinethione **9** in 58% yield. However, PtO_2 and K_2CO_3[15] in EtOH provided a hydrogenation medium which did not lead to loss of the side chain. This modification of our original reduction–"capping" methodology afforded benzodiazepinethione **2**.

Biological Screening–Results

Analogs **2** and **7**–**10** were evaluated in the preliminary screen of the In Vitro Anti-AIDS Drug Discovery Program at the National Cancer Institute.[16] This assay measures the suppression of viral cytopathic effects with a colorimetric assay. Data are reported as effective concentration (EC_{50}, protection in infected cells), inhibitory concentration (IC_{50}, cytotoxicity in uninfected cells), and "in vitro therapeutic index" ($TI_{50} = IC_{50}/EC_{50}$).

TIBO analogs **7**–**9** which do not have a side-chain at the N-7 position are inactive against the HIV-1. Compound **10**, with a modified prenyl side chain, is also inactive. However agent **2**, in which there is a simple prenyl side chain at N-7 and which closely resembles TIBO R82150 (**1**) in structure, inhibits viral replication in the submicromolar range. It is approximately 10-fold less potent than the corresponding (+)-TIBO compound.[17] It has an EC_{50} of 1.1×10^{-6} M, an $IC_{50} > 1.00 \times 10^{-4}$ M, and therefore an "in vitro therapeutic index" (TI_{50}) of > 90.

IsoTIBO **2** was also tested against several drug-resistant HIV strains. It showed good activity against the wild types RF and IIIB and against AZT-sensitive and AZT-resistant viral strains. However, it was inactive against HIV-2 (as are all of the NNRTI's tested so far) and also against the strains which show general NNRTI-resistance.

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[12] Heck, R. F.; Terpko, M. O. *J. Org. Chem.* **1980**, *45*, 4992.

[13] Wulfman, D. S.; Cooper, C. F. *Synthesis* **1978**, 924.

[14] Ziv, J.; Knapp, S.; Rosen, J. D. *Synth. Commun.* **1988**, *18*, 973.

[15] Skiles, J. W.; Cava, M. P. *J. Org. Chem.* **1979**, *44*, 409.

[16] Weislow, O. W.; Kiser, R.; Fine, D.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 577. The protocol involves the killing of T-4 lymphocytes in a CEM-IW or CEM-SS cell line by HIV-1 and is designed to detect antiviral agents acting at any stage of the virus reproductive cycle.

[17] (+)-TIBO (**1**) is reported to have an effective concentration of 9.70×10^{-8} M; see: Kukla, M. J.; Breslin, H. J.; Diamond, C. J.; Grous, Philip, P.; Ho, C. Y.; Miranda, M.; Rodgers, J. D.; Sherrill, R. G.; De Clercq, E.; Pauwels, R.; Andries, K.; Moens, L. J.; Janssen, M. A. C.; Janssen, P. A. J. *J. Med. Chem.* **1991**, *34*, 3187–3197. For the (+)-S enantiomer, TIBO R82150 (**1**), the effective concentration is reported as 2.80×10^{-8} M and the cytotoxic concentration as $>8.70 \times 10^{-4}$ M; see: Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymakers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. *Nature* **1990**, *343*, 470.

Table 1. Results of Biological Screening

strain	IC_{50}(M)[e]	EC_{50}(M)[f]	TI_{50} (IC/EC)[g]
HIV-1_{RF}	6.10×10^{-5}	1.40×10^{-6}	4.57×10^{1}
HIV-1_{IIIB}	2.45×10^{-5}	4.98×10^{-7}	4.93×10^{1}
HIV-1_{6S}[a]	2.44×10^{-5}	1.29×10^{-6}	1.89×10^{1}
HIV-1_{6R}[b]	2.16×10^{-5}	8.50×10^{-7}	2.48×10^{1}
HIV-2_{ROD}	7.12×10^{-5}	inactive	
N_{119}[c]	2.72×10^{-5}	inactive	
HIV-1_{A-17}[d]	2.62×10^{-5}	inactive	

[a] AZT-sensitive. [b] AZT-resistant. [c] Y181C, selected in the presence of neviripine. [d] K103N, Y181C, selected in the presence of pyridinones. [e] 50% inhibitory concentration. [f] 50% effective concentration. [g] Therapeutic index.

Screening data are shown in Table 1. The reported values represent the mean of at least two experiments.

Conclusions

The structure−activity profile for the isoTIBO's examined is very similar to that of the original TIBO compounds. In both series, the lack of a side chain or the presence of a side chain that is different from a simple prenyl moiety results in compounds with diminished activity or no activity.[11] The continuing assembly of structure−activity data on compounds which act as NNRTI's may lead to improved drugs and/or drug combinations.

Experimental Section

General. High-resolution mass spectra were obtained under EI, CI, or FAB conditions. Thin layer chromatography (TLC) was performed on precoated silica gel 60 F-254 glass-supported plates, 0.25 mm thick. Column chromatography was performed with silica gel 60 (230−400 mesh). Ethyl ether, tetrahydrofuran, and benzene were distilled from sodium−benzophenone ketyl. Dimethyl sulfoxide, dimethyl formamide, pyridine, methylene chloride, methanol, and toluene were distilled from calcium hydride. All reactions were perfomed under argon unless otherwise stated.

3-[(2,6-Dinitrophenyl)amino]-2-methylpropanoic Acid (4a). To a slurry of 2.4 g (29.7 mmol) of $NaHCO_3$ in 20 mL of EtOH were added 1.0 g (4.9 mmol) of 2,6-dinitrochlorobenzene (**5**) and 770 mg (7.4 mmol) of β-aminoisobutyric acid (**6**). The reaction mixture was stirred at reflux for 6 h, cooled, quenched with 2 N HCl to pH 1, and extracted with EtOAc (9 × 40 mL). The combined organic solution was dried over Na_2SO_4 and evaporated to 1.3 g (98%) of a yellow solid. **4a**: mp 150−151 °C; IR (KBr pellet) 3337, 3120, 1696, 1615 cm^{-1}; 400 MHz ^1H NMR (acetone-d_6) 1.19 (d, 3H, $J = 7.2$ Hz), 2.88 (m, 1H), 3.13 (dd, 1H, $J = 12.9$, 4.7 Hz), 3.18 (dd, 1H, $J = 12.9$, 7.9 Hz), 6.94 (t, 1H, $J = 8.2$ Hz), 8.23 (d, 2H, $J = 8.2$ Hz), 8.41 (bs, 1H); 100.6 MHz ^{13}C NMR (acetone-d_6) 14.9, 40.2, 50.2, 50.3, 115.8, 132.9, 139.4, 140.7, 175.8; HRMS (EI) for $C_{10}H_{11}N_3O_6$ (M^+) calcd 269.0647, found 269.0645.

3-[(2,6-Dinitrophenyl)amino]-2-methylpropanoic Acid Methyl Ester (4b). To a solution of 1.30 g of acid **4a** in 30 mL of methanol was added 2 mL of 98% H_2SO_4. The reaction mixture was stirred at reflux for 4 h, cooled to rt, and diluted with $CHCl_3$. The organic solution was washed with saturated aqueous $NaHCO_3$ until basic pH and brine (2 × 30 mL) and dried over Na_2SO_4. Solvent evaporation resulted in 1.34 g (98%) of a yellow solid. **4b**: mp 123−124 °C; IR (KBr pellet) 3328, 3090, 1720, 1615 cm^{-1}; 250 MHz ^1H NMR ($CDCl_3$) 1.22 (d, 3H, J =7.2 Hz), 2.80 (m, 1H), 3.12 (m, 2H), 3.71 (s, 3H), 6.81 (t, 1H, $J = 8.2$ Hz), 8.18 (d, 2H, $J = 8.2$), 8.55 (bs, 1H); 100.6 MHz ^{13}C NMR ($CDCl_3$) 14.8, 39.9, 49.5, 52.2, 114.7, 132.3, 138.3, 139.9, 174.4; HRMS (EI) for $C_{11}H_{13}N_3O_6$ ($M^+ + H$) calcd 284.0879, found 284.0879.

3-[(2-Amino-6-nitrophenyl)amino]-2-methylpropanoic Acid Methyl Ester (12). A slurry of 1.2 g (4.2 mmol) of methyl ester **4b**, 1.7 g (2.4 mL, 16.9 mmol) of Et_3N and 800 mg of 10% Pd on activated carbon in 80 mL of CH_3CN was prepared. To this was added dropwise 0.86 mL (774 mg, 16.1 mmol) of 90% HCO_2H at rt. After 15 min the catalyst was removed by filtration and the solvent was evaporated. The residue was subjected to silica gel column chromatography with 3% MeOH in $CHCl_3$ to afford 886 mg (82%) of a red oil. **12**: IR (neat) 3443, 3362, 1731, 1620 cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$) 1.20 (d, 3H, $J = 7.0$ Hz), 2.69 (m, 1H), 3.12 (bd, 1H, $J = 12.4$ Hz), 3.37 (bm, 1H), 3.73 (s, 3H), 4.32 (bs, 2H), 5.84 (bs, 1H), 6.38 (m, 2H), 7.41 (m, 1H); 100.6 MHz ^{13}C NMR (CDCl3) 15.1, 40.6, 48.1, 51.8, 114.7, 120.0, 122.4, 131.2, 142.7, 143.1, 176.1; HRMS (EI) for $C_{11}H_{15}N_3O_4$ (M^+) calcd 253.1059, found 253.1071.

Nitrobenzodiazepinone 13 (from 12). To a solution of 830 mg (3.28 mmol) of methyl ester **12** in 65 mL of CH_3CN was added 0.5 mL of 98% H_2SO_4. The resulting orange mixture, after being stirred at reflux for 30 min, yielded a red solution which was quenched with 500 mg of solid K_2CO_3. After filtration of the solids, the solvent was concentrated to a brown-red solid which was successively triturated with of EtOH (1 × 3 mL) and Et_2O (3 × 3 mL). This afforded 600 mg (82%) of a bright red solid.

13 from 4b. To a solution of 20 mg (0.07 mmol) of compound **4b** in 3 mL of glacial acetic acid was added 31 mg (0.56 mmol) of Fe dust. The slurry was stirred at reflux for 40 min and then was poured into 3 mL of H_2O. The acidic solution was neutralized (pH 10) with 10% aqueous KOH and was extracted with EtOAc (7 × 5 mL). The organic layer was dried over Na_2SO_4 and then concentrated to a red residue. Silica gel column chromatography afforded 6 mg (38%) of red solid. **13**: mp 185 °C (dec.); IR (KBr pellet) 3317, 3207, 1675, 1609, cm^{-1}; 250 MHz ^1H NMR ($CDCl_3$) 1.25 (d, 3H, $J = 7.0$ Hz), 2.95 (m, 1H), 3.67 (m, 2H), 6.70 (t, 1H, 8.6 Hz), 7.05 (dd, 1H, 7.8, 1.3 Hz), 7.64 (bs, 1H), 8.01 (dd, 1H, 8.6, 1.5 Hz), 8.42 (bs, 1H); 100.6 MHz ^{13}C NMR (CDCl3) 13.6, 38.9, 50.1, 116.4, 123.6, 127.2, 128.1, 135.9, 138.4, 175.7; HRMS (EI) for $C_{10}H_{11}N_3O_3$ (M^+) calcd 221.0800, found 221.0808.

TIBO Analog 7. To a solution of 110 mg (0.50 mmol) of benzodiazepinone **13** in 10 mL of EtOH was added 90 mg of 10% Pd on activated carbon. The reaction vessel was flushed with H_2 at atmospheric pressure. After 1 h, the catalyst was removed by filtration under argon, and 215 mg (2.56 mmol) of $NaHCO_3$ and 134 mg (1.76 mmol, 107 μL) of carbon disulfide were added to the filtrate. After 16 h at 37 °C, the reaction was quenched with water and extracted with EtOAc (7 × 15 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to a solid residue. Tirturation and then recrystalization from EtOH yielded 43 mg (37%) of product, a white solid. **7**: mp >270 °C; IR (KBr pellet) 3162, 3122, 1658, 1624 cm^{-1}; 250 MHz ^1H NMR (DMSO-d_6) 1.10 (d, 3H, $J = 6.9$ Hz), 2.95 (m, 1H), 4.00 (bs, 1H), 4.49 (bd, 1H, $J = 13.4$ Hz), 6.85 (apparent t, presumably two closely situated d, 2H), 7.11 (t, 1H, $J = 7.7$ Hz), 10.32 (s, 1H), 12.89 (bs, 1H); 100.6 MHz ^{13}C NMR (DMSO-d_6) 14.1, 46.7, 104.2, 111.8, 122.2, 122.2, 123.5, 132.0, 167.3, 173.1.

TIBO Analog 8. To a solution of 80 mg (0.36 mmol) of **13** in 10 mL of EtOH was added 80 mg of 10% Pd on activated carbon and the reaction vessel was flushed with H_2 at atmospheric pressure. After 45 min the catalyst was removed by filtration under argon and then 420 mg (1.44 mmol) of triphosgene was added to the filtrate. After 12 h, the reaction mixture was poured into aqueous saturated $NaHCO_3$ and extracted with EtOAc (7 × 15 mL). The organic layer was dried over Na_2SO_4 and concentrated to afford a beige solid. This was chromatographed on a silica gel coloumn with a 2−5% MeOH in $CHCl_3$ gradient. Finally 20 mg (26%) of a white solid was isolated. **8**: mp > 270 °C; IR (KBr pellet) 3218, 1697, 1665, 1629 cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6) 1.11 (d, 3H, $J = 7.0$ Hz), 2.97 (m, 1H), 3.63 (apparent t, 1H), 3.98 (dd, 1H, $J = 11.9$, 1.3 Hz), 6.69 (apparent dd, presumably two overlapping d, 2H), 6.93 (t, 1H, $J = 7.9$ Hz), 10.09 (bs, 1H), 10.98 (bs, 1H); 100.6 MHz ^{13}C NMR (DMSO-d_6) d 14.0, 40.0, 43.8, 103.7, 111.1, 119.3, 121.1, 121.3, 129.2, 153.0, 173.2; LRMS (FAB) for $C_{11}H_{11}N_3O_2$ ($M^+ + H$) calcd 218, found 218.

Nitrobenzodiazepine 14. To a solution of 370 mg (1.7 mmol) of **13** in 15 mL of dry THF was added 6.2 mL (6.2 mmol, 3.6 equiv) of a 1 M solution of $BH_3 \cdot THF$ complex. The reaction mixture was stirred under reflux for 2.5 h and then was poured in H_2O. The aqueous layer was extracted with $CHCl_3$ (5 × 10 mL). The combined organic layer was dried over Na_2SO_4 and was evaporated to a black-red residue, which after silica gel column chromatography with 3% MeOH in $CHCl_3$ yielded in 300 mg (86%) of a dark red to black solid: mp 103−104 °C; IR

(CHCl$_3$) 3382, 3020, 1604, 1510 cm^{-1}; 250 MHz ^{1}H NMR (CDCl$_3$) 1.00 (d, 3H, $J = 6.8$ Hz), 2.18 (m, 1H), 3.02 (dd, 1H, $J = 12.9$, 6.4 HZ), 3.23 (m, 1H), 3.57 (dd, 1H, $J = 13.3$, 6.2 Hz), 3.69 (m, 2H), 6.51 (dd, 1H, $J = 8.6$, 7.6 Hz), 6.65 (ddd, 1H, $J = 7.4$, 1.4, 0.6 Hz), 7.61 (dd, 1H, $J = 8.6$, 1.4 Hz), 7.89 (bs, 1H); 100.6 MHz ^{13}C NMR (CDCl$_3$) 17.1, 32.8, 49.9, 51.8, 116.9, 117.9, 121.9, 135.4, 139.9, 142.1; HRMS (EI) for $C_{10}H_{13}N_3O_2$ (M$^+$) calcd 207.1005, found 207.1010.

TIBO Analog 9. To a solution of 75 mg (0.36 mmol) of nitrobenzodiazepine **14** in 10 mL of EtOH was added 70 mg of 10% Pd on carbon, and the reaction vessel was flushed with H$_2$ at atmospheric pressure. After 50 min the catalyst was removed by filtration under argon, and 182 mg (2.17 mmol) of NaHCO$_3$ and 83 mg (65 μL, 1.1 mmol) of CS$_2$ were added to the filtrate. After 16 h at 37 °C the suspension was filtered and the solvent was evaporated. The residual yellow solid was titurated with Et$_2$O (5 × 1 mL) and then subjected to chromatograpy on a silica gel column with a 2/5/10% MeOH in CHCl$_3$ gradient. This afforded 36 mg (45%) of a yellow solid. **9**: mp 255 °C (dec); IR (KBr pellet) 3331, 3113, 1624 cm^{-1}; 250 MHz ^{1}H NMR (CDCl$_3$) 1.12 (d, 3H, $J = 7.1$ Hz), 2.38 (m, 1H), 3.24 (dd, 1H, $J = 13.1$, 8.1 Hz), 3.41 (distorted d, 1H, $J = 13.9$ Hz), 3.82 (dd, 1H, $J = 13.9$, 9.1 Hz), 4.32 (bs, 1H), 4.53 (dd, 1H, $J = 12.9$, 1.9 Hz), 6.41 (d, 1H, $J = 8.1$ Hz), 6.60 (d, 1H, $J = 7.8$ Hz), 6.99 (t, 1H, $J = 7.9$ Hz), 9.40 (s, 1H); 100.6 MHz ^{13}C NMR (DMSO-d_6) 16.1, 34.1, 51.5, 54.7, 97.1, 107.8, 119.5, 123.5, 132.5, 134.8, 166.7; HRMS (EI) for $C_{11}H_{13}N_3S$ (M$^+$) calcd 219.0830, found 219.0843.

Nitrobenzodiazepine 15. To a suspension of 150 mg (0.72 mmol) of compound **14**, 244 mg (2.90 mmol) of solid NaHCO$_3$, and a catalytic amount of DMAP was added 258 mg (0.24 mL, 2.18 mmol) of 3,3-dimethylacryloyl chloride. After 3 h, the reaction mixture was poured into aqueous saturated NaHCO$_3$. The aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 15 mL) and the combined organic layer was dried over Na$_2$SO$_4$. The solvent was evaporated to leave a bright orange residue. Silica gel column chromatography with 2% MeOH in CHCl$_3$ resulted in 204 mg (98%) of a bright orange oil. **15**: IR (CHCl$_3$) 3370, 3014, 1645, 1624 cm^{-1}; 250 MHz ^{1}H NMR (CDCl$_3$) 0.70 × 3H), 2.42 (m, 0.30 × 1H), 2.61 (m, 0.70 × 1H), 2.80 (dd, 0.70 × 1H, $J = 13.3$, 10.5 Hz), 2.98 (dd, 0.70 × 1H, $J =14.5$, 7.3 Hz), 3.19 (m, 0.30 × 1H), 3.35 (dd, 0.30 × 1H, $J = 13.4$, 7.1 Hz), 3.60 (apparent t, 0.30 × 1H), 3.87 (dt, 0.7H, $J = 13.8$, 3.7 Hz), 4.56 (dd, 0.30 × 1H, $J = 13.4$, 6.7 Hz), 4.68 (dd, 0.70 × 1H, $J = 13.4$, 7.2 Hz), 5.47 (bs, 0.30 × 1H), 5.78 (s, 0.70 × 1H), 6.59 (t, 1H, $J = 7.7$ Hz), 7.09 (distorted t, presumably two closely situated d), 8.05 (distorted t, presumably two closely situated d, 1H), 8.30 (bs, 0.70 × 1H), 8.38 (bs, 0.30 × 1H); 100.6 MHz ^{13}C NMR (CDCl$_3$) 16.1, 18.1, 20.3, 27.2, 27.3, 32.0, 34.5, 48.1, 49.0, 51.1, 52.2, 115.0, 115.1, 116.9, 117.1, 125.2, 126.3, 131.3, 133.0, 134.0, 134.3, 135.0, 136.2, 143.1, 143.6, 152.4, 152.9, 166.2, 167.8; HRMS (CI) for $C_{15}H_{19}O_3N_3$ (M$^+$ + H): calcd 290.1501, found 290.1483.

TIBO Analog 10. To a solution of 110 mg (0.38 mmol) of nitrodiazepine **15** in 10 mL of EtOH was added 80 mg of 10% Pd on carbon and then the reaction vessel was flushed with H$_2$ at atmospheric pressure. After 1 h, the catalyst was removed by filtation under argon, and 130 mg (1.55 mmol) of NaHCO$_3$ and 86 mg (1.13 mmol, 68 μL) of CS$_2$ were added to the filtrate. After 16 h at 37 °C the reaction mixture was poured into H$_2$O. The aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic layer was dried over Na$_2$SO$_4$ and concentrated to a brownish solid. Silica gel column chromatography with a 15−40% EtOAc in hexanes gradient afforded 60 mg (52%) of a white solid. **10**: mp 193−194 °C; IR (CHCl$_3$) 3250, 3019, 2965, 1652, 1612 cm^{-1}; 250 MHz ^{1}H NMR (CDCl$_3$) 1.16 (d, 3H, $J = 6.9$ Hz), 1.82 (s, 3H), 2.10 (s, 3H), 2.72 (m, 1H), 4.37 (bs, 4H), 5.90 (s, 1H), 6.93 (bd, 1H, $J = 5.9$ Hz), 7.11 (m, 2H), 11.09 (bs, 1H); 100.6 MHz ^{13}C NMR (CDCl$_3$) 17.7, 20.4, 27.3, 32.5, 48.9, 53.1, 107.7, 116.7, 120.2, 123.5, 128.3, 128.6, 131.3, 153.3, 166.5, 169.2; HRMS (FAB) for $C_{16}H_{19}N_3SO$ (M$^+$ + H) calcd 302.1327, found 302.1341.

***N*-Prenylbenzodiazepine 16.** To a slurry of 300 mg (1.4 mmol) of compound **14** and 1.0 g (7.3 mmol) of K$_2$CO$_3$ in 10 mL of DMF was added 650 mg (4.4 mmol, 0.5 mL) of prenyl bromide at room temperature. After 8 h, the reaction mixture was poured into H$_2$O and extracted with CH$_2$Cl$_2$ (5 × 15 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated to a black residue. Preparative TLC chromatography on silica gel with 15% EtOAc in hexanes resulted in 370 mg (93%) of a black solid. **15**: mp 64−65 °C; IR (CH$_2$Cl$_2$) 3358, 3051, 1595, 1527, 1503 cm^{-1}; 250 MHz ^{1}H NMR (CDCl$_3$) 0.99 (d, 3H, $J = 6.8$ Hz), 1.71 (s, 3H), 1.78 (s, 3H), 2.15 (m, 1H), 3.04 (dd, 1H, $J = 13.8$, 8.0 Hz), 3.35 (m, 2H), 3.75 (m, 3H), 5.23 (bt, 1H), 6.53 (t, 1H, $J = 8.2$ Hz), 6.73 (bs, 1H), 7.61 (d, 1H, $J = 8.9$ Hz), 8.30 (bs, 1H); 100.6 MHz ^{13}C NMR (CDCl$_3$) 16.9, 18.1, 25.8, 31.9, 49.5, 51.6, 56.9, 116.3, 117.5, 120.5, 121.1, 134.2, 135.4, 142.4, 143.5; HRMS (EI) for $C_{15}H_{21}N_3O_2$ (M$^+$) calcd 275.1634, found 275.1634.

TIBO Analog 2. To a solution of 111 mg (0.40 mmol) of prenylnitrobenzodiazepine **16** in 10 mL of EtOH were added 18 mg of PtO$_2$ and 330 mg (2.40 mmol) of K$_2$CO$_3$. The reaction vessel was then flushed with H$_2$ at atmospheric pressure. After 20 min, the solids were removed by filtration under argon, and 168 mg (0.20 mmol) of NaHCO$_3$ and 91 mg (1.20 mmol, 72 μL) of CS$_2$ were added to the filtrate. After 16 h at 37 °C the filtrate was concentrated to a beige solid. Silica gel column chromatography with a 5/15/40 EtOAc in hexanes gradient afforded in 61 mg (53%) of an off-white solid. **2**: mp 173−174 °C; IR (KBr pellet) 3111, 3039, 1618, 1597, 1524 cm^{-1}; 250 MHz ^{1}H NMR (CDCl$_3$) 1.11 (d, 3H, $J = 7.0$ Hz), 1.72 (s, 3H), 1.78 (s, 3H), 2.38 (m, 1H), 3.17 (dd, 1H, $J = 13.8$, 7.7 Hz), 3.38 (dd, 1H, $J = 13.4$, 2.7), 3.92 (m, 3 H), 4.56 (dd, 1H, $J = 13.7$, 4.0 Hz), 5.28 (bt, 1H), 6.47 (d, 1H, $J = 8.2$ Hz), 6.68 (d, 1H, $J = 7.8$ Hz), 7.05 (t, 1H, $J = 8.0$), 10.51 (s, 1H); 100.6 MHz ^{13}C NMR (CDCl$_3$) 17.3, 18.1, 25.7, 33.5, 51.3, 53.7, 60.1, 99.9, 107.9, 120.4, 122.7, 124.3, 131.8, 135.6, 136.9, 166.6; HRMS (EI) for $C_{16}H_{21}N_3S$ (M$^+$) calcd 287.1456, found 287.1459.

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Supporting Information Available: IR, ^{1}H, and ^{13}C spectra for compounds **2**, **4a**, **4b**, **7**−**10**, and **12**−**16** (37 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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