Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 2345–2381

BIOORGANIC & MEDICINAL CHEMISTRY

Preparation and Biological Activity of Novel Tricyclic GPIIb/IIIa Antagonists[1]

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Received 14 May 1998; accepted 30 July 1998

Abstract—Antagonists of the glycoprotein GPIIb/IIIa are a promising class of antithrombotic agents offering potential advantages over present antiplatelet agents (i.e., aspirin and ticlopidine). Novel tricyclic nonpeptidal GPIIb/IIIa antagonists have been prepared and evaluated in vitro as antagonists of fibrinogen binding to the purified GPIIb/IIIa receptor and as inhibitors of platelet aggregation. The work presented demonstrates the robustness of the benzodiazepinedione (BZDD) scaffold, which can be functionalized at the N^1–C^2 amide as well as at C^7, to provide structural diversity and allow optimization of the physiochemical and pharmacological properties of the BZDD based GPIIb/IIIa antagonists. In addition, the resulting new class of tricyclic GPIIb/IIIa antagonists could be used to probe for additional binding interactions on the GPIIb/IIIa receptor and perhaps lead to BZDD based GPIIb/IIIa antagonists with increased potency. The tricyclic molecules reported herein demonstrate that a heterocyclic ring can be fused to the benzodiazepinedione scaffold with retention of anti-aggregatory potency and in the case of tetrazole **30i**, increased potency relative to the bicyclic analogue **1c**.

Introduction

The final common pathway for platelet aggregation, regardless of the activating stimulus, involves fibrinogen (Fg) bridging of GPIIb/IIIa on activated platelets.[2,3] Aggregation of circulating platelets in certain circumstances is the primary cause of a variety of human cerebral and cardiovascular diseases.[4–8] Binding of fibrinogen to GPIIb/IIIa (integrin α II_b β_3) is mediated through the 12 amino acid sequences (HHL GGAKQAGDV) at the carboxy terminus on the γ-chain (γ400–411).[9,10] However, polypeptides isolated from snake venoms, leeches, and ticks, as well as linear and cyclic peptides that contain the RGD tripeptide sequence, inhibit fibrinogen binding to GPIIb/IIIa and platelet aggregation.[11] Therefore, nonpeptidal GPIIb/IIIa antagonists, which mimic the RGD sequence of these peptides, represent a novel class of promising antithrombotics.[11–14]

A de novo design approach, based in part on a rigid cyclic RGD peptide (G4120), led to the discovery of the potent, selective, nonpeptidal GPIIb/IIIa antagonists based on the benzodiazepinedione (BZDD) scaffold (**1**, Fig. 1).[15,16] As has been reported,[1,15,17–22] a dramatic increase in potency was obtained by replacing a flexible 'arginine' side chain by a phenylamidine. Compound **1a** is equipotent with the cyclic peptide (G4120) from which it was designed.[15]

The structure–activity relationship observed with cyclic RGD peptides demonstrated that the N-terminus of the arginine and the C-terminus of the aspartic acid could be connected via a diverse set of linkages with retention of potency.[23–27] The SAR from the cyclic RGD peptides suggested we could use the N^1–C^2 amide functionality of the benzodiazepinedione to append a third ring and

Key words: Tricyclic GPIIb/IIIa antagonists.
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PII: S0968-0896(98)00192-8

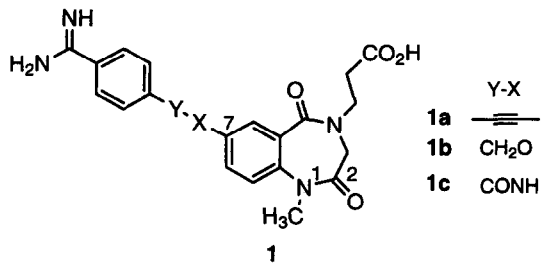

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Figure 1. Benzodiazepines **1a–c**.

probe for additional binding interactions on the GPIIb/IIIa receptor while retaining the orientation of the phenylamidine and carboxylate required for tight binding. Nitrogen heterocycles were chosen as the third ring since they contained a heteroatom which could act as a potential H-bond acceptor and in that respect mimic the carbonyl oxygen of the N^1–C^2 amide in the benzodiazepinediones. The resulting new class of tricyclic GPIIb/IIIa antagonists (Fig. 2) would be novel,[28] explore new space in the context of the rigid benzodiazepinedione scaffold and perhaps lead to BZDD based GPIIb/IIIa antagonists with increased potency.

It was less clear based on the RGD peptide SAR what effect modification of the ethynyl group at C^7 would have on potency. SAR studies of other rigid, centrally constrained nonpeptide GPIIb/IIIa antagonists[29–33] have shown that additional functional groups in a similar position to the C^7 acetylene could be incorporated and potency retained or improved. To explore the range of structural diversity allowed within the same class of antagonists, first we explored the effect of replacing the two atom ethynyl linker at C^7 with an ether (CH_2O) and an amide (CONH) and secondly the effect of replacing the N^1–C^2 amide group with a variety of heterocycles (Fig. 2).

Chemistry

The syntheses of the ether-linked compound **1b** and amide-linked compound **1c** are detailed in Schemes 1 and 2, respectively.[16]

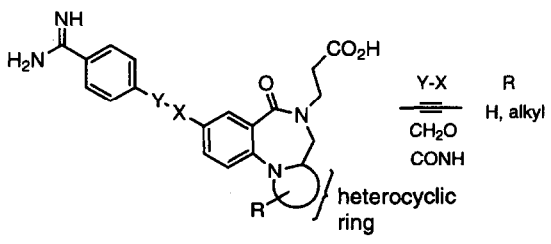


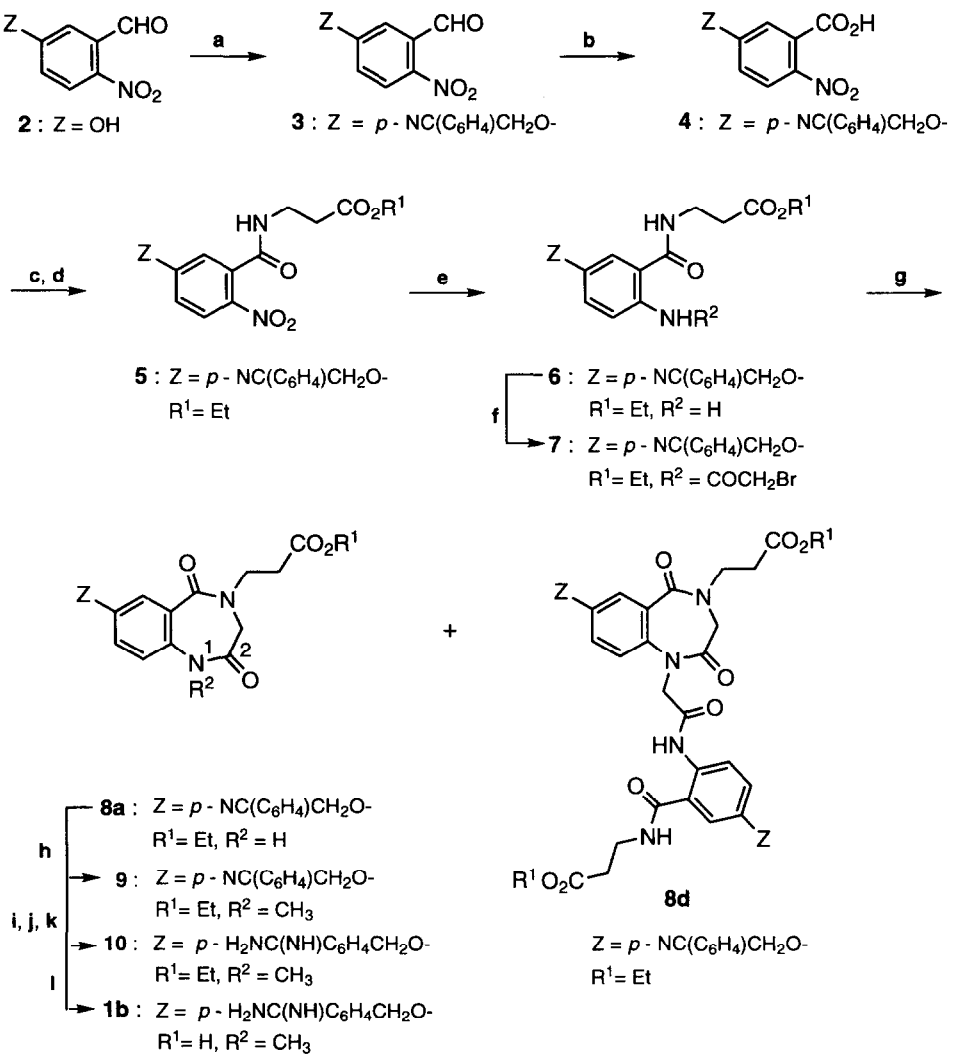
Figure 2. Nonpeptidal tricyclic GPIIb/IIIa antagonists.

Compound **1b** was prepared starting with the commercially available phenol **2**, which was alkylated with α-bromo-*p*-tolunitrile[34,35] to give a quantitative yield of nitrobenzaldehyde benzyl ether **3**. Oxidation with potassium permanganate afforded the nitrobenzoic acid benzyl ether **4** along with varying amounts of *p*-cyano benzoic acid (from oxidation at the benzylic position). Conversion of **4** into the benzoyl chloride, reaction with β alanine ethyl ester, and reduction of the nitro group with tin(II) chloride afforded the aniline **6**. Treatment of **6** with bromoacetylbromide under biphasic conditions gave **7**, and potassium carbonate mediated ring closure in DMF (50 °C) under high dilution conditions gave the benzodiazepinedione **8a**. Although reaction conditions were optimized to minimize competing dimer formation, dimer formation to yield **8d** (Scheme 1) could not be completely avoided. The dimer was formed via alkylation of the N^1 nitrogen of **8a** with **7**. However, the undesired dimer (**8d**) could be easily removed by trituration with benzene to yield **8a** of high chemical purity. Alkylation of the N^1 nitrogen with methyl iodide gave the benzonitrile **9**, which was converted into the benzamidine **10** via a modified Pinner reaction.[21] Saponification of the ethyl ester gave the amidino acid **1b**, which was evaluated in vitro in receptor binding and platelet aggregation assays (Table 4).[1,16]

The synthesis of the C^7 amide linked analogue **1c** is detailed in Scheme 2 and begins with a highly efficient, high yielding one pot conversion of *N*-methyl isatoic anhydride[36] into the benzodiazepinedione **11**. Nitration with fuming nitric acid followed by catalytic transfer hydrogenation gave the aniline **13** which was acylated with *p*-cyano benzoyl chloride to afford benzonitrile ethyl ester **14**. Conversion of **14** into the benzamidine ethyl ester **15**[21] and saponification yielded **1c** that was evaluated in vitro for activity (Table 4).[1,16]

After demonstrating that both ether and amide modifications to the linker at C^7 are tolerated with retention and increased inhibition of platelet aggregation, respectively relative to **1a** (Table 4), we then explored the effect of replacing the N^1–C^2 amide with a heterocyclic ring. The tricyclic analogues (**30a–j**, Table 4) further increased the structural diversity of BZDD based GPIIb/IIIa antagonists and allowed us to probe for additional binding interactions with the GPIIb/IIIa receptor in the hope of increasing the potency exhibited by this class of compounds relative to the comparative bicyclic analogues.

Retrosynthetic analysis (Fig. 3) of the tricyclic analogues indicates secondary amides **8a–c** are the required precursors for the synthesis of the benzyloxy, ethynyl and amide series of tricyclic GPIIb/IIIa antagonists. The synthesis of **8a** has been detailed in Scheme 1 and the

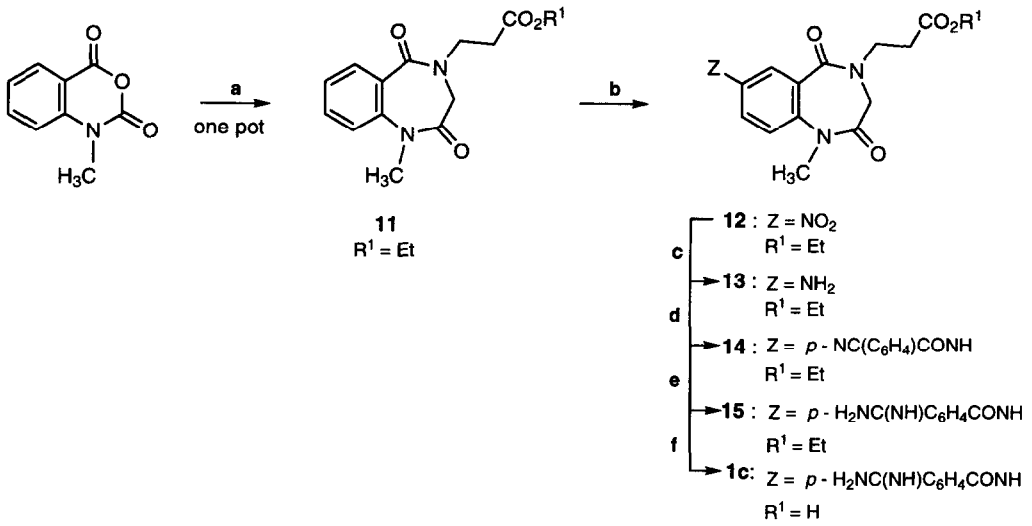


Scheme 1. Synthesis of benzodiazepinediones **8a** and **1b**. Reagents and conditions: (a) K_2CO_3, DMF, *p*-$NC(C_6H_4)CH_2Br$, 104%; (b) $KMnO_4$, Bu_4NBr, H_2O, C_5H_5N, 45% (ratio of **4**/*p*-$NC(C_6H_4)CO_2H$, ca. 5/1); (c) $(COCl)_2$, C_6H_6, DMF, 65 °C; (d) $ClH_3NCH_2CH_2CO_2Et$, $NaHCO_3$, THF/H_2O (1/2), 94%; (e) $SnCl_2$, H_2O, EtOAc/EtOH; (f) $BrCOCH_2Br$, H_2O/CH_2Cl_2, triturate w/ EtOH to purify (or recrystallize from EtOH), 50–70%; (g) powdered K_2CO_3/DMF (0.018–0.026 M), 50 °C, 2–3 h, 53–77%; (h) MeI, Cs_2CO_3, DMF, rt, 75%; (i) pyridine/Et_3N (1/1), H_2S, 50 °C, 4 h; (j) CH_2Cl_2/MeI (5/1), sealed tube, 50 °C, 1 h; (k) NH_4OAc(xs), MeOH, 50 °C, 12 h, RP HPLC, 64% (overall from **9**); (l) THF/NaOH (aq. 4.2 equiv.), RP HPLC, 75%.

syntheses of **8b** and **8c** are shown in Schemes 3 and 4, respectively.

Secondary amide **8b** (Scheme 3) was prepared starting from the reaction of iodo isatoic anhydride with β-alanine ethyl ester to yield **17**. The aniline **17** was protected at N^1 with a diphenylmethane group to completely suppress dimer formation during the base promoted ring closure to form the BZDD scaffold. Compound **18** was acylated with bromoacetyl bromide and cyclized to the benzodiazepinedione with sodium hydride.[16] Removal of the N^1 diphenylmethane-protecting group by hydrofluoric acid or TFA/Et_3SiH (3/1) gave **8b**. When HF cleavage conditions were used, the overall yield from **16** was higher (19%) compared to the overall yield when TFA/Et_3SiH was used to effect deprotection (10%). However, we found it more practical and safer for large-scale preparations of **8b**, to use the TFA/Et_3SiH deprotection conditions.

Bis amide **8c** (Scheme 4) was prepared starting from commercially available 5-nitro isatoic anhydride[37] which in a one pot procedure was hydrogenated, acylated with *p*-cyano benzoyl chloride, and then treated



Scheme 2. Synthesis of benzodiazepinedione **1c**. Reagents and conditions: (a) $ClH_3NCH_2CH_2CO_2Et$, CH_2Cl_2, Et_2N, 40 °C; CH_2Cl_2, KPhos pH 7.0, $BrCH_2COBr$, 5–25 °C; CH_2Cl_2, DBU, rt, 93% from *N*-methyl isatoic anhydride; (b) HNO_3, 0–25 °C, $NaHCO_3$/EtOAc, 73%; (c) CH_3CN, Et_3N, Pd–C, HCO_2H, 5–25 °C, then reflux, 98%; (d) CH_2Cl_2, Et_3N, *p*-$CN(C_4H_4)COCl$, 0–25 °C, 73%; (e) pyridine/Et_3N (1.4/1), H_2S, 70 °C, 24 h; CH_2Cl_2, MeI (xs), reflux; NH_4OAc (xs), EtOH, 50 °C, 24 h, 24% (three steps); (f) THF 50% aq. NaOH (xs), 57%.

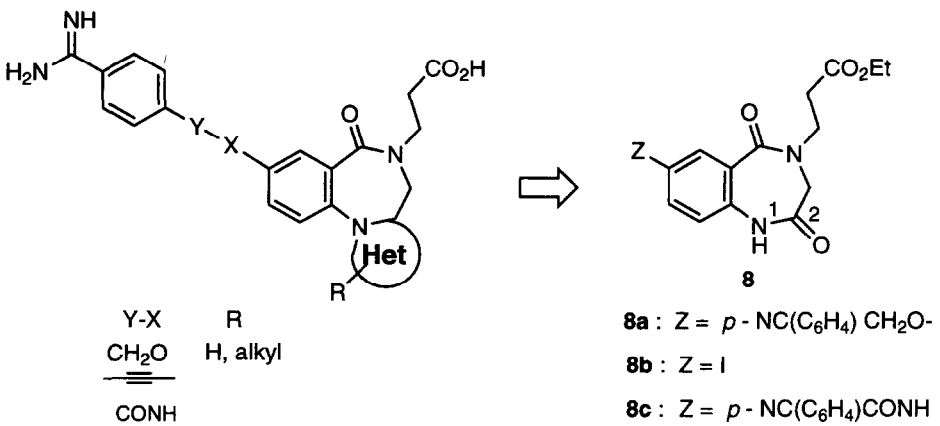


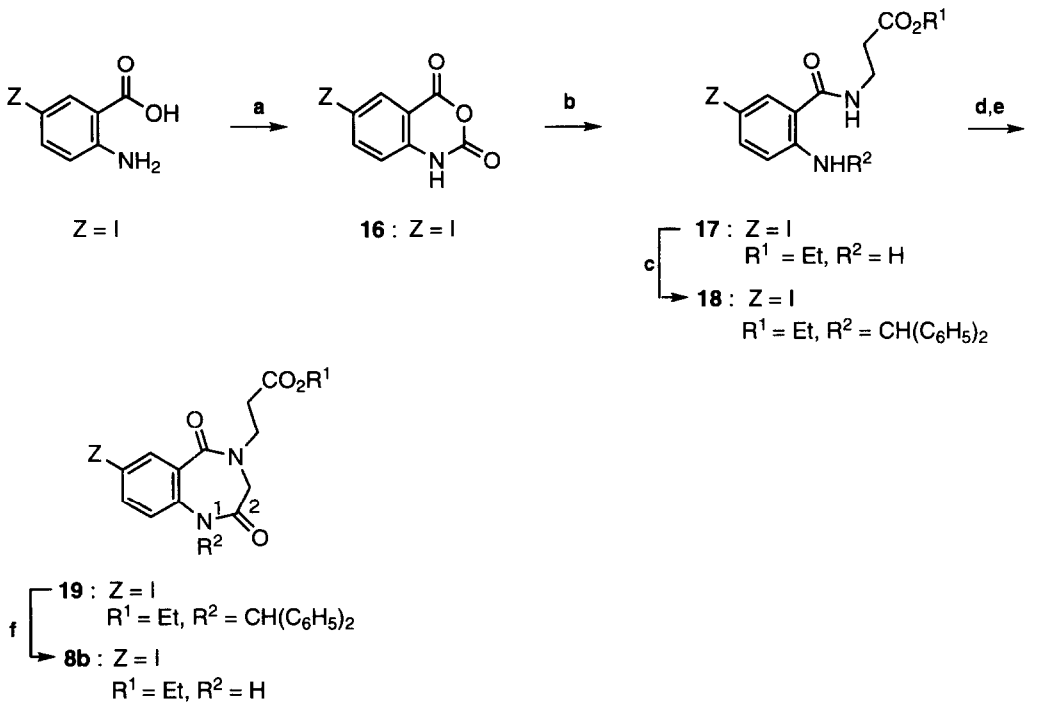
Figure 3. Retrosynthetic analysis to give bicyclic precursors to tricyclic GPIIb/IIIa antagonists.

with β-alanine ethyl ester to yield **20** in a 54% overall yield. The anilino nitrogen was protected with a diphenylmethane group, treated with bromoacetyl bromide, and cyclized to **22** with cesium carbonate in DMF (25 °C). Deprotection of the diphenylmethane-protecting group with hydrofluoric acid yielded the required bicyclic benzodiazepinedione **8c**.

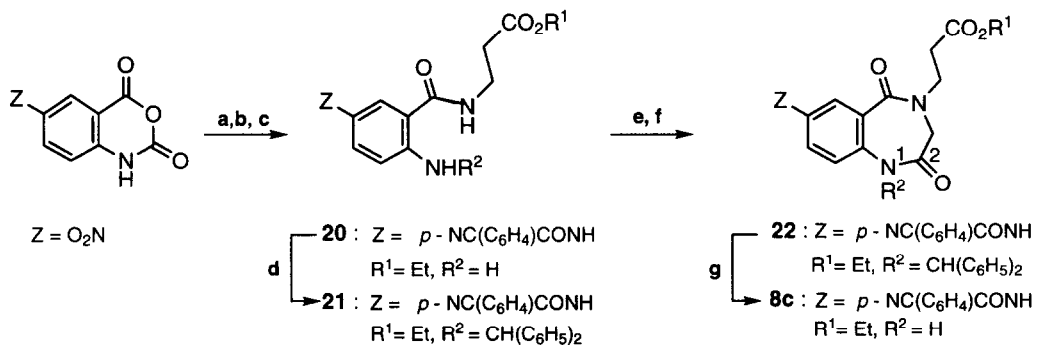
The N^1–C^2 amides of **8a–c** could be converted into an imidazole,[38–40] triazole,[38–40] or tetrazole[41] heterocyclic ring by known literature procedures. The imidazole, triazole, and quinazoline heterocycles (Table 1) were prepared from the thioamides **23a–c** as detailed in Scheme 5.

Since initial attempts to form a tetrazole heterocycle from the reaction of the S–Me thiaimidate derived from **23b** with sodium azide were unsuccessful, the tetrazole heterocycles were prepared directly from the N^1–C^2 amide of the benzodiazepinedione benzonitriles **8a**, **8c**, and **25**. Benzonitrile **25** was prepared from the reaction of ethynylbenzonitrile[42] with aryl iodide **8b** via a palladium/CuI catalyzed Heck coupling[43,44] (Fig. 4).

Tricyclic benzonitrile **26**, precursor to amidino ester **29g** and amidino acid **30g**, was similarly prepared (Fig. 4). Benzodiazepinedione benzonitriles **8a**, **8c**, and **25** were converted into tricyclic tetrazoles **27a–c** by treatment with



Scheme 3. Synthesis of benzodiazepinedione **8b**. Reagents and conditions: (a) K_2CO_3/H_2O, 0 °C, $COCl_2$, 84%; (b) Cl $H_3NCH_2CH_2CO_2Et$, DMF, Et_3N, DMAP (cat.), 90%; (c) Ph_2CHCl, 2,6-lutidine, DMF, 50 °C, 56%; (d) $BrCH_2COBr$, CH_2Cl_2/H_2O, rt; (e) NaH, DMF, 0 °C, 48% from **18**; (f) HF (g), anisole, $H_3CCH_2SCH_3$, −196 °C, 80% or TFA/Et_3SiH (3/1), reflux, 16 h, 40%.

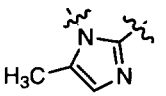
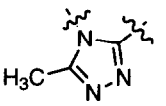
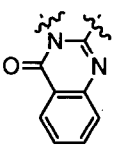
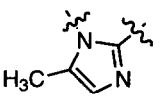
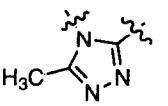
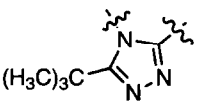
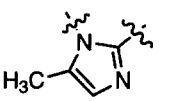


Scheme 4. Synthesis of benzodiazepinedione **8c**. Reagents and conditions: (a) H_2, 5% Pd–C, DMA; (b) *p*-$NC(C_6H_4)COCl$, NEt_3, DMAP; (c) Cl $H_3NCH_2CH_2CO_2Et$ NEt_3, DMAP; 56% overall for the three steps; (d) 2,6-lutidine, $ClCH_2CH_2Cl$, Ph_2CHBr, 60 °C, 3 h, 86%; (e) $BrCH_2COBr$, CH_2Cl_2/H_2O; (f) Cs_2CO_3, DMF, 93% overall for the two steps; (g) HF, anisole, $H_3CCH_2SCH_3$, −196–0 °C, 56%.

triphenylphosphine, DEAD, and trimethylsilylazide[41] as depicted in Figure 5 (Table 2).

It is interesting to note that in the syntheses of tricyclic GPIIb/IIIa antagonists containing an amide linker, both thioamide and tetrazole formation took place exclusively at the N^1–C^2 amide of the benzodiazepinedione. The origin of the selectivity is dictated by the electronic environment of the two secondary amides.[45–47] The amide in the linker in **8c** is conjugated to a *p*-cyano phenyl group and the phenyl ring of the benzodiazepinedione, an extended pi system, while the N^1–C^2 amide of the benzodiazepinedione is not. Reaction with Lawessons reagent[47] and PPh_3/DEAD/$TMSiN_3$ takes place preferentially at the more accessible and electron rich N^1–C^2 amide carbonyl. Similar selectivity was observed in the alkylation of **8c** with one equivalent of ethyl bromide (Fig. 6). RP HPLC analysis of the crude reaction mixture revealed an 85/15 mixture of the mono alkylated product **28** and **8c**. The *N*-ethyl benzodiazepinedione benzonitrile **28** was identical in all respects to 1-ethyl-4-(2-carboxyethyl)-7-(4-cyano)benzamido-3,4-dihydro-1H-1,4-

Table 1. Synthesis of thioamides **23a–c** (Scheme 5) and tricyclic intermediates **24a–g**

Thioamide	Method	Product	Z	Het	Yield
23a	**A**	**24a**	*p*-NC(C_6H_4)CH_2O		22%
23a	**B**	**24b**	*p*-NC(C_6H_4)CH_2O		63%
23a	**C**	**24c**	*p*-NC(C_6H_4)CH_2O		33%
23b	**A**	**24d**	I		66%
23b	**B**	**24e**	I		91%
23b	**B**	**24f**	I		81%
23c	**D**	**24g**	*p*-NC(C_6H_4)CONH		23%

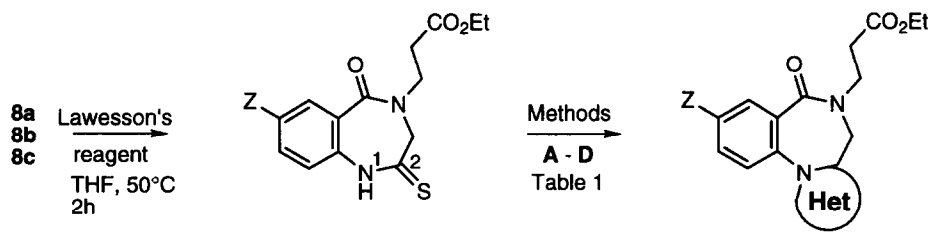
Conditions and reagents: (**A**) i. MeI, H_2O, Bu_4NHSO_4 (cat.), NaOH; ii. propargyl amine, C_5H_5N(HCl), $PhCH_3$, reflux, 5 h; (**B**) i. MeI, H_2O, Bu_4NHSO_4 (cat.), NaOH; ii. $H_3CCONHNH_2$ or $(H_3C)_3CONHNH_2$, C_5H_5N(HCl), $C_6H_5CH_3$, reflux, 5 h; (**C**) i. MeI, H_2O, Bu_4NHSO_4 (cat.), NaOH; ii. methyl anthranilate, C_5H_5N(HCl), $C_6H_5CH_3$, reflux, 6 h; (**D**) i. MeI, CH_3CN (anhyd.), sealed tube, charge w/MeI at t = 6, 7, and 8 h after reflux. Reflux for 8.5 h; ii. propargyl amine, C_5H_5N(HCl), $C_6H_5CH_3$, reflux, 6 h.

benzodiazepine-2,5-dione ethyl ester that was prepared via the reaction sequence described in Scheme 2 starting from *N*-ethyl isatoic anhydride. This indicates alkylation took place exclusively at the more nucleophilic N^1–C^2 amide.

Amidino esters (**29c–e**, **29f**, **29g**, **29h–j**) were prepared from corresponding benzonitriles (**24a–c**, **24g**, **26**, **27a–c**) via a modified Pinner reaction[21] (Table 3) as depicted in Scheme 6 or in the case of aryl iodides **24e** and **24f** by a palladium/CuI catalyzed Heck arylation with 4-ethynylbenzamidine (Fig. 7).[42] In the course of this work, it was determined to be a convergent and higher yielding process to convert 4-ethynylbenzonitrile into 4-ethynylbenzamidine prior to Heck arylation for the preparation of amidino esters containing an acetylene linker.

Saponification of amidino esters **29a–j** (Scheme 6, Table 3, Fig. 7 and Scheme 7) gave the tricyclic molecules (**30a–j**) that were evaluated in vitro for receptor binding (ELISA assay, Table 4) and for inhibition of platelet aggregation (platelet aggregation assay, Table 4).

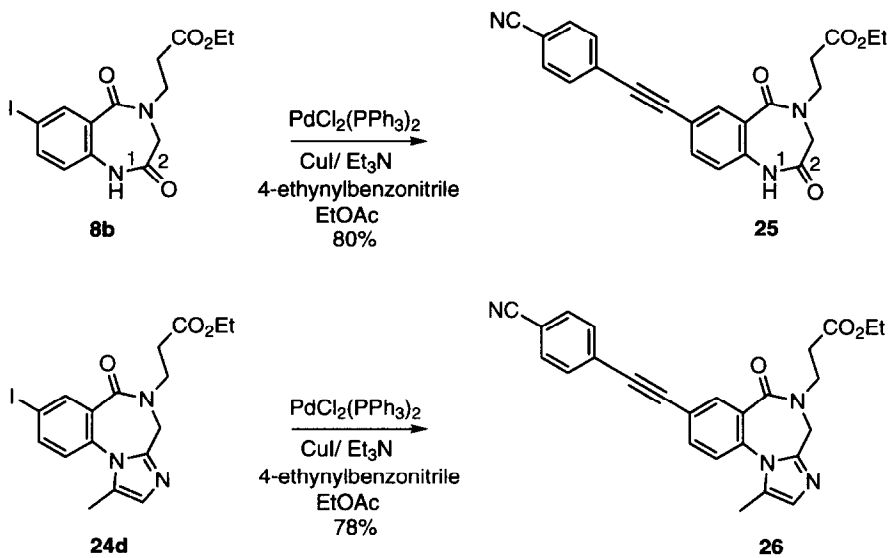
A more practical synthesis of **30i** which avoids the use of HF, features an improved method for the conversion of



23 24

Thioamide	yield
23a : Z = *p* - NC(C_6H_4)CH_2O	96%
23b : Z = I	91%
23c : Z = *p* - NC(C_6H_4)CONH	70%

Scheme 5. Syntheses of thioamides **23a–c** and tricyclic intermediates **24a–g**. Conditions and reagents: (**A**) i. MeI, H_2O, Bu_4NHSO_4 (cat.), NaOH; ii. propargyl amine, C_5H_5N(HCl), $PhCH_3$, reflux, 5 h; (**B**) i. MeI, H_2O, Bu_4NHSO_4 (cat.), NaOH; ii. $H_3CCONHNH_2$ or $(H_3C)_3CONHNH_2$, C_5H_5N(HCl), $C_6H_5CH_3$, reflux, 5 h; (**C**) i. MeI, H_2O, Bu_4NHSO_4 (cat.), NaOH; ii. methyl anthranilate, C_5H_5N(HCl), $C_6H_5CH_3$, reflux, 6 h; (**D**) i. MeI, CH_3CN (anhyd.), sealed tube, charge w/Mel at t = 6, 7, and 8 h after reflux,. Reflux for 8.5 h; ii. propargyl amine, C_5H_5N(HCl), $C_6H_5CH_3$, reflux, 6 h.



8b 25 24d 26

Figure 4. Synthesis of benzonitriles **25** and **26**.

a benzonitrile into a benzamidine,[32] and utilizes a common intermediate (**38**) which would allow functionalization of the aromatic ring of the benzodiazepinedione is detailed in Scheme 7.[48]

Results and Discussion

Biological assays

Bicyclic and tricyclic GPIIb/IIIa antagonists (**1a–c** and **30a–j**, respectively) were evaluated in ELISA and platelet aggregation assays[23,42] to determine potencies. With one exception, the assay data in Table 4 indicates that a five-membered heterocyclic ring can be fused to the benzodiazepinedione scaffold containing an ethynyl, ether, or amide linker to the benzamidine without a significant loss in potency.

In the series with an ether linker, potency (relative to **1b**) was retained (i.e. less than twofold decrease) in the tricyclics containing triazole and tetrazole heterocycles (**30d** and **30h**). Fusing an imidazole ring resulted in a fourfold decrease in potency in the platelet aggregation assay while the tetracyclic quinazoline (**30e**) exhibited over a 100-fold decrease in potency. Appending a quinazoline ring may not be optimal for steric reasons and

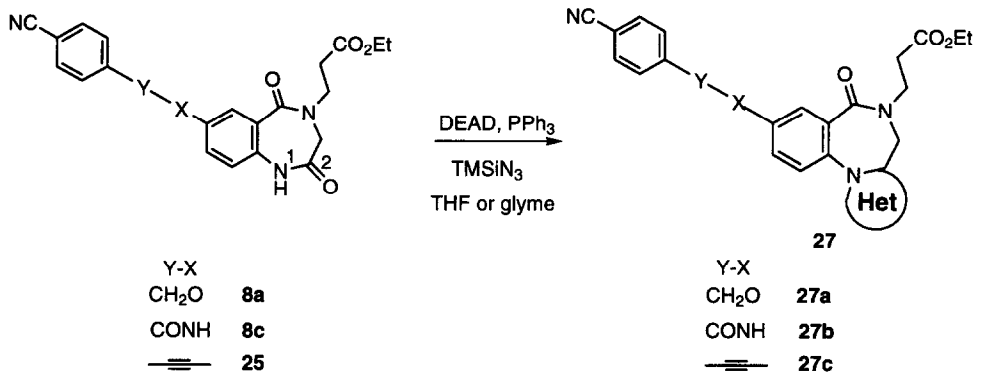
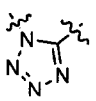
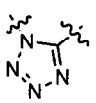


Figure 5. Synthesis of tricyclic tetrazoles **27a–c**.

Table 2. Synthesis of tricyclic tetrazoles **27a–c**

Reactant	Method	Prod.	Y-X	Het	Yield
8a	E	**27a**	CH_2O		72%
8c	F	**27b**	CONH		52%
25	E	**27c**			68%

(**E**) i. DEAD (1.0 equiv.), PPh_3 (1.0 equiv.), $TMSiN_3$ (1.0 equiv.), THF (anhyd.), rt, 24 h; ii. charge rx. with additional 1.0 equiv. of reagents, rt, 48 h; (**F**) DEAD (2.0 equiv.), PPh_3 (2.0 equiv.), $TMSiN_3$ (2.0 equiv.), glyme (anhyd.), rt, 16 h.

indicated that tricyclic molecules in which incorporated five membered heterocyclic rings would be preferred as probes for additional binding interactions with the GPIIb/IIIa receptor. In the tricyclic IIbIIIa antagonists which retain activity in the platelet aggregation assay and have a substituent on the heterocyclic ring, the substituent is located alpha to the N^1 nitrogen of the N^1–C^2 amide of the benzodiazepinedione from which the heterocycle was derived. Even a bulky substituent such as a *t*-butyl group (i.e. **30b**) is tolerated when it is placed on the heterocycle alpha to the N^1 nitrogen. In the quinazoline heterocycle **30e**, the aromatic ring of the heterocycle is appended at the beta and gamma position relative to the N^1 nitrogen resulting in a significant loss of potency. Based on the data obtained for **30e**, the tetracyclic quinazoline analogues derived from **8a** and **8b** were not prepared.

In the ethynyl linker series, anti-aggregatory potency was similar (less than twofold loss of potency relative to **1a**) with the tricyclic compounds containing triazole and tetrazole heterocycles. In this series, the imidazole containing tricyclic compound was equipotent with **1a** in the platelet aggregation assay. Since it appeared in the case of the imidazole heterocycle that retention or loss of potency may be dependent on the nature of the linker, the imidazole heterocycle containing an amide linker (**30f**) was prepared. The tetrazole heterocycle **30i** (amide linker) was prepared since the loss of potency exhibited by the tetrazole analogues **30h** (ether linker) and **30j** (ethynyl linker) was less than twofold, relative to their respective bicyclic compounds (**1b** and **1a**).

To determine what, if any, conformational effects occur in the seven member ring when the N^1–C^2 amide is converted into a heterocyclic ring and what impact such effects may have on the activity of the newly designed agents (**30a–j**), ab initio geometry optimizations at the HF/6-31G* level using the Gaussian 94 program[49] were conducted on the tetracyclic core of **30e** as well as the tricyclic cores **30a–c** and **30h–j**. The resulting minimized tri- and tetracyclic cores were overlaid with the minimized bicyclic core of benzodiazepinediones **1a–c** (Figure 8, part A) and the X-ray crystal structure of atropisomer 1-*tert*-butyl-4-(3(*S*)-butanoic acid)-7-[(4-cyanophenyl)-ethynyl]-9-chloro-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione, ethyl ester[42] (Figure 8, part B). It can be concluded that fusion of a heterocyclic ring to the BZDD scaffold has a minor effect on the conformational mobility of the seven-membered ring. However, what appears to have a dramatic effect on potency is how far the heterocyclic ring extends out from the bicyclic nucleus. With the exception of imidazole **30c**, the tricyclic GPIIb/IIIa inhibitors which retained (i.e. exhibited $\leq$ twofold decrease in potency) or improved potency all stayed within the consensus volume defined by the BZDD nucleus, N^1*t*-butyl group, and C^9 chloro substituent of the atropisomer depicted in Figure 8, part B.

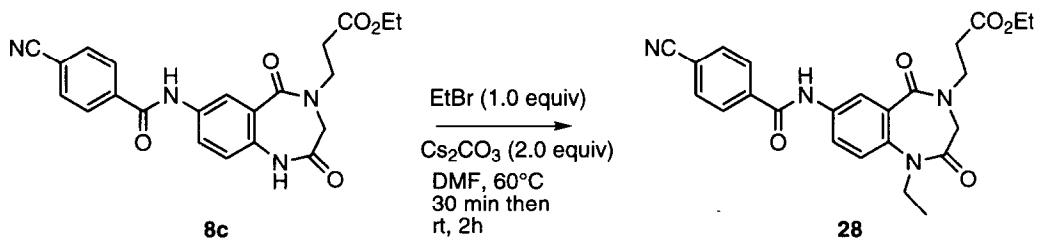
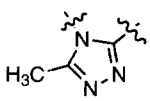
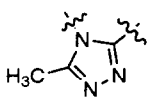
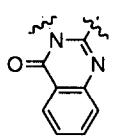
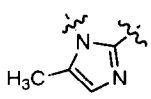
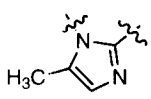
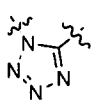
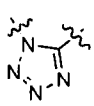
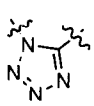
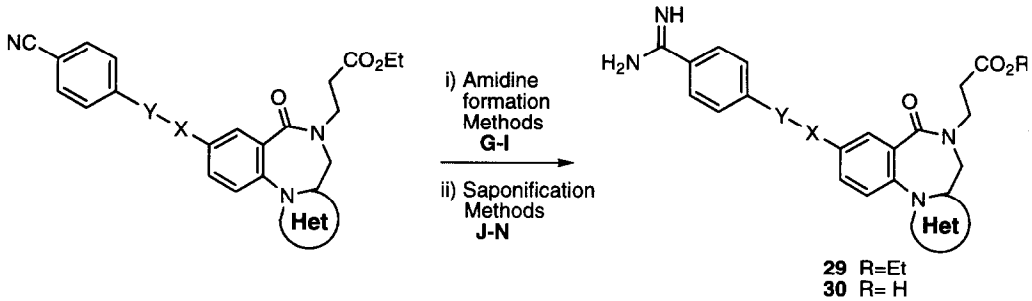


Figure 6. Selective alkylation of **8c**.

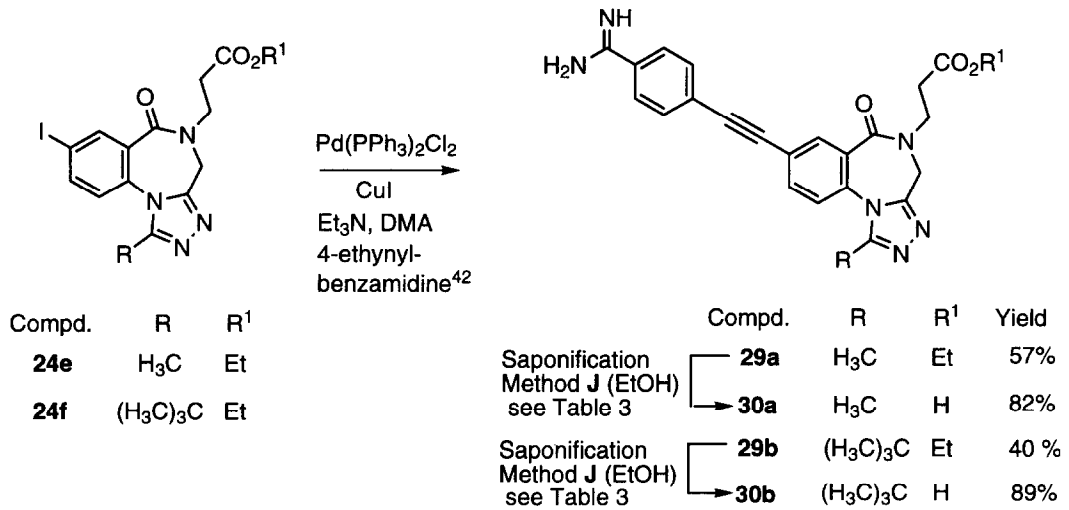
Table 3. Conversion of tricyclic benzonitriles **24a–c**, **24g**, **26**, and **27b** into amidino acids **29c–i**

NCAr	Y-X	Het.	Method[a]	Prod.	Yield	Method[b]	Prod.	Yield
24a	CH_2O		**G**	**29c**	nd	**J**(MeOH)	**30c**	5% overall from **24a**
24b	CH_2O		**G**	**29d**	26%	**J**(EtOH)	**30d**	10% overall from **24b**
24c	CH_2O		**G**	**29e**	71%	**K,L**	**30e**	10% and 39%, 46%
24g	CONH		**H**	**29f**	24%	**M**	**30f**	24%
26	—≡—		**G**	**29g**	16%	**J**(MeOH)	**30g**	85%
27a	CH_2O		**G**	**29h**	66%	**J**(MeOH)	**30h**	83%
27b	CONH		**I**	**29i**	18%	**N**	**30i**	65%
27c	—≡—		**G**	**29j**	34%	**J**(MeOH)	**30j**	95%

[a](**G**) i. H_2S, Et_3N or Et_2NH/C_5H_5N (1/1), rt, 2 h; ii. MeI, CH_2Cl_2, 50 °C, 30 min; iii. NH_4OAc, EtOH or MeOH, 50 °C, 30 min; (**H**) i. H_2S, Et_3N/C_5H_5N (1/1), 50 °C, 90 min; ii. 50 °C, 90 min; ii. MeI, CH_3CN (anhyd.) sealed tube, 85 °C, 1 h; iii. NH_4OAC, EtOH, rt, 18 h; (**I**) i. H_2S, Et_3N/C_5H_5N (1/1), 50 °C, 90 min; ii. MeI, N–Me pyrrolidinone (anhyd.), rt, 24 h; iii. NH_4OAc (anhyd.), EtOH, 18 h.
[b](**J**) NaOH (aq.), EtOH or MeOH, rt; (**K**) $LiOH/H_2O_2$, THF/H_2O, rt; (**L**) 50% TFA/H_2O, 60 °C, 3 h; (**M**) NaOH, $THF/MeOH/H_2O$ (3/2/1), rt, 18 h; (**N**) LiOH, THF/H_2O (3/1), rt, 40 h.



Scheme 6. Conversion of tricyclic benzonitriles **24a–c**, **24g**, **26**, and **27b** into amidino acids **29c–i**. Conditions and reagents: (**G**) i. H_2S, Et_3N or Et_2NH/C_5H_5N (1/1), rt, 2 h; ii. MeI, CH_2Cl_2, 50 °C, 30 min; iii. NH_4OAc, EtOH or MeOH, 50 °C, 30 min; (**H**) i. H_2S, Et_3N/C_5H_5N (1/1), 50 °C, 90 min; ii. MeI, CH_3CN (anhyd.) sealed tube, 85 °C, 1 h; iii. NH_4OAc, EtOH, rt, 18 h; (**I**) i. H_2S, Et_3N/C_5H_5N (1/1), 50 °C, 90 min; ii. MeI, N–Me pyrrolidinone (anhyd.), rt, 24 h; iii. NH_4OAc (anhyd.), EtOH, 18 h; (**J** NaOH (aq.), EtOH or MeOH, rt; (**K**) $LiOH/H_2O_2$, THF/H_2O, rt; (**L**) 50% TFA/H_2O, 60 °C, 3 h; (**M**) NaOH, $THF/MeOH/H_2O$ (3/2/1), rt, 18 h; (**N**) LiOH, THF/H_2O (3/1), rt, 40 h.



Compd.	R	R^1
24e	H_3C	Et
24f	$(H_3C)_3C$	Et

	Compd.	R	R^1	Yield
Saponification Method **J** (EtOH) see Table 3	**29a**	H_3C	Et	57%
	30a	H_3C	H	82%
Saponification Method **J** (EtOH) see Table 3	**29b**	$(H_3C)_3C$	Et	40 %
	30b	$(H_3C)_3C$	H	89%

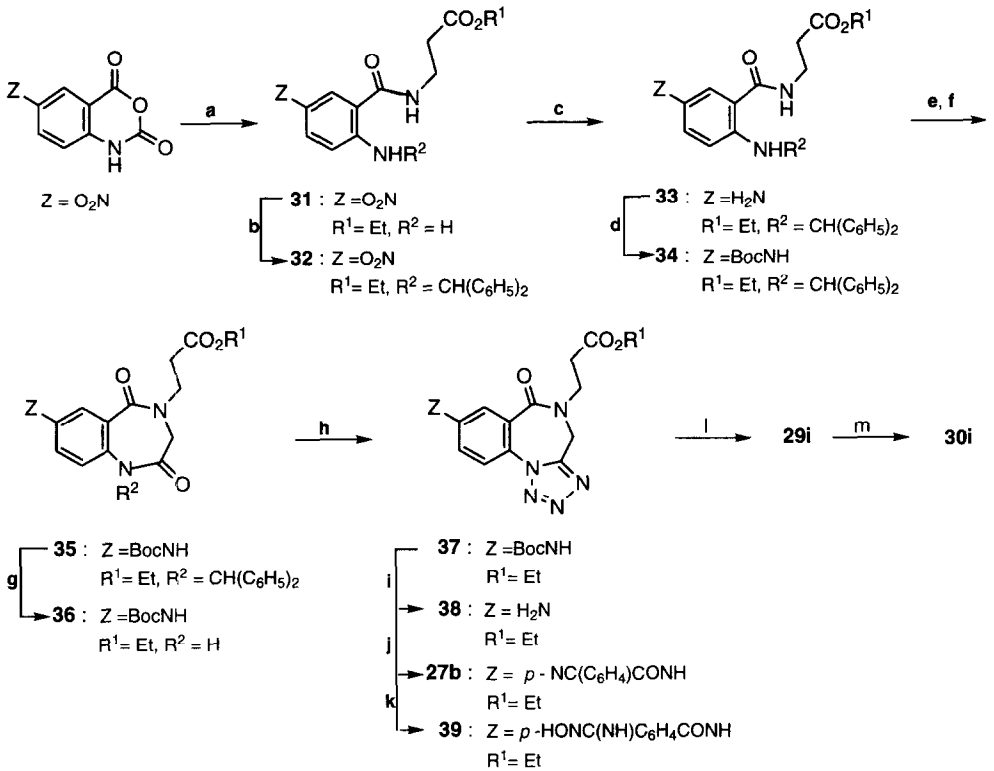
Figure 7. Synthesis of triazole tricyclic GPIIb/IIIa antagonists **30a** and **30b**.

Conclusion

A diverse group of novel tricyclic GPIIb/IIIa antagonists have been prepared and evaluated for potency. In general, replacement of the N^1–C^2 amide with a heterocycle to yield tricyclic GPIIb/IIIa antagonists **30a**, **30b**, **30d**, and **30f–j** resulted in retention of potency (i.e. less than a twofold decrease) relative to the comparative bicyclic progenitor. The exception was tricyclic imidazole **30c**, which exhibited a fourfold loss in potency relative to **1b**. The dramatic reduction of activity exhibited by the tetracyclic quinazoline **30e** suggests that a tricyclic scaffold may be optimal for steric reasons. For improved potency, the tricyclic molecule containing an amide linker and a tetrazole heterocycle (**30i**) was the most potent, exhibiting an $IC_{50} = 54$ nM in the platelet aggregation assay compared to, and $IC_{50} = 66$ nM for the analogous bicyclic BZDD (**1c**).

In addition, the effect on potency due to modification of the linker from C^7 of the benzodiazepinedione to the phenylamidine has been investigated. The linker can be modified to an ether (**1b**) and an amide (**1c**) with retention and increased anti-aggregatory potency, respectively, relative to the ethynyl linker (**1a**). For increasing potency, the amide linker to the phenylamidine is optimal.

Finally, due to the poor oral absorption of the active compounds described herein, prodrugs of the compounds with equal or increased potency in inhibiting platelet aggregation relative to **1a** have been prepared to increase oral bioavailability. The syntheses and in vivo



Scheme 7. Improved synthesis of tricyclic tetrazole **30i**. Reagents and conditions: (a) Cl $H_3NCH_2CH_2CO_2Et$, NEt_3, DMAP, CH_2Cl_2, rt, 39%; (b) 2,6-lutidine, K_2CO_3, $ClCH_2CH_2Cl$, Ph_2CHBr, 83 °C, 91%; (c) NEt_3, HCO_2H, 5% Pd–C (4% by wt), rt, 81%; (d) BOC-ON = $(CH_3)_3COCO_2N=C(C_6H_5)CN$, DMAP, THF, reflux, 90%; (e) $BrCH_2COBr$, KPhos/CH_2Cl_2, rt, 71%; (f) DBU, CH_2Cl_2, rt, 71% from **33**; (g) $Pd(OH)_2$, HOAc, 40 psi. 60 °C, 77%; (h) PPh_3, DEAD, $TMSiN_3$, rt, 57%; (i) TFA, CH_2Cl_2, $NaHCO_3$, 81%; (j) *p*-$CN(C_6H_4)COCl$, $NaHCO_3$, THF, 50 °C, 70%; (k) $H_2NOH(HCl)$, NaOEt, 60 °C, 79%; (l) Ac_2O, HOAc, 5% Pd–C (6% by wt), H_2, 1 atm, rt, 55%; (m) LiOH, THF/H_2O (3/1), rt, 65%.

evaluation of these tricyclic prodrugs will be reported in due course.

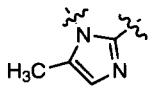
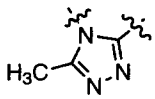
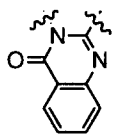
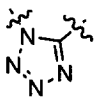
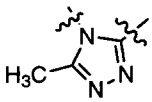
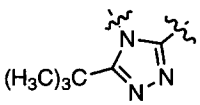
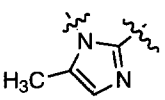
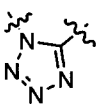
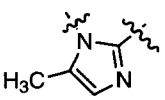
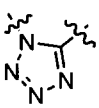
Experimental

General methods

Melting points were determined on a Laboratory Devices Mel-Temp II melting point apparatus and are uncorrected. All proton spectra except compounds **29e**, **30d**, **30e**, and **30g** were recorded on a Varian VXR-300S spectrometer at 293 K. Spectra for compounds **29e**, **30d**, **30e**, and **30g** were recorded on a Varian Unity Inova 400 NMR spectrometer at 293 K. All carbon NMR were recorded on a Varian VXR-300S spectrometer (75 MHz) at 293 K. NMR samples were prepared in either $CDCl_3$, CD_3OD, DMSO-d_6, CD_3COCD_3 or deuterium oxide (99.9% ^2H atoms) purchased from either Aldrich or Cambridge Isotope. Chemical shifts for ^1H NMR in $CDCl_3$, CD_3OD, and CD_3COCD_3 were measured relative to tetramethylsilane (TMS) at 0.0 ppm. Chemical shifts for ^1H NMR in DMSO-d_6 were measured relative to either tetramethylsilane (TMS) at 0.0 ppm when DMSO-d_6 containing 0.05% TMS (v/v) was used as the ^1H NMR solvent or a pentuplet at 2.49 ppm when DMSO-d_6 that did not contain TMS was used as the ^1H NMR solvent. ^1H NMR in D_2O were measured relative to HOD at 4.85 ppm. Chemical shifts for ^{13}C NMR used 77.0, 49.0, and 39.5 ppm as the internal lock for solvents $CDCl_3$, CD_3OD, and DMSO-d_6, respectively. The ^1H NMR assignments are described with the abbreviations s (singlet), d (doublet), t (triplet), q (quartet), p (pentuplet), m (multiplet), and br (broad). All coupling constants are reported in hertz.

Low-resolution mass spectra were obtained on either a PE Sciex API electrospray (ES) mass spectrometer or on a Jeol JMS-HX110HF/HX110HF tandem FAB MS/MS. High-resolution mass spectra were obtained on a Jeol JMS-HX110HF/HX110HF tandem FAB MS/MS instrument. C, H, and N analyses were conducted by Oneida Research Services, Whitesboro, NY. Elemental analyses observed within ±0.4% of calculated are

Table 4. Comparison of the biological activities of tricyclic (**30a–j**, Scheme 6 and Figure 7) with bicyclic (**1a–c**) GPIIb/IIIa antagonists

Compd	X-Y	Het	ELISA[a]	IC_{50} (nM) PA (huPRP, ADP)[b]
1b	CH_2O	none	**15**[c]	**91**[c]
30c	CH_2O		**20**	**357**
30d	CH_2O		**14.5**	**159**
30e	CH_2O		**62**	**10,000**[d]
30h	CH_2O		**13**	**140**[d]
1a	—≡—	none	**14.5**[e,f]	**120**[g]
30a	—≡—		**50**[e]	**191**
30b	—≡—		**50**[e]	**173**
30g	—≡—		**38**	**123**
30j	—≡—		**21**	**218**[d]
1c	CONH	none	**11**[h]	**66**[d,h]
30f	CONH		**12**	**86**
30i	CONH		**9**	**54**[d]

[a]ELISA = Enzyme Linked Immunofluorescent Assay. ELISA assay data are presented as an average of n = 2, unless otherwise noted. The coefficient of variance (% cv) was always ± 15% of the mean for the ELISA assay.

[b]PA = Platelet Aggregation. Platelet aggregation data is reported as n = 1, unless otherwise noted. The coefficient of variance (% cv) was ± 20% for the platelet aggregation assay.

[c]Previously reported IC_{50} (**1b**) ELISA = 5 nM (Ref. 16). PA data taken from Ref. 16.

[d]Average of n = 2.

[e]Average of n = 3.

[f]Previously reported IC_{50} (**1a**) ELISA = 11 nM (Refs. 15 and 42).

[g]PA data taken from Refs. 15, 16 and 42.

[h]Previously reported IC_{50} (**1c**) ELISA = 8 nM and IC_{50} (**1c**) PA 59 nM (see Ref. 16).

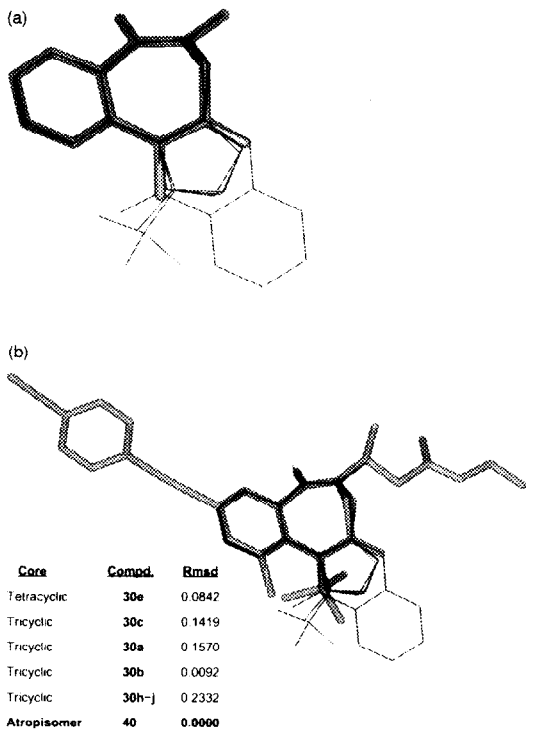


Figure 8. (A) Geometry optimized tetracyclic core of **30e**, tricyclic cores **30a–c** and **30h–j** overlaid with the geometry optimized bicyclic core of benzodiazepinediones **1a–c**. The tetracyclic and tricyclic cores are depicted in black and the bicyclic core is depicted in grey. (b) Geometry optimized tetracyclic core of **30e**, tricyclic cores **30a–c** and **30h–j** overlaid with the X-ray crystal structure of atropisomer 1-*tert*-butyl-4-(3(*S*)butanoic acid)-7-(cyanophenyl)-ethynyl]-9-chloro-3,4 dihydro-1*H*-1,4 benzodiazepine-2,5-dione, ethyl ester (**40**).[42] The tetracyclic and tricyclic cores are depicted in black and the atropisomer is depicted in grey.

included in the experimental details. All concentrations were performed on a Büchi rotary evaporator under reduced pressure.

Analytical thin-layer chromatography (TLC) was conducted on Whatman silica gel 60A M_{254} MK6F glass coated plates and visualized by UV and/or charring with either phosphomolybdic acid (20% wt in ethyl alcohol) or ninhydrin (0.2% solution in ethyl alcohol). Silica gel 60 (SiO_2, 230–400 mesh, E. Merck) was used for preparative (flash) column chromatography. Prepacked Lobar silica columns were used when MPLC was performed and the chromatography was monitored by both refractive index and UV detection (254 nM). Analytical high-pressure liquid chromatography was used to monitor reaction progress and to assess chemical purity of the compound (UV detection at 214 or 254 nM). The analytical HPLC methods are listed using the syntax: Method: column, solvent gradient, flow rate (mL/min), wavelength, time (min), (% CH_3CN). Analytical method **A1**: Microsorb Short-One 80-200-C3 C_{18}, 4.6×100 mm, 0–100% CH_3CN/H_2O (0.1% TFA), 1.5 mL/min, 254 nM, 0:00 (0%), 9:00 (100%), 9:10 (100%), 11:00 (0%). Analytical method **A2**: Dynamax 300AC8, 83-303-C5, 4.6×250 mm, 30–100% CH_3CN/H_2O (0.1% TFA), 1.5 mL/min, 214 nM, 0:00 (30%), 5:00 (37%), 10:00 (70%), 11:00 (70%), 11:30 (0%). Analytical method **A3**: Dynamax 300AC8, 83-303-C5, 4.6×250 mm, 0–50% CH_3CN/H_2O w/0.1% TFA, 1.5 mL/min, 214 nM, 0:00 (0%), 8:02 (50.5%), 10:50 (50%), 11:36 (0%), 12:00 (0%). Analytical method **A4**: Dynamax 300AC8, 83-303-C5, 4.6×250 mm, 0–50% CH_3CN/H_2O w/0.1% TFA, 1.5 mL/min, 214 nM, 0:00 (0%), 10:03 (50%), 14:27 (50%), 14:51 (50%), 15:00 (0%). Analytical method **A5**: Microsorb Short-One 80-200-C3 C_{18}, 4.6×100 mm, 0–100% CH_3CN/H_2O (0.1% TFA), 1.5 mL/min, 214 nM, 0:00 (0%), 9:00 (100%), 9:10 (100%), 11:00 (0%). Analytical method **A6**: Dynamax 300AC8, 83-303-C5, 4.6×250 mm, 30–100% CH_3CN/H_2O w/0.1% TFA, 1.5 mL/min, 214 nM, 0:00 (30%), 1:00 (40%), 2:00 (58%), 3:00 (70%), 4:00 (80%), 5:00 (90%), 6:00 (100%), 8:00 (100%). Analytical method **A7**: Microsorb C18 80-225-C5, 4.6×250 mm, 30% isocratic CH_3CN/H_2O w/0.1% TFA, 1.5 mL/min, 214 nM, 0:00 (0%), 0:42 (30%), 4:00 (30%), 11:57 (30%), 12:36 (60%), 13:24 (60%), 14:00 (30%), 15:00 (30%). Semipreparative and preparative high-pressure liquid chromatography were used to purify products which were not amenable to purification via preparative silica gel chromatography (UV detection at 214 or 254 nM). The semipreparative and preparative HPLC methods are listed using the syntax: Method: column, solvent gradient, flow rate (mL/min), wavelength, time (min), (% CH_3CN). Semipreparative HPLC method **SP1**: Microsorb 80-299-C5 C18, 10 mm (ID)×25 cm, 0–100% CH_3CN/H_2O (0.1% TFA) then 50% CH_3CN (0.1% TFA), 12 mL/min, 254 nM, 0:00 (0%), 10:00 (100%), 10:05 (50%), 40:05 (50%). Preparative HPLC method **P1**: Dynamax-60A 83-241-C C18, 41.4 mm (ID)×25 cm, 30–70% CH_3CN/H_2O (0.5% HOAc), 15 mL/min, 254 nM, 0:00 (30%), 10:00 (30%), 50:00 (70%). Preparative method **P2**: Dynamax-60A 83-241-C C18, 41.4 mm (ID)×25 cm, 25–60% CH_3CN/H_2O (0.5% HOAc), 15 mL/min, 254 nM, 0:00 (0%), 10:00 (25%), 50:00 (60%). Preparative method **P3**: Dynamax-60A 83-221-C C18, 21.4 mm (ID)×25 cm, 25–60% CH_3CN/H_2O (0.5% HOAc), 20 mL/min, 254 nM, 0:00 (0%), 10:00 (25%), 50:00 (60%). Preparative method **P4**: Dynamax-60A 83-221-C C18, 21.4 mm (ID)×25 cm, 5–90% MeOH/H_2O (0.1% TFA), 20 mL/min, 254 nM, 0:00 (5%), 15:00 (5%), 70:00 (90%). Preparative method **P5**: Dynamax-60A 83-221-C C18, 21.4 mm (ID)×25 cm, 0–24% MeOH/H_2O (0.1% TFA), 20 mL/min, 254 nM, 0:00 (0%), 20:00 (0%), 60:00 (24%). Preparative method **P6**: Dynamax-60A 83-221-C C18, 21.4 mm

(ID)×25 cm, 15–50% CH_3CN/H_2O (0.1% TFA), 20 mL/min, 254 nM, 0:00 (15%), 10:00 (20%), 50:00 (38%), 75:00 (50%). Preparative method **P7**: Dynamax-60A 83-221-C C18, 21.4 mm (ID)×25 cm, 0–50% CH_3CN/H_2O (0.1% TFA), 10 mL/min, 214 nM, 0:00 (0%), 12:00 (0%), 46:17 (50%), 66:51 (50%), 70:00 (0%), 72:00 (0%). Preparative method **P8**: Vydac C18 300 Å, 5×26 cm, 30–75% CH_3CN/H_2O (0.5% HOAc), 20 mL/min, 254 nM, 0:00–2:00 Load, 2:00 (30%), 42:00 (60%), 48:00 (75%), 58:00 (75%), 60:00 (30%). Preparative method **P9**: Dynamax-60A 83-241-C C18, 41.4 mm (ID)×25 cm, 20–70% CH_3CN/H_2O (0.5% HOAc), 20 mL/min, 254 nM, 0:00 (20%), 10:00 (20%), 60:00 (70%), 65:00 (70%), 70:00 (20%). Preparative method **P10**: Dynamax-60A 83-221-C C18, 21.4 mm (ID)×25 cm, 10–50% CH_3CN/H_2O (0.1% TFA), 10 mL/min, 254 nM, 0:00 (10%), 10:00 (10%), 20:00 (20%), 30:00 (30%), 40:00 (40%), 50:00 (50%), 55:00 (50%), 60:00 (10%).

Solvents and reagents were purchased from commercial sources and used as received. Anhydrous solvents were purchased from Aldrich (Sure Seal) and beta alanine ethyl ester hydrochloride was purchased from Sigma.

3-Formyl-4-nitrophenoxy *p*-tolunitrile (3). Commercially available 5-hydroxy-nitrobenzaldehyde (102.90 g, 0.616 mol), α-bromo-*p*-tolunitrile (126.75 g, 0.647 mol, 1.05 equiv.) were dissolved in DMF (anhyd., 500 mL) and treated at room temperature with anhydrous potassium carbonate (93.61 g, 0.677 mol, 1.05 equiv.). The reaction mixture was heated at 50 °C for 2.5 h after which time it was determined that ≥95% of **2** had been consumed. The solution was poured into a 2-L separatory funnel containing 1 L of water. Extraction with EtOAc (600 mL) yielded insoluble material partitioning into the organic layer. The aqueous layer was removed and the EtOAc layer washed with 1 N $NaHCO_3$ (1 L) and the insoluble material remained in the organic layer. Washing with water (1 L) resulted in an emulsion so solid sodium chloride (57 g) was added to effect separation of the layers. However, solids remained in the EtOAc layer. The EtOAc layer was diluted with additional EtOAc (approx. 1.5 L) and then acetone (approx. 1.5 L) until complete dissolution of the solids. The layers were separated; the organic layer dried (Na_2SO_4) and filtered. The aqueous layers were back extracted with EtOAc (600 mL) and the EtOAc dried (Na_2SO_4) and filtered. The organic extracts were combined and concentrated in vacuo to afford **3** (175.8 g, 104%) as a tan solid: mp 148–151 °C; TLC R_f = 0.76 (50% EtOAc/hexane, UV positive). ^{1}H NMR ($CDCl_3$, TMS) δ 10.48 (1H, s, **CHO**), 8.18 (1H, d, $^3J_{HH}$ = 9.0, C5 ArH), 7.72 (2H, d, $^3J_{HH}$ = 8.60, NC ArH), 7.55 (2H, d, $^3J_{HH}$ = 8.60, NC ArH), 7.41 (1H, d, $^4J_{HH}$ = 3.0, C2 ArH), 7.23 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 3.0, C6 ArH), 5.27 (2H, s, NCAr CH_2O). ^{13}C NMR ($CDCl_3$) 188.11, 162.34, 140.19, 134.23, 132.60, 127.67, 127.36, 119.32, 118.28, 113.99, 112.50, 69.79. LRMS (FAB, $M+H^+$) 283.07. HRMS (FAB) *m/z* calcd for $C_{15}H_{11}N_2O_4$, 283.0719; found: 283.0731. Anal. calcd for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.92. Found: C, 63.57; H, 3.49; N, 9.79.

3-Carboxy-4-nitrophenoxy *p*-tolunitrile (4). To a mixture of **3** (175.8 g, 0.623 mol), tetrabutylammonium bromide (20.08 g, 0.062 mol), water (12 mL), and pyridine (1 L) (the reaction mixture is heterogeneous at this point) was added potassium permanganate (78.75 g, 0.24 mol) over a five minute period. The reaction mixture exothermed and because of the temperature increase the reaction mixture became homogeneous. The reaction was monitored by TLC (50% EtOAc/hexane) and after 1 h was >50% complete. Additional water (12 mL) was added, the reaction stirred for 1 h, and by TLC, it did not appear that the oxidation was proceeding. The reaction mixture was quite viscous so the stirring bar was removed, replaced by a mechanical stirrer, mechanically stirred for another 1 h and checked by TLC (t = 3 h). It did not appear that the reaction had proceeded any further than at t = 1 h so to facilitate dissolution more pyridine (700 mL) was added and the reaction stirred overnight at room temperature. One half of the pyridine solution was transferred to a round-bottomed flask and the solvent removed in vacuo. The crude residue was diluted with EtOAc/acetone (500 mL of each) and washed with 10% sodium bisulfite (ca. 1 L). A thick black precipitate formed which was collected via vacuum filtration in a Büchner funnel. The aqueous layer was acidified to a pH = 1 with 1.0 N HCl and extracted with ethyl acetate (ca. 500 mL). The organic layer was checked by TLC, the desired acid **4** was present so the EtOAc extract was dried (Na_2SO_4), filtered, and concentrated. ^{1}H NMR confirmed the material in the EtOAc extract was the acid **4**. The black solid that had been collected via vacuum filtration was treated with 1 N HCl and extracted with ethyl acetate to give impure **4** as determined by TLC and ^{1}H NMR. The impure **4** could be further purified via treatment with 1.0 N NaOH, washing with EtOAc, reacidifying with 1.0 M HCl, and extracting with ethyl acetate to yield pure **4**. The initial EtOAc/acetone extract was concentrated in vacuo to give a mixture of unreacted **3** as well as the desired acid **4**. The other one half of the reaction mixture was treated with EtOAc/acetone (1/1, ca. 400 mL of each), washed with 1 N HCl, 10% sodium bisulfite, water, dried ($MgSO_4$), filtered into a flask containing the mixture of unreacted **3** and **4** isolated previously. Concentration in vacuo gave 120 g of crude material. This residue (120 g) was dissolved in pyridine (500 mL) and 50 mL was removed, transferred to another flask, diluted to 100 mL with additional pyridine and assuming 12 g of **3**, treated with tetrabutyl

ammonium bromide (1.37 g, 4.25 mmol, 0.1 equiv.) and potassium permanganate (5.38 g, 34 mmol, 0.8 equiv.). Water (1.0 mL) was added and the reaction mixture exothermed. TLC (50% EtOAc/hexane) after 15 min indicated complete consumption of **3** and clean transformation into the desired acid **4**. The remaining pyridine solution (450 mL) was treated with tetrabutyl ammonium bromide (13.7 g), potassium permanganate (53.8 g), and water (5.0 mL). The reaction mixture became very exothermic and began to reflux. A water bath was used to cool the reaction and the TLC after 10 min showed complete conversion of **3** into **4**. **Note—the order of addition of reagents is important in driving the oxidation to completion. It is optimal to add the water last.** The pyridine solution (100 mL) was combined with the 450-mL pyridine solution and concentrated in vacuo. The crude residue was dissolved in EtOAc/acetone (1/1, ca. 400 mL each), washed with 1.0 N HCl (approx. 800 mL), 10% sodium bisulfite (ca. 800 mL), water (ca. 1 L), brine (500 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. To further purify **4**, the crude product was dissolved in EtOAc (600 mL), washed with 1.0 N NaOH (500 mL), and the basic solution acidified with concd HCl (50 mL). The acidified solution was extracted with ethyl acetate (600 mL), the organic layer washed with water (500 mL), brine (500 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. This material was combined with **4** isolated from the workup of the first half of the reaction mixture to yield 82.8 g (45% total) of **4**, which contained a small amount of *p*-cyanobenzoic acid (ca. 25%).

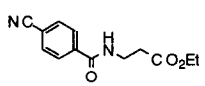
This potassium permanganate oxidation worked better on a small scale (0.0062 mol, 72% yield, ratio **4**/*p*-cyano benzoic acid, 8/1) and when scaled 100-fold (0.62 mol **3**), the reaction did not proceed to completion (ca. 50% conversion). As described, the unreacted nitro benzaldehyde could be recovered and resubjected to oxidation. In the scaleup, the ratio of **4**/*p*-cyano benzoic acid was ca. 3/1. The 3/1 mixture was typically taken on to the next reaction without further purification. ^{1}H NMR (DMSO-d_6, TMS) δ 8.08 (1H, d, $^3J_{HH}$ = 9.0, C5ArH), 7.90 (2H, d, $^3J_{HH}$ = 8.55, NC ArH), 7.68 (2H, d, $^3J_{HH}$ = 8.55, NC ArH), 7.35 (1H, d, $^4J_{HH}$ = 3.0, C2 ArH), 7.31 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 3.0, C6 ArH), 5.4 (2H, br s, NCAr**CH_2**O).

***N*-(5-(4-Cyanobenzyloxy)-2-nitrobenzoyl)-β-alanine ethyl ester (5).** To a vigorously stirring suspension of the **4**/*p*-cyano benzoic acid (82.8 g, 0.28 mol) mixture in benzene (anhyd., 500 mL) and DMF (10 mL) under an atmosphere of nitrogen was added oxalyl chloride (46.87 g, 32.2 mL, 0.369 mol, 1.33 equiv.) over 20 min. The reaction mixture exothermed and towards the end of the addition the solution was almost completely homogeneous. Immediately after completion of the addition of oxalyl chloride a precipitate formed. When the gas evolution ceased, the reaction mixture was heated to 65 °C (oil bath) for 15 min and the solvent was removed in vacuo. The residue was dissolved in THF (anhyd., 500 mL) and the THF solution added over 25 min to a vigorously stirred suspension of β-alanine ethyl ester hydrochloride (46.91 g, 0.305 mol, 1.1 equiv.) and sodium bicarbonate (116.61, 1.39 mol, 5.0 equiv.) in 2/1 (THF/H_2O, 1.5 L) at 0 °C. After completion of the addition of the acid chloride, the ice bath was removed, the reaction mixture allowed to warm to room temperature, and stirred at room temperature for 18 h at which time TLC (CH_2Cl_2/MeOH 96/4, UV positive) indicated complete conversion of **4** into **5**. The reaction mixture was transferred into a separatory funnel and the layers separated. The aqueous layer was extracted with EtOAc and the combined organic layers were concentrated in vacuo. The residue was dissolved in ethyl acetate (500 mL), washed with 1 N sodium bicarbonate (800 mL), 1 N $NaHSO_4$ (1 L), water (800 mL), and brine (600 mL). The aqueous layers were back extracted with ethyl acetate, the ethyl acetate extracts combined, dried ($MgSO_4$), filtered and concentrated in vacuo to yield 103.68 g (94% mass recovery) of a tan solid of which **5** was the major component. Typically, the crude product was used in the next reaction without additional purification. A portion of the tan solid (1.0 g) was subjected to preparative column chromatography (CH_2Cl_2/MeOH 96/4 eluent) to yield 800 mg of pure **5**: mp 115–116 °C (dec). TLC R_f = 0.70 (CH_2Cl_2/MeOH 96/4, UV positive); ^{1}H NMR ($CDCl_3$, TMS) δ 8.12 (1H, d, $^3J_{HH}$ = 9.5, C3 ArH), 7.72 (2H, d, $^3J_{HH}$ = 8.55, NC ArH), 7.53 (2H, $^3J_{HH}$ = 8.55, NC ArH), 7.03 (1H, dd, $^3J_{HH}$ = 8.0, $^4J_{HH}$ = 2.0, C4 ArH), 7.02 (1H, d, $^4J_{HH}$ = 2.0, C6 ArH), 6.42 (1H, br t, $^3J_{HH}$ = 6.0, CONH$CH_2CH_2CO_2$), 5.21 (2H, s, NCAr**CH_2**O), 4.16 (2H, q, $^3J_{HH}$ = 7.0, CO_2**CH_2**CH_3), 3.71 (2H, apparent q, $^3J_{HH}$ = 6.0, CONH**CH_2**CH_2CO_2), 2.72 (2H, t, $^3J_{HH}$ = 6.0, **CH_2**CO_2), 1.28 (3H, t, $^3J_{HH}$ = 7.0, CO_2CH_2**CH_3**); ^{13}C NMR ($CDCl_3$) 172.85, 166.43, 162.15, 140.37, 139.32, 135.57, 132.59, 127.66, 127.26, 118.32, 115.52, 114.63, 69.67, 60.86, 35.45, 33.12, 14.12; LRMS (FAB, M + H$^+$) 398.13; HRMS (FAB) *m/z* calcd for $C_{20}H_{20}N_3O_6$ 398.1352; found: 398.1342. Anal. calcd for $C_{20}H_{19}N_3O_6$ (0.25 H_2O): C, 59.77; H, 4.89; N, 10.45. Found: C, 59.75; H, 4.66; N, 10.34.

***N*-(5-(4-Cyanobenzyloxy)-2-aminobenzoyl)-β-alanine ethyl ester (6).** Compound **5** (103.68 g, 0.261 mol) was suspended in a mixture of ethyl acetate (1 L) and absolute ethanol (750 mL) and treated with tin chloride dihydrate (294.32 g, 1.30 mol, 5.0 equiv.). After completion of addition of the tin chloride, the reaction mixture became homogeneous. The solution was heated to 70 °C (oil bath) and stirred at 70 °C for 3.5 h at which time TLC (CH_2Cl_2/MeOH 96/4, UV positive) indicated

complete consumption of **5**. The reaction mixture was cooled to ca. 30 °C, then poured onto ice (2 L). The quenched solution was neutralized by slow addition of solid $NaHCO_3$ (ca. 125 g) until a pH of 7–8 was obtained. A thick precipitate of tin oxide formed which was collected in a Büchner funnel via vacuum filtration (very slow filtration). The filtrate was transferred into a separatory funnel and the layers separated. The organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo to give a white crystalline solid. The white paste of tin oxide in the Büchner funnel was washed with ethanol (again, very slow filtration), the ethanolic solution dried (Na_2SO_4), filtered, and concentrated. The resulting residue was contaminated with a thick, white inorganic material so the residue was redissolved in EtOAc, dried ($MgSO_4$), filtered into a flask containing **6** (the first crop of the white crystalline solid) and concentrated to yield 92.41 g (96% mass recovery) of material containing **6** as the major component. Typically, the white crystalline product was used in the next reaction without additional purification. A portion of the white solid (1.0 g) was crystallized from absolute ethanol and the crystals collected via vacuum filtration to yield 700 mg of pure **6**: mp 117–118 °C; TLC R_f = 0.59 ($CHCl_3$/MeOH 95/5, UV positive). ^{1}H NMR ($CDCl_3$, TMS) δ 7.65 (2H, d, $^3J_{HH}$ = 8.3, NC ArH), 7.51 (2H, d, $^3J_{HH}$ = 8.1, NC ArH), 6.96 (1H, d, $^4J_{HH}$ = 2.7, C6 ArH), 6.88 (1H, dd, $^3J_{HH}$ = 8.8, $^4J_{HH}$ = 2.7, C4 ArH), 6.85 (1H, br t, CONHCH$_2$), 6.44 (1H, d, $^3J_{HH}$ = 8.8, C3 ArH), 4.14 (2H, q, $^3J_{HH}$ = 7.1, $CO_2\mathbf{CH_2}CH_3$), 3.65 (2H, apparent q, $^3J_{HH}$ = 6.0, CONH$\mathbf{CH_2}CH_2CO_2$), 2.60 (2H, t, $^3J_{HH}$ = 6.0, $\mathbf{CH_2}CO_2$), 1.25 (3H, t, $^3J_{HH}$ = 7.1, $CO_2CH_2\mathbf{CH_3}$); ^{13}C NMR ($CDCl_3$) 172.66, 168.51, 149.52, 142.96, 142.55, 132.22, 127.52, 120.21, 118.60, 116.99, 113.54, 111.49, 69.90, 60.69, 34.98, 33.84, 14.07; LRMS (FAB, M^+) 367.15; LRMS (FAB, M^+H^+) 368.15; HRMS (FAB) *m/z* calcd for $C_{20}H_{21}N_3O_4$ 367.1532. Found: 367.1517; HRMS (FAB) *m/z* calcd for $C_{20}H_{22}N_3O_4$ 368.1610. Found: 368.1601.

The nitro compound **5** and the aniline **6** were contaminated with ca. 25% of *N*-ethyl-[carboxy ethyl]-4-cyanobenzamide



that was removed after the reaction of **6** with bromoacetylbromide. The dichloromethane layer after drying ($MgSO_4$) and concentration in vacuo gave a solid, which was triturated with ethanol to remove the *N*-ethyl-[carboxy ethyl]-4-cyanobenzamide and yield **7** of very high chemical purity. Recrystallization of **6** from EtOH also removed the *N*-ethyl-[carboxy ethyl]-4-cyanobenzamide impurity.

***N*-(5-(4-Cyanobenzyloxy)-2-(bromoacetyl)aminobenzoyl)-β-alanine ethyl ester (7).** Compound **6** (2.38 g, 6.5 mmol, 1.0 equiv.) was dissolved in dichloromethane (100 mL), cooled to 0 °C (ice/H_2O bath) and treated with water (30 mL). To the vigorously stirred cold (0 °C) biphasic solution was added over 10 min a solution of bromoacetyl bromide (1.44 g, 7.15 mmol, 1.1 equiv.) in dichloromethane (10 mL). Vigorous stirring was maintained for 30 min at which time the reaction was determined to be complete by TLC (CH_2Cl_2/MeOH, 96/4, R_f (**7**) = 0.76). The reaction mixture was transferred to a separatory funnel and partitioned between dilute aqueous sodium bicarbonate and methylene chloride. The organic phase was dried over sodium sulfate, filtered and concentrated to give 3.11 g (98%) of off-white crystals of **7**, which could be taken directly into the ring closure. Typically the crystalline solid was purified by trituration with ethanol or by recrystallization from either ethanol or ethyl acetate/hexane prior to subjecting to the ring closure: mp 173–174 °C; TLC R_f = 0.71 (CH_2Cl_2/MeOH 96/4, UV positive); ^{1}H NMR ($CDCl_3$, TMS) δ 8.41(1H, d, $^3J_{HH}$ = 8.5 C3 ArH), 7.70 (2H, d, $^3J_{HH}$ = 7.0, NC ArH), 7.50 (2H, d, $^3J_{HH}$ = 7.0, NC ArH), 7.02 (2H, d, $^3J_{HH}$ = 8.5 C4 ArH), 7.02 (1H, br s, C6 ArH), 6.98 (1H, br t, CO**NH**), 5.12 (2H, s, Ar$\mathbf{CH_2}$O), 4.16 (2H, q, $^3J_{HH}$ = 7.0, O$\mathbf{CH_2}$), 3.97 (2H, s, $\mathbf{CH_2}$Br), 3.68 (2H, q, $^3J_{HH}$ = 6.0 N$\mathbf{CH_2}CH_2CO_2$) 2.61 (2H, t, $^3J_{HH}$ = 6.0 $\mathbf{CH_2}CO_2$), 1.25 (3H, t, $^3J_{HH}$ = 7.0 OCH$_2\mathbf{CH_3}$); IR (KBr): 3389 (NH), 3355 (NH), 2226 (CN), 1735 (ester), 1675, 1642 (amides) 1609, 1523, 1423, 1244, 1184, 819; LRMS (FAB, M + H$^+$) 488.08 (98.5%), 489.08 (25.7%), 490.08 (100%), 491.08 (25.5%); HRMS (FAB) *m/z* calcd for $C_{22}H_{23}BrN_3O_5$ 490.0804 (100%); found. 490.0808: Anal. calcd for $C_{22}H_{22}BrN_3O_5$ (0.25 H_2O): C, 53.62; H, 4.60; N, 8.53. Found: C, 53.66; H, 4.43; N, 8.54.

7-[[4-Cyanophenyl]methoxy]-4-propanoic acid, 3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (8a). A mixture of **7** (3.11 g, 6.37 mmol, 1.0 equiv.), powdered potassium carbonate (1.32 g, 9.55 mmol, 1.5 equiv.) and DMF (anhyd., 350 mL) were combined and stirred at 50 °C for 2 h at which time TLC (70% EtOAc/hexane, R_f(**8a**) = 0.31, R_f(**7**) = 0.85) indicated complete reaction. The reaction mixture was partitioned between ethyl acetate and water, the organic phase washed twice with water, once with brine, dried (Na_2SO_4) and evaporated to yield a crystalline solid. The solid was slurried in ether/ethyl acetate and filtered to give 1.5 g of **8a**. The residual ether/ethyl acetate solution was concentrated and chromatographed (70% EtOAc/hexane) to recover an additional 0.5 g of **8a** (total 2.0 g of **8a**, 77%). In addition to the desired benzodiazepinedione **8a**, 0.5 g (19%) of a dimer (**8d**) was isolated from the chromatography of the mother liquor: (**8a**) mp 164–165 °C; TLC R_f = 0.31 (70% EtOAc/hexane); ^{1}H NMR (acetone-d_6, TMS) δ 9.32 (1H, s, **NH**), 7.84 (2H, d, $^3J_{HH}$ = 7.0, NC ArH), 7.78 (2H, d, $^3J_{HH}$ = 7.0, NC ArH), 7.41 (1H, d, $^3J_{HH}$ = 2.0, C6 ArH), 7.20 (1H, d, $^3J_{HH}$ = 7.0, C8 ArH), 7.15 (1H, d,

$^3J_{HH}$ = 7.0, C9 ArH), 5.24 (2H, s, Ar**CH$_2$**O), 4.10 (2H, q, $^3J_{HH}$ = 6.0, **OCH$_2$**), 3.98 (2H, s, **NCH$_2$**C=O), 3.88 (2H, t, $^3J_{HH}$ = 6.0, **NCH$_2$**CH$_2$CO$_2$) 3.77 (HOD), 2.66 (2H, t, $^3J_{HH}$ = 6.0 **CH$_2$**CO$_2$), 2.03 (acetone), 1.20 (3H, t, $^3J_{HH}$ = 6.0, OCH$_2$**CH$_3$**); IR (KBr) 3521 (NH), 2226 (CN), 1735 (ester), 1682, 1642, 1609, 1503, 1277, 1204, 819; HRMS (FAB) *m/z* calcd for $C_{22}H_{22}N_3O_5$, 408.1559; found: 408.1538; Anal. calcd for $C_{22}H_{21}N_3O_5$ (0.25 H_2O): C, 64.15; H, 5.26; N, 10.20. Found: C, 64.22; H, 5.21; N, 10.29.

TLC R_f (dimer, **8d**) = 0.23 (70% EtOAc/hexane); (**8d**) ^{1}H NMR (acetone-d_6, TMS) δ 11.60 (1H, s, **CONH**), 8.46 (1H, d, $^3J_{HH}$ = 10.0, ArH), 8.10 (1H, br t, **CONH**CH$_2$), 7.78 (3H, m, ArH), 7.64 (4H, m, ArH), 7.39 (1H, m, ArH), 7.38 (1H, br s, ArH), 7.30 (1H, s, ArH), 7.18 (3H, m, ArH), 5.60 (2H, s, N**CH$_2$**CO), 5.24 (2H, s, O**CH$_2$**), 5.20 (2H, O**CH$_2$**), 4.70 (1H, d, $^2J_{HH}$ = 15.0, N**CH**HC=O), 4.30 (1H, dd, $^3J_{HH}$ = 6.0, N**CH**HCH$_2$CO$_2$), 4.06 (4H, m, two overlapping quartets, $^3J_{HH}$ could not be determined, O**CH$_2$**CH$_3$), 3.90 (1H, d, $^2J_{HH}$ = 15.0, N**CH**HC=O), 3.80 (1H, m, NCH**H**CH$_2$CO$_2$), 3.50 (2H, apparent q, $^3J_{HH}$ = 6.0, CONH**CH$_2$**CH$_2$CO$_2$), 2.64 (2H, m, CONH CH$_2$**CH$_2$** CO$_2$ or NCH$_2$**CH$_2$**CO$_2$), 2.56 (2H, m, CONHCH$_2$**CH$_2$**CO$_2$ or NCH$_2$**CH$_2$**CO$_2$), 1.10 (6H, m, two overlapping triplets, $^3J_{HH}$ could not be determined, OCH$_2$**CH$_3$**).

1-Methyl-4-(2-carboxyethyl)-7-(4-cyanobenzyloxy)-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione (9). An oven dried 24/40 500-mL round-bottomed flask was cooled to room temperature under an atmosphere of argon and charged with **8a** (5.0 g, 12 mmol), DMF (anhyd., 30 mL, 0.4 M), cesium carbonate (7.82 g, 24 mmol, 2.0 equiv.) and iodomethane (5.11 g, 2.24 mL, 3.0 equiv.). The reaction mixture was stirred at room temperature under an atmosphere of argon for 7 h. TLC (70% EtOAc/hexane, 2×elute, UV positive) indicated complete consumption of **8a** to give a less polar new product. The reaction mixture was concentrated and the residue dissolved in ethyl acetate (300 mL). The ethyl acetate solution was transferred to a separatory funnel and washed once with water (200 mL). The aqueous layer was extracted additionally with ethyl acetate (3×100 mL), the ethyl acetate extracts combined and washed with satd $NaHCO_3$, brine, dried ($MgSO_4$), filtered and concentrated in vacuo to yield a viscous yellow syrup which was purified via preparative column chromatography (67% EtOAc/hexane, then 100% EtOAc gradient elute) to yield 3.88 g (75%) of a colorless syrup which solidified upon drying under high vacuum (1.0 mm Hg overnight): mp 97–99 °C; TLC R_f = 0.14 (2×elute, 70% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, TMS) δ 7.70 (2H, d, $^3J_{HH}$ = 8.3, NC ArH), 7.55 (2H, d, $^3J_{HH}$ = 7.8, NC ArH), 7.39 (1H, d, $^4J_{HH}$ = 0.9, C6 ArH), 7.13 (2H, apparent ABq, $^3J_{HH}$ = 5.0, C8 ArH, C9 ArH), 5.17 (2H, ABq, $^2J_{HH}$ = 8.0, $\delta\nu_{AB}$ = 7.4, Ar**CH$_2$**O), 4.15 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 4.04 (1H, d, $^2J_{HH}$ = 15.0, N**CH**HC=O), 3.94 (2H, m, N**CH$_2$**CH$_2$CO$_2$) 3.85 (1H, d, $^2J_{HH}$ = 15.0 NCHHC=O), 3.35 (3H, s, N**CH$_3$**), 2.70 (2H, m, **CH$_2$** CO$_2$), 1.23 (3H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) 171.17, 168.70, 166.64, 155.37, 141.61, 134.98, 132.41, 129.64, 127.54, 122.62, 119.99, 118.48, 114.61, 111.94, 69.22, 60.72, 52.19, 45.12, 34.92, 32.75, 14.11; HRMS (FAB) *m/z* calcd for $C_{23}H_{24}N_3O_5$ 422.1716, found: 422.1744; Anal. calcd for $C_{23}H_{23}N_3O_5$ (0.25 H_2O): C, 64.85; H, 5.56; N, 9.86. Found: C, 64.57; H, 5.30; N, 9.85.

1-Methyl-4-(2-carboxyethyl)-7-(4-amidinobenzyloxy)-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester trifluoroacetate (10). Compound **9** (83.6 mg, 0.198 mmol) was dissolved in 3 mL of pyridine/triethylamine (1/1) and hydrogen sulfide gas bubbled into the homogeneous solution for 15 min. The reaction mixture was warmed to 50 °C (oil bath) and the temperature maintained at 50 °C for 4 h. The solvent was evaporated, the residue dissolved in methylene chloride/iodomethane (5/1, total volume 6 mL), and warmed to 50 °C for 1 h. After cooling to room temperature, the solution was again concentrated, the crude S–Me imidate dissolved in methanol (3 mL), treated with ammonium acetate (0.5 g) and the reaction mixture warmed to 50 °C. After stirring at 50 °C for 12 h, the solvent was concentrated to a volume of 1.0 mL and the crude amidino ester purified by semipreparative reverse phase chromatography to yield 70 mg (65%) of **10** as a colorless amorphous solid after lyophilization from acetonitrile/water (0.1% TFA). RP HPLC (method **SP1**) t_R = 35.2 min; RP HPLC (method **A4**) t_R = 8.45 min (≥ 98.5% purity); RP HPLC (method **A7**) t_R = 5.55 min (> 99% purity); ^{1}H NMR (D_2O/acetone-d_6, 1/1) δ 7.85 (2H, d, $^3J_{HH}$ = 7.5, H_2NC=N ArH), 7.68 (2H, d, $^3J_{HH}$ = 7.5, H_2NC=N ArH), 7.40–7.25 (3H, m, C6 ArH, C8 ArH, and C9 ArH), 5.24 (2H, s, Ar**CH$_2$**O), 4.07 (1H, d, $^2J_{HH}$ = 15.1, N**CH**HC=O), 4.04 (1H, m, N**CH**HCH$_2$CO$_2$), 4.02 (2H, q, ^3J = 7.3, **OCH$_2$**), 3.82 (1H, d, $^2J_{HH}$ = 15.0, NCH**H**C=O), 3.71 (1H, ddd, $^2J_{HH}$ = 14.2, $^3J_{HH}$ = 5.9, N**CH**H CH$_2$CO$_2$), 3.29 (3H, s, N**CH$_3$**), 2.65 (2H, m, **CH$_2$**CO$_2$), 1.11 (3H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**); HRMS (FAB) *m/z* calcd for $C_{23}H_{27}N_4O_5$, 439.1981; found: 422.1977.

1-Methyl-4-(2-carboxyethyl)-7-(4-amidinobenzyloxy)-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione trifluoroacetate (1b). The amidino ester **10** (37.3 mg, 0.067 mmol) was suspended in tetrahydrofuran (1.0 mL), cooled to 0 °C, and treated with 1 N aqueous sodium hydroxide (0.28 mL, 4.2 equiv.). The reaction mixture was allowed to warm to room temperature over a period of 1 h, acidified with glacial acetic acid (5 mL), concentrated to dryness, redissolved in 20% acetonitrile/water containing 0.1% TFA (1.0 mL) and purified by semipreparative reverse phase chromatography to yield

26.6 mg (75%) of **1b** as a colorless amorphous solid after lyophilization from acetonitrile/water (0.1% TFA): RP HPLC (method **SP1**) $t_R = 32.4$ min; RP HPLC (method **A4**) $t_R = 7.10$ min (≥ 99.0% purity); RP HPLC (method **A7**) $t_R = 4.45$ min (≥99% purity); ^{1}H NMR (D_2O/acetone-d_6, 1/1) δ 7.75 (2H, d, $^3J_{HH} = 8.3$, $H_2NC=NH$ ArH), 7.60 (2H, d, $^3J_{HH} = 8.3$, $H_2NC=NH$ ArH), 7.32–7.18 (3H, m, C6 ArH, C8 ArH, and C9 ArH), 5.16 (2H, s, Ar**CH$_2$**O), 4.04 (1H, ddd, $^2J_{HH} = 14.2$, $^3J_{HH} = 7.3$, NC**H**HCH_2CO_2), 4.01 (1H, d, $^2J_{HH} = 15.1$, NC**H**H$C=O$), 3.77 (1H, d, $^2J_{HH} = 15.1$ NC**H**H$C=O$), 3.67 (1H, ddd, $^2J_{HH} = 14.2$, $^3J_{HH} = 5.8$, NC**H**H CH_2CO_2), 3.20 (3H, s, N**CH$_3$**), 2.59 (2H, m, **CH$_2$**CO_2); HRMS (FAB) m/z calcd for $C_{21}H_{23}N_4O_5$, 411.1668; found: 411.1701.

1-Methyl-4-(2-carboxyethyl)-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (11). β-Alanine ethyl ester hydrochloride (100 g, 0.65 mol, 1.15 equiv.) was placed in a 3-L three-neck round-bottom flask equipped with an overhead stirrer, a thermometer, and a solid addition funnel. Dichloromethane (anhyd., 800 mL) was added followed by triethylamine (91 mL, 0.65 mol, 1.15 equiv.) and the reaction placed under a nitrogen atmosphere. *N*-methyl isatoic anhydride (100 g, 0.565 mol, >98% purity, TCI)[36a] was added to the suspension as a solid over a period of 1 h using a powder addition funnel. A slight exotherm was noted and the rate of addition adjusted to keep the reaction temperature below reflux. After addition of *N*-methyl isatoic anhydride was complete, the reaction mixture was allowed to cool to room temperature and stirred at room temperature for 1.5 h, at which time TLC (50% EtOAc/hexane) indicated a small amount of *N*-methyl isatoic anhydride still present. The reaction mixture was allowed to stir an additional 14 h at room temperature at which time TLC indicated complete conversion to a single, higher R_f spot ($R_f = 0.85$, 50% EtOAc/hexane, UV positive). The reaction was poured into a 2-L separatory funnel and washed once with water (100 mL). The dichloromethane layer was separated, dried over anhydrous sodium sulfate and poured back into the 3-L three-necked flask. Potassium phosphate buffer (1500 mL, 1 M, pH 7)[36b] was added and the biphasic mixture cooled to 5 °C using an ice bath. Bromoacetyl bromide (65 mL, 0.75 mol, 1.33 equiv.) was added dropwise to the vigorously stirring mixture over 15 min. The reaction was allowed to stir for 45 min and determined to be complete at that time by TLC (product $R_f = 0.15$, 50% EtOAc/hexane, UV positive; Note—if the reaction is not complete, additional bromoacetyl bromide should be added to push the reaction to completion. However, the pH of the aqueous phase may need to be adjusted to maintain a pH 7 if additional bromoacetylbromide is added). The reaction mixture was poured into a separatory funnel and the aqueous layer removed. The organic layer was dried (Na_2SO_4), poured back into a 3-L three-necked round-bottom flask and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU, 95 mL, 0.64 mol, 1.13 equiv.) was added dropwise to the stirred reaction at such a rate as to keep the reaction below reflux temperature (32–35 °C). The reaction was allowed to stir at room temperature for 1 h at which time TLC (dichloromethane/acetone, 90/10) indicated complete conversion to a slightly higher R_f product ($R_f = 0.5$, 90/10 dichloromethane/acetone; it is necessary to spot the TLC plate lightly (dilute sample) in order to see an R_f difference versus the bromoacetanilide). The reaction mixture was poured into a large separatory funnel and the organic layer washed twice with water (ca. 50 mL), once with 1 N citric acid (100 mL), and twice with 1 N HCl (100 mL). The combined aqueous layers were back extracted once with dichloromethane (100 mL), the combined organic layers dried (Na_2SO_4), filtered, and concentrated to a residue which solidified upon standing to yield 152 g (93%) of **11** as a light tan solid. The crude product from the three-step process was determined by ^{1}H NMR, HPLC and GC to be >90% pure and could be used in the next step without additional purification. The crude solid could be further purified by recrystallization from hexanes/ethyl acetate at −20 °C: mp (recrystallized **11**) 118–121 °C; RP HPLC (method **A5**, t_R (**11**, not recrystallized) = 4.62 min (90% purity); ^{1}H NMR ($CDCl_3$, TMS) δ 7.86 (1H, dd, $^4J_{HH} = 1.5$, $^3J_{HH} = 8.0$ ArH), 7.52 (1H, dt, $^4J_{HH} = 1.5$, $^3J_{HH} = 6.0$ ArH), 7.29 (1H, $^3J_{HH} = 6.0$, ArH), 7.18 (1H, d, $^3J_{HH} = 9.0$, ArH), 4.15 (2H, q, $^3J_{HH} = 9.0$, **OCH$_2$**), 4.05 (1H, d, ABq, $^2J_{HH} = 15.0$, NC**H**H$C=O$), 3.92 (2H, t, $^3J_{HH} = 6.0$, N**CH$_2$**CH_2CO_2), 3.86 (1H, d, ABq, $^2J_{HH} = 15.0$, NC**H**H$C=O$), 3.39 (s, 3H, N**CH$_3$**), 2.72 (2H, m, complex, **CH$_2$**CO_2), 1.26 (3H, t, $^3J_{HH} = 6.0$, OCH_2**CH$_3$**); ^{13}C NMR ($CDCl_3$) 171.10, 168.86, 166.96, 140.81, 131.96, 130.62, 128.46, 125.44, 120.75, 60.57, 51.99, 44.88, 34.71, 32.69, 13.99; LRMS (FAB, $M+H^+$) 291; Anal. calcd for $C_{15}H_{18}N_2O_4 \cdot 0.25\ H_2O$: C, 61.11; H, 6.32; N, 9.50. Found: C, 61.00; H, 6.12; N, 9.59.

1-Methyl-4-(2-carboxyethyl)-7-nitro-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (12). Fuming nitric acid (200 mL) was cooled to 0 °C in an ice-water bath and treated portionwise with **11** (4×10.75 g = 43 g, 0.148 mol). After dissolution of the solids, the 24/40 round-bottomed 500-mL flask was stoppered and the homogeneous dark-yellow reaction mixture gradually warmed to room temperature. After stirring at room temperature for 18 h, the nitric acid solution was carefully poured into a 2-L beaker containing ice (ca. 700 mL) and solid $NaHCO_3$. After the effervescence subsided, an equiv. volume of ethyl acetate (700 mL) was added and the quenched reaction mixture transferred to a 2-L separatory funnel. The layers were separated and the aqueous layer extracted additionally with ethyl acetate (3×400 mL). The combined organic layers were washed

once with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to yield 36 g of an orange oil (73%): TLC (R_f = 0.53, 80% EtOAc/hexane eluent, UV positive); RP HPLC (method **A4**) t_R = 7.19 min (96% purity); ^{1}H NMR ($CDCl_3$, TMS) δ 8.75 (1H, d, $^4J_{HH}$ = 3.0, C6 ArH), 8.35 (1H, dd, $^4J_{HH}$ = 3.0, $^3J_{HH}$ = 9.0, C8 ArH), 7.34 (1H, d, $^3J_{HH}$ = 9.0, C9 ArH), 4.15 (2H, q, $^3J_{HH}$ = 7.0, **OCH_2**), 4.03 (2H, br s, **NCH_2C**=O), 3.95 (2H, m, **$NCH_2CH_2CO_2$**), 3.44 (3H, s, **NCH_3**), 2.77 (1H, m, NCH_2**CH**HCO_2), 2.70 (1H, m, NCH_2CH **H**CO_2), 1.26 (3H, t, $^3J_{HH}$ = 7.0, OCH_2**CH_3**); ^{13}C NMR ($CDCl_3$) δ 194.51, 191.54, 188.52, 168.96, 167.69, 152.58, 150.31, 150.05, 145.12, 84.22, 75.21, 68.61, 58.39, 55.97, 37.44; LRMS (FAB, $M+H^+$) 336.0; HRMS (FAB) *m/z* calcd for $C_{15}H_{17}N_3O_6$, 336.1196; found: 336.1197.

1-Methyl-4-(2-carboxyethyl)-7-amino-3,4-dihydro-1*H*-1, 4-benzodiazepine-2,5-dione ethyl ester (13). A 1-L three-necked round-bottomed flask equipped with an overhead stirrer, a thermometer, and a dropping funnel, was charged with **12** (59.0 g, 0.176 mol), acetonitrile (anhyd., 200 mL) and triethylamine (103 mL, 0.748 mol, 4.25 equiv.). The reaction mixture was placed under an atmosphere of nitrogen, cooled to 5 °C (ice/H_2O bath) and a suspension of 5% Pd–C (2.5 g) in acetonitrile (anhyd., 20 mL) added. A solution of formic acid (28 mL, 0.748 mol, 4.25 equiv.) in acetonitrile (anhyd., 50 mL) was added via dropping funnel to the reaction mixture over a period of 20 min. The reaction temperature rose to 10 °C and a white fume was observed. After the addition of the formic acid was complete, the ice bath was removed and the dropping funnel was replaced by a reflux condenser. The reaction was brought to reflux over a period of 45 min and refluxed with stirring for one hour. TLC (80/20, dichloromethane/acetone) indicated incomplete reaction (less than 50%) so the reaction mixture was allowed to cool to 55 °C, charged with an additional suspension of 5% Pd–C (1.0 g) in acetonitrile (anhyd., 10 mL), and again heated to reflux. After refluxing additionally for 2 h, TLC indicated complete conversion **12** into a single new product (R_f (**13**) = 0.4, 80/20, dichloromethane/acetone, UV positive). The reaction mixture was allowed to cool to room temperature, filtered through Celite to remove the catalyst, and the filtrate concd. The resulting residue was dissolved in a small amount of 80/20 dichloromethane/acetone, filtered through a plug of silica gel (4×20 cm, 80/20 dichloromethane/acetone with 1% triethylamine eluent) and the solvent removed in vacuo to yield 50.3 g of a yellow solid (88%). The crude product was greater than 90% pure by analytical RP HPLC and could be used directly in the next reaction. A portion of the solid (25 g) was further purified by dissolution in refluxing toluene (250 mL) and allowing the toluene to cool to room temperature overnight. The crystals were isolated via filtration, washed once with cold toluene, and dried in a vacuum oven at 50 °C to yield 16.2 g of **13** (65% recovery): mp 116–118 °C; RP HPLC (method **A5**) t_R = 3.54 min (85% purity); ^{1}H NMR ($CDCl_3$, TMS) δ 7.09 (1H, d, $^4J_{HH}$ = 3.0, C6 ArH), 6.98 (1H, d, $^3J_{HH}$ = 8.5, C9 ArH), 6.81 (1H, dd, $^3J_{HH}$ = 8.5, $^4J_{HH}$ = 3.0, C8 ArH), 4.15 (2H, q, $^3J_{HH}$ = 7.0, **OCH_2**), 4.05 (1H, d, $^2J_{HH}$ = 14.0, **N**$CHHC$=O), 3.92 (2H, t, $^3J_{HH}$ = 7.0, **$NCH_2CH_2CO_2$**), 3.83 (2H, br s, **$ArNH_2$**), 3.79 (1H, d, $^2J_{HH}$ = 14.0, **N**$CHHC$=O), 3.32 (3H, s, **NCH_3**), 2.71 (2H, m, **CH_2CO_2**), 1.26 (3H, t, $^3J_{HH}$ = 7.0, OCH_2**CH_3**); ^{13}C NMR ($CDCl_3$) δ 171.13, 168.71, 167.04, 144.29, 132.02, 129.25, 122.07, 118.48, 115.15, 60.54, 52.12, 44.83, 34.71, 32.66, 13.98; LRMS (FAB, M^+) 305.14, LRMS (FAB, $M+H^+$) 306.14. HRMS (FAB) *m/z* calcd for $C_{15}H_{20}N_3O_4$, 306.1454; found: 306.1445. Anal. calcd for $C_{15}H_{19}N_3O_4$: C, 59.01; H, 6.27; N, 13.76. Found: C, 58.84; H, 6.40; N, 13.76.

1-Methyl-4-(2-carboxyethyl)-7-(4-cyano)benzamido-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (14). The aniline **13** (32.3 g, 0.105 mol) was dissolved in dichloromethane (anhyd., 250 mL), cooled to 0 °C (ice bath), treated with triethylamine (17.5 mL, 0.126 mol, 1.2 equiv.) and *p*-cyanobenzoyl chloride (20.86 g, 0.126 mol, 1.2 equiv.). The resulting mixture was stirred under nitrogen at room temperature for 24 h, transferred to a 500-mL separatory funnel, washed with water, brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to yield a light brown solid which was purified by recrystallization from dichloromethane/hexane to yield 33 g (76%) of a white granular solid: mp 158–160 °C; ^{1}H NMR ($CDCl_3$, TMS) δ 8.76 (1H, s, **CONH**), 8.0 (1H, dd, $^4J_{HH}$ = 3.0, $^3J_{HH}$ = 9.0, C8 ArH), 7.72 (2H, d, $^3J_{HH}$ = 9.0, NC ArH), 7.58 (1H, d, $^4J_{HH}$ = 3.0, C6 ArH), 7.47 (2H, d, $^3J_{HH}$ = 9.0, NC ArH), 6.94 (1H, d, $^3J_{HH}$ = 9.0, C9 ArH), 3.83 (2H, q, $^3J_{HH}$ = 6.0, **OCH_2**), 3.77 (1H, d, $^2J_{HH}$ = 15.0, **N**$CHHC$=O), 3.57 (1H, d, $^2J_{HH}$ = 15.0, **N**$CHHC$=O), 3.53 (2H, t, $^3J_{HH}$ = 6.0, **$NCH_2CH_2CO_2$**), 3.09 (3H, s, **NCH_3**), 2.34 (2H, m, **CH_2CO_2**), 0.95 (3H, t, $^3J_{HH}$ = 7.0, OCH_2**CH_3**); ^{13}C NMR ($CDCl_3$) δ 171.04, 168.57, 166.90, 164.31, 138.24, 137.34, 135.51, 132.47, 128.60, 128.05, 124.64, 121.85, 117.83, 115.51, 60.94, 52.09, 45.01, 34.92, 32.60, 14.13; LRMS (FAB, $M+H^+$) 435.17; HRMS (FAB) *m/z* calcd for $C_{23}H_{23}N_4O_5$, 435.1668; found: 435.1684.

1-Methyl-4-(2-carboxyethyl)-7-(4-amidino)benzamido-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester trifluoroacetate (15). The benzonitrile ethyl ester (**14**, 10.0 g, 0.023 mol) was dissolved in triethylamine (50 mL)/pyridine (70 mL), saturated with H_2S (perform in an efficient fume hood) and heated at 70 °C for 24 h. The triethylamine and pyridine was removed in vacuo, the residue suspended in dichloromethane (200 mL) and treated with iodomethane (10 mL). The flask was equipped with a reflux condenser and the solution heated

to reflux. After refluxing for 24 h, the reaction mixture was cooled to room temperature, the volatiles removed in vacuo, the residue dissolved in absolute ethanol (100 mL) and treated with ammonium acetate (3.2 g). The resulting suspension was heated to 50 °C with stirring for 24 h, concentrated to a residue which was dissolved in 30% acetonitrile/water containing 0.5% HOAc and purified by preparative RP HPLC to yield 2.45 g (23.5%) of the desired amidino ester (**15**) as a white powder after lyophilization from acetonitrile/water (0.5% HOAc). A portion of the acetate salt (100 mg) was converted to the more soluble trifluoroacetate salt by dissolution in a 50/50 solution of H_2O/TFA and concentrating in vacuo (2×). The resulting TFA salt was then dissolved in 10% acetonitrile/H_2O (0.1% TFA) and lyophilized to give **15** (TFA salt form, 100 mg) as a white powder: RP HPLC (method **P8**) t_R (**15**, HOAc salt form) = 26.9 min; RP HPLC (method **A5**) t_R (**15**, TFA salt) = 6.91 min (≥98.0% purity); RP HPLC (method **A7**) t_R (**15**, TFA salt) = 6.53 min (≥98% purity); ^{1}H NMR (D_2O, TFA salt form) δ 7.72 (2H, d, $^3J_{HH}$ = 12.0, H_2NC=NH ArH), 7.65 (2H, d, $^3J_{HH}$ = 12.0, H_2NC=NH ArH), 7.63 (1H, d, $^4J_{HH}$ = 3.0, C6 ArH), 7.53 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 3.0, C8 ArH), 7.12 (1H, d, $^3J_{HH}$ = 12.0, C9 ArH), 4.40 (1H, d, $^2J_{HH}$ = 15.0, NCHHC=O), 4.40 (1H, m, NCHH CH_2CO_2), 3.91 (2H, q, $^3J_{HH}$ = 6.0, **OCH_2**), 3.68 (1H, d, $^2J_{HH}$ = 15.0, NCHHC=O), 3.48 (1H, m, NCHHCH_2 CO_2), 3.18 (3H, s, **NCH_3**), 2.54 (2H, m, **CH_2CO_2**), 0.98 (3H, t, $^3J_{HH}$ = 6.0, OCH_2 **CH_3**); LRMS (FAB, M + H$^+$) 452.20; HRMS (FAB) *m/z* calcd for $C_{23}H_{26}N_5O_5$, 452.1934; found: 452.1956.

1-Methyl-4-(2-carboxyethyl)-7-(4-amidino)benzamido-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione acetate (1c). Amidino ester **15** (0.2 g, 0.456 mmol) was suspended in THF (20 mL) and treated with 50% aqueous NaOH (3.0 mL). The reaction mixture was stirred at room temperature for 24 h, quenched by the addition of glacial acetic acid, concentrated in vacuo, and residue purified by preparative RP HPLC to yield 122 mg (57%) of **1c** as a white foam after lyophilization from CH_3CN/H_2O (0.5% HOAc): RP HPLC (method **P8**) t_R = 21.0 min; RP HPLC (method **A4**) t_R = 6.29 min (≥99% purity); RP HPLC (method **A7**) t_R = 5.57 min (≥99% purity); ^{1}H NMR (D_2O) δ 7.73 (2H, d, $^3J_{HH}$ = 6.0, H_2NC=NH ArH), 7.63 (2H, d, $^3J_{HH}$ = 6.0, H_2NC=NH ArH), 7.62 (1H, m, C6 ArH), 7.54 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 3.0, C8 ArH), 7.12 (1H, d, $^3J_{HH}$ = 9.0, C9 ArH), 3.87 (1H, d, $^2J_{HH}$ = 15.0, NCHHC=O), 3.84 (1H, m, NCHHCH_2 CO_2), 3.20 (1H, d, $^2J_{HH}$ = 15.0, NCHHC=O), 3.48 (1H, m, NCHHCH_2CO_2), 3.03 (3H, s, **NCH_3**), 2.50 (2H, m, **CH_2CO_2**); HRMS (FAB) *m/z* calcd for $C_{21}H_{22}N_5O_5$, 424.1620; found: 424.1647.

***N*-(2-Diphenylmethylamino-5-iodobenzoyl)-β-alanine ethyl ester (18).**[16] Compound **17**[15] (1.1 g, 3.1 mmol) was dissolved in dimethylformamide (anhyd., 10.0 mL, 0.31 M), treated with 2,6-lutidine (0.48 mL, 4.1 mmol, 1.32 equiv.), chlorodiphenylmethane (1.2 g, 4.7 mmol, 1.52 equiv.) and heated to 50 °C for 1 h. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. The resulting oil was dissolved in dichloromethane (35 mL), transferred to a 125 mL separatory funnel, washed with 10% citric acid (2×50 mL), water, dried (Na_2SO_4), filtered, and concentrated. The resulting oil was purified by preparative column chromatography (10% EtOAc/hexane to 25% EtOAc/hexane gradient elute) to yield 0.92 g (56%) of **18**. TLC R_f = 0.82 (50% EtOAc/hexane, UV positive). ^{1}H NMR ($CDCl_3$ TMS) δ 8.40 (1H, d, $^3J_{HH}$ = 5.0, **NHCH**Ar_2), 7.56 (1H, d, $^4J_{HH}$ = 2.0, C6 ArH), 7.36–7.18 (11H, m, $^4J_{HH}$ = 2.0, CH(**C_6H_5**)$_2$ and C4 ArH), 6.73 (1H, t, $^3J_{HH}$ = 6.0, CONHCH$_2$), 6.29 (1H, d, $^3J_{HH}$ = 9.0, C3 ArH), 5.52 (1H, d, $^3J_{HH}$ = 5.0, NH**CH**Ar_2), 4.15 (2H, q, $^3J_{HH}$ = 7.0, **OCH_2**), 3.61 (2H apparent q, $^2J_{HH}$ = $^3J_{HH}$ = 6.0, **$CH_2CH_2CO_2$**), 2.58 (2H, t, $^3J_{HH}$ = 6.0, **CH_2** CO_2), 1.25 (3H, t, $^3J_{HH}$ = 6.0, OCH_2**CH_3**).

1-(Diphenylmethyl)-4-(2-carboxyethyl)-7-iodo-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (19). The aniline **18** (0.93 g, 1.8 mmol) was dissolved in dichloromethane (15 mL) and treated with water (15 mL). With vigorous stirring, bromoacetylbromide (424 mg, 0.183 mL, 2.1 mmol, 1.17 equiv.) was added to the biphasic solution, the reaction mixture stirred overnight at room temperature and transferred to a separatory funnel. The layers were separated and the aqueous layer extracted additionally with dichloromethane (2×15 mL). The dichloromethane layers were combined, washed with satd sodium bicarbonate (1×20 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The resulting residue was dissolved in dimethylformamide (anhyd., 7.0 mL) and added via an addition funnel to a slurry of sodium hydride (95%, 57 mg, 2.1 mmol, 1.17 equiv.) in dimethylformamide (anhyd., 5 mL) that had been cooled to 0 °C (ice/H_2O bath). After stirring for 2 h at 0–10 °C, the reaction was quenched by pouring into a flask of cold 10% citric acid (5 °C, 40 mL) and extracted with dichloromethane (3×40 mL). The combined dichloromethane layers were washed with 10% citric acid (2×50 mL), water (1×50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo to yield a viscous syrup which was purified by preparative column chromatography (50% EtOAc/hexane eluent) to yield 474 mg (48%) of **19**: mp 131–132 °C; TLC R_f = 0.61 (50% EtOAc/hexane, UV positive); ^{1}H NMR ($CDCl_3$, TMS) δ 8.03 (1H, d, $^4J_{HH}$ = 2.0, C6 ArH), 7.45 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 2.0, C8 ArH), 7.4–7.1 (10H, m, CH (**C_6H_5**)$_2$), 6.79 (1H, d, $^3J_{HH}$ = 9.0, C9 ArH), 6.70 (1H, s, ArN (**CH**Ar_2)CO), 4.15 (d, 1H, $^2J_{HH}$ = 15.6, **NCH** HC=O), 4.13 (2H, q, $^3J_{HH}$ = 7.0, **OCH_2**), 3.94 (1H, m, $^3J_{HH}$ = 6.2, $^3J_{HH}$ = 8.0, NCHHCH$_2$CO$_2$), 3.82 (2H, 1H,

d, $^2J_{HH}$=15.0, NCHHC=O and 1H, m, NCHH CH_2CO_2), 2.55 (2H, m, $^3J_{HH}$=4.0, $^3J_{HH}$=8.0, **CH_2** CO_2), 1.27 (3H, t, $^3J_{HH}$=7.0, OCH_2**CH_3**); ^{13}C NMR ($CDCl_3$) δ 171.2, 168.0, 165.6, 139.9, 139.3, 138.8, 138.1, 137.5, 132.0, 129.1, 128.5, 128.4, 128.0, 127.9, 127.6, 125.4, 90.6, 67.2, 60.8, 53.1, 45.0, 32.6, 14.2; LRMS (FAB, M+H$^+$) 569.10; HRMS (FAB) *m/z* calcd for $C_{27}H_{26}IN_2O_4$, 569.0937; found: 569.0959. Anal. calcd for $C_{27}H_{25}IN_2O_4$: C, 57.05; H, 4.43; N, 4.93; I, 22.23. Found: C, 57.12; H, 4.53; N, 4.95; I, 21.88.

4-(2-Carboxyethyl)-7-iodo-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (8b). A slurry of **19**[16] (15 g, 0.0264 mol), 15 mL anisole, and 3 mL ethyl methyl sulfide in a Teflon tube was cooled to −196 °C (N_2) and hydrogen fluoride (70 mL) condensed into the Teflon tube. The reaction was stirred for 2 h and concd in vacuo. The resulting residue was purified via preparative column chromatography (30% ethyl acetate/hexane to 80% ethyl acetate/hexane gradient elute) followed by crystallization using a minimum volume of ethyl acetate (heating was required to dissolve the material) and addition of an equal volume of hexanes to yield 8.5 grams (80%) of **8b**: mp 118–120 °C; ^{1}H NMR ($CDCl_3$, TMS) δ 9.60 (1H, s, NH), 8.21 (1H, d, $^4J_{HH}$=2.0, C6 ArH), 7.72 (1H, dd, $^4J_{HH}$=2.0, $^3J_{HH}$=9.0, C8 ArH), 6.81 (1H, d, $^3J_{HH}$=9.0, C9 ArH), 4.12 (2H, q, $^3J_{HH}$=7.0, **OCH_2**), 3.97 (2H, s, **NCH_2**C=O), 3.91 (2H, t, $^3J_{HH}$=7.0, **$NCH_2CH_2CO_2$**), 2.72 (2H, t, $^3J_{HH}$=7.0, **CH_2CO_2**), 1.23 (3H, t, $^3J_{HH}$=7.0, OCH_2**CH_3**). ^{13}C NMR ($CDCl_3$) δ 171.3, 170.7, 165.7, 141.1, 140.1, 135.6, 127.9, 122.4, 88.5, 60.8, 51.5, 45.7, 32.8, 14.1; LRMS (FAB, M+H$^+$), 403.01; HRMS (FAB) *m/z* calcd for $C_{14}H_{16}IN_2O_4$, 403.0155; found: 403.0142. Anal. calcd for $C_{14}H_{15}IN_2O_4$ (0.25 $C_4H_8O_2$): C, 42.47; H, 4.03; N, 6.60; I, 29.91. Found: C, 42.84; H, 3.80; N, 6.92; I, 30.03.

As an alternative to HF cleavage, the diphenyl methane-protecting group could be cleaved using TFA/Et_3SiH (3/1) using the following procedure. A dry 100 mL round-bottom flask equipped with a stirring bar was charged with **19** (10 g, 17.6 mmol). The solid was suspended in a 3/1 mixture of TFA/Et_3SiH (15 mL/5.0 mL, 20 mL total volume) and the mixture heated to reflux. Upon heating to reflux, the reaction mixture became homogeneous and the solution was refluxed for 16 h. The excess TFA was removed by simple distillation and the residue was purified by two sequential preparative column chromatographs (10% acetone/CH_2Cl_2) to yield 2.8 g (40%) of pure **8b**.

2-Amino-5-[4-cyanobenzoyl]amino-1-benzoyl-(ethyl-3-aminopropanoate) (20). A 1-L Paar hydrogenation flask was charged with 5-nitro isatoic anhydride[37] (25 g, 120 mmol), dimethylacetamide (200 mL), and flushed with nitrogen. Palladium catalyst (10% Pd–C, 0.8 g, 3.2% by wt) was added, the Paar flask placed on the Paar apparatus, flushed with nitrogen, evacuated, and charged with hydrogen (evacuation/fill×3). Hydrogenation proceeded at 45 psi for 2 h (renewing hydrogen charge to 45 psi every 20 min) at which time TLC (100% EtOAc) indicated complete conversion of the nitro isatoic anhydride (R_f=0.56, 100% EtOAc) to a new lower R_f product (R_f=0.44, 100% EtOAc). The reaction mixture was filtered through a pad of Celite and the Celite rinsed with additional DMA (200 mL). The crude product was stored as a solution in dimethylacetamide. The reaction was repeated (same scale) and the DMA solutions of the aniline combined (ca. 240 mmol of 5-amino isatoic anhydride, total volume of DMA approx. 800 mL). A dry 2-L round-bottomed flask equipped with a stir bar was charged with *p*-cyano benzoyl chloride (41.4 g, 250 mmol), DMA (300 mL), triethylamine (26.5 g, 36.5 mL, 262 mmol, 1.08 equiv.), DMAP (1.0 g, 8.2 mmol, 0.034 equiv.), and cooled to 5 °C (ice/H_2O bath). The DMA solution (ca. 800 mL) of the crude 5-amino isatoic anhydride was added dropwise over 1.5 h and the reaction mixture allowed to warm to room temperature. After stirring at room temperature for 48 h, the reaction mixture was treated with beta alanine ethyl ester hydrochloride (38.5 g, 251 mmol, 1.05 equiv.), triethylamine (35 g, 48.2 mL, 347 mmol, total 2.54 equiv.), and DMAP (1.0 g, 8.2 mmol, 0.068 equiv.). After stirring additionally for 24 h, the DMA was removed in vacuo and the crude residue dissolved in ethyl acetate (500 mL), transferred to a separatory funnel, washed twice with brine, dried ($MgSO_4$), filtered, and concentrated to give a crude brown residue. The residue was dissolved in EtOAc and filtered through silica (800 g, EtOAc eluent). Attempts to recrystallize **20** after chromatographic filtration were unsuccessful so the material was further purified by flash chromatography (50% EtOAc/hexane, then 80% EtOAc/hexane gradient elute). Two separate chromatographs were performed. In the first chromatography one third of the crude residue was purified and the remaining material was purified in the second chromatography. Fractions which were a single homogeneous spot were pooled and concentrated to give 75 g of **20** (batch no. 1). Fractions highly enriched in the desired product (ca. 90% purity) but containing small amounts of impurities were pooled to give an additional 50 g of **20** (batch no. 2). Each individual batch was further purified by recrystallization from ethyl acetate/hexane to obtain 93 g of pure **20** as a pale yellow solid (56%). The mother liquors were combined and concentrated to yield an additional 32 g of **20** as a brown residue which was of suitable purity to be used in the next reaction if desired: mp 144–146 °C dec.: TLC R_f=0.5 (80% EtOAc/hexane, UV positive); ^{1}H NMR (DMSO-d_6, TMS) δ 10.26 (1H, s, ArCO**NH** Ar), 8.27 (1H, br t, $^3J_{HH}$=6.85, CO**NH**CH_2), 8.13 (2H, d, $^3J_{HH}$=8.1, *p*-NC ArH), 8.04 (2H, d, $^3J_{HH}$=8.3, *p*-NC

ArH), 7.77 (1H, d, $^4J_{HH}=2.2$, C6 ArH), 7.44 (1H, dd, $^3J_{HH}=8.8$, $^4J_{HH}=2.2$, C4 ArH), 6.74 (1H, d, $^3J_{HH}=8.8$, C3 ArH), 6.24 (2H, br s, Ar**NH$_2$**), 4.11 (2H, q, $^3J_{HH}=7.1$, **OCH$_2$**), 3.48 (2H, apparent q, $^3J_{HH}=6.85$, CONH**CH$_2$**CH$_2$CO$_2$), 2.59 (t, $^3J_{HH}=6.85$, **CH$_2$**CO$_2$), 1.22 (3H, t, $^3J_{HH}=7.1$, OCH$_2$**CH$_3$**); ^{13}C (DMSO-d_6) 171.40, 168.80, 163.49, 146.47, 138.95, 132.43, 128.28, 126.37, 121.79, 118.36, 116.29, 115.0, 113.71, 59.98, 35.27, 33.87, 14.08; LRMS (FAB, M$^+$) 380.15; LRMS (FAB, M+H$^+$) 381.15; HRMS (FAB) *m/z* calcd for $C_{20}H_{20}N_4O_4$, 380.1484; found: 380.1486. Anal. calcd for $C_{20}H_{20}N_4O_4 \cdot 0.25\ H_2O$: C, 62.41; H, 5.37; N, 14.56; Found: C, 62.63; H, 5.37; N, 14.72.

***N*-(2-Diphenylmethylamino)-5-[4-cyanobenzoyl]-amino-1-benzoyl-(ethyl-3-aminopropanoate) (21).** A slurry of **20** (8 g, 2.12 mmol) in 1,2-dichloroethane (100 mL) was treated with 2,6-lutidine (2.5 mL, 21.2 mmol, 10 equiv.) and heated to 60 °C. Diphenyl methyl-bromide (10 g, 40 mmol, 18.9 equiv.) dissolved in 1,2-dichloroethane (50 mL) was added in four equal portions over a 3 h period. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. The residue was dissolved in ethyl acetate (50 mL), transferred to a separatory funnel, washed with water (50 mL), 5% sodium bicarbonate solution (50 mL), and brine (50 mL). The ethyl acetate layer was dried ($MgSO_4$), filtered, concentrated in vacuo and purified by preparative column chromatography (50% EtOAc/hexane elute) to yield 10 g (86%) of **21**: ^{1}H NMR ($CDCl_3$, TMS) δ 8.25 (1H, s, Ar-CONH -Ar), 7.88 (2H, d, $^3J_{HH}=7.8$, *o*-NC ArH), 7.74 (1H, br s, C6 ArH), 7.65 (2H, d, $^3J_{HH}=7.8$, *m*-NC ArH), 7.35–7.15 (12H, m, **ArCHAr**, Ar**NH**(CH)Ar$_2$, and C4 ArH), 6.96 (1H, br t, $^3J_{HH}=6.0$, ArCO**NH**CH$_2$CH$_2$CO$_2$), 6.48 (1H, d, $^3J_{HH}=9.0$, C3 ArH), 5.54 (1H, s, ArNH (**CH**)Ar$_2$), 4.11 (2H, q, $^3J_{HH}=6.8$, **OCH$_2$**), 3.59 (2H, apparent q, $^2J_{HH}=^3J_{HH}=5.9$, N**CH$_2$**CH$_2$CO$_2$Et), 2.56 (2H, t, $^3J_{HH}=6.0$, **CH$_2$**CO$_2$Et), 1.23 (3H, t, $^3J_{HH}=6.8$, OCH$_2$ **CH$_3$**); ^{13}C NMR ($CDCl_3$) 172.6, 169.2, 163.9, 146.2, 142.4, 138.7, 132.3, 128.8, 128.4, 127.8, 127.7, 127.3, 127.2, 126.3, 125.6, 120.8, 118.0, 115.2, 114.9, 113.5, 62.1, 60.8, 35.2, 34.0, 14.1; HRMS (FAB) *m/z* calcd for $C_{33}H_{30}N_4O_4$, 547.2346; found: 547.2309.

1-(Diphenylmethyl)-4-(2-carboxyethyl)-7-[4-(cyano)benzoyl]-amino]-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (22). (a) To a biphasic CH_2Cl_2/H_2O (50 mL/50 mL) solution of **21** (9 g, 16 mmol), potassium carbonate (4.14 g, 30 mmol, 1.875 equiv.) and bromoacetyl-bromide (5.90 g, 29.2 mmol, 1.82 equiv.) were added in three equal portions over 25 min (pH maintained at 7–8). After stirring at room temperature for 1 h, the biphasic solution was transferred to a separatory funnel and the layers separated. The organic phase was dried ($MgSO_4$), filtered, concentrated in vacuo and purified by preparative column chromatography (67% EtOAc/hexane) to yield 6.68 g (61%) of *N*-[2-diphenyl methyl amino]-*N*-bromoacetyl-5-[4-cyanobenzoyl] amino-1-benzoyl-(ethyl-3-aminopropanoate): ^{1}H NMR ($CDCl_3$, TMS) δ 9.75 (1H, s, Ar-CO**NH**-Ar), 8.05 (2H, d, $^3J_{HH}=7.2$, *o*-NC ArH), 7.98 (1H, br s, C6 ArH), 7.71, (2H, d, $^3J_{HH}=7.2$, *m*-NC ArH), 7.58 (1H, br d, $^3J_{HH}=8.7$, C4 ArH), 7.30–7.00 (10H, m, **ArCHAr**), 6.82 (1H, s, ArN(**CH**Ar$_2$)CO), 6.74 (1H, d, $^3J_{HH}=8.7$, C3 ArH), 5.73 (1H, br t, $^3J_{HH}=5.4$, Ar CO**NH**CH$_2$CH$_2$CO$_2$), 4.18 (2H, q, $^3J_{HH}=7.2$, **OCH$_2$**), 3.83 (2H, br s, NCO**CH$_2$**Br) 3.47 (1H, m, N**CH**HCH$_2$CO$_2$Et), 3.13 (1H, m, NCH**H**CH$_2$CO$_2$Et), 2.46 (2H, br t, **CH$_2$**CO$_2$Et), 1.28 (3H, t, $^3J_{HH}=7.2$, OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) δ 172.0, 169.3, 166.5, 164.5, 139.6, 139.3, 138.1, 136.6, 135.4, 132.7, 132.3, 131.3, 128.3, 128.2, 128.0, 127.4, 127.2, 122.6, 119.2, 118.0, 115.0, 64.4, 60.8, 35.3, 33.5, 29.9, 14.1; LRMS (FAB, M+H$^+$) 667.2; HRMS (FAB) *m/z* calcd for $C_{35}H_{32}BrN_4O_5$, 667.1556; found: 667.1538.

(b) To a stirring room temperature DMF (400 mL) solution of *N*-[2-diphenyl methyl amino]-*N*-bromoacetyl-5-[4-cyanobenzoyl]amino-1-benzoyl-(ethyl-3-aminopropanoate) (6.56 g, 9.83 mmol), solid cesium carbonate (6.41 g, 19.6 mmol, 2.0 equiv.) was added in one portion. The heterogeneous solution was stirred at room temperature for 2 h, then poured into a separatory funnel containing ethyl acetate (500 mL) and water (500 mL). After thorough mixing, the aqueous phase was removed and the organic phase washed with water (200 mL), brine (300 mL), dried ($MgSO_4$), filtered and concentrated in vacuo to give 5.36 g (93%) of **22**. ^{1}H NMR ($CDCl_3$, TMS) δ 9.47 (1H, s, ArCO**NH**Ar), 7.83 (2H, d, $^3J_{HH}=7.8$, *o*-NC ArH), 7.77 (1H, s, C6 ArH), 7.46 (2H, d, $^3J_{HH}=8.4$, *m*-NC ArH), 7.25 7.0 (11H, m, **Ar CHAr**, upon expansion of this region C8 ArH appears at 7.09 as a dd, $^3J_{HH}=7.8$, $^4J_{HH}=2.0$), 6.97 (1H, d, $^3J_{HH}=8.4$, C9 ArH), 6.62 (1H, s, ArN (**CH**Ar$_2$)CO), 4.09 (1H, d, $^2J_{HH}=14.4$, N**CH**HC=O), 3.96 (2H, q, $^3J_{HH}=7.2$, **OCH$_2$**), 3.81 (1H, m, N**CH**HCH$_2$CO$_2$), 3.70 (1H, d, $^2J_{HH}=14.4$, NCH**H**C=O), 3.55 (1H, m, (N**CH**HCH$_2$CO$_2$), 2.39 (2H, br t, **CH$_2$**CO$_2$Et), 1.10, (3H, t, $^3J_{HH}=7.2$, OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) 170.8, 168.2, 166.6, 164.2, 138.1, 138.0, 137.6, 136.4, 135.5, 132.0, 130.3, 129.1, 128.2, 128.1, 128.0, 127.9, 127.6, 127.4, 124.4, 123.1, 121.9, 117.8, 114.9, 67.0, 60.6, 44.6, 32.0, 14.0; HRMS (FAB) *m/z* calcd for $C_{35}H_{30}N_4O_5$, 587.2295; found: 587.2324.

4-(2-Carboxyethyl)-7-[4-(cyano)benzoyl]-amino]-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (8c). A slurry of **22** (5.36 g, 9.14 mmol), anisole (5 mL) and ethyl methyl sulfide (5 mL) in a Teflon tube was cooled to −196 °C (N_2) and hydrogen fluoride (30 mL) condensed into the Teflon tube. The reaction was stirred for 30 min at −196 °C, allowed to warm to 0 °C and concentrated in vacuo over a period of 2 h. The resulting

residue was triturated with ethyl ether, collected via vacuum filtration and dried under high vacuum (0.5 mm Hg) to give 3.22 g (56%) of **8c**: mp 253–257 °C dec.; TLC R_f = 0.46 (50% EtOAc/hexane, UV positive); ^{1}H NMR (DMSO-d_6, TMS) δ 10.63 (1H, s, ArCONHAr), 10.45 (1H, s, ArNHCOCH$_2$N), 8.16 (1H, d, $^4J_{HH}$ = 3.3, C6 ArH), 8.12 (2H, d, $^3J_{HH}$ = 8.0, *o*-NC ArH), 8.03 (2H, d, $^3J_{HH}$ = 8.0, *m*-NC ArH), 7.95 (1H, dd, $^3J_{HH}$ = 8.7, $^4J_{HH}$ = 3.3, C8 ArH), 7.10 (1H, d, $^3J_{HH}$ = 8.7, C9 ArH), 4.06 (2H, q, $^3J_{HH}$ = 7.2, **OCH$_2$**), 3.91 (2H, s, CON**CH$_2$**C=O), 3.81 (2H, t, $^3J_{HH}$ = 7.2, **NCH$_2$**CH$_2$CO$_2$Et), 2.62 (2H, t, $^3J_{HH}$ = 7.2, **CH$_2$**CO$_2$Et), 1.18 (3H, t, $^3J_{HH}$ = 7.2, OCH$_2$**CH$_3$**); ^{13}C NMR (DMSO-d_6) δ 170.9, 170.0, 166.2, 164.0, 138.6, 134.8, 133.2, 132.5, 128.5, 126.2, 124.4, 122.3, 121.2, 118.3, 114.0, 60.1, 50.9, 44.5, 32.4, 14.0; LRMS (FAB, M + H$^+$) 421.4; HRMS (FAB) *m/z* calcd for $C_{22}H_{21}N_4O_5$, 421.1512; found: 421.1501; Anal. calcd for $C_{22}H_{20}N_4O_5 \cdot 1.0\ H_2O$: C, 60.27; H, 5.06; N, 12.78. Found: C, 60.47; H, 4.64; N, 12.95.

General procedure for thioamide preparation. Thioamides **23a–c** were prepared using the procedure described for the preparation of **23a**. Compound **23b** was purified as described for **23a**.

7-[[4-Cyanophenyl]methoxy]-4-propanoic acid, 3,4-dihydro-1*H*-1,4-benzodiazepine-2-thione-5-one, ethyl ester (23a). A THF solution (anhyd., 20 mL) of **8a** (1.0 g, 2.45 mmol) under an atmosphere of nitrogen was treated with Lawessons reagent ((2,4-bis(4-methoxyphenyl) 1,2,3,4-dithiadiphosphetane-2,4-disulfide, 1.0 g, 2.49 mmol, 1.02 equiv.), heated to 50 °C for 2 h, cooled to room temperature, concentrated in vacuo, and purified by preparative column chromatography (40% EtOAc/hexane to 60% EtOAc/hexane gradient elute) to yield 1.0 g (96%) of **23a**: TLC R_f = 0.67 (50% EtOAc/hexane, UV positive); ^{1}H NMR (CDCl$_3$, TMS) δ 9.65 (1H, s, NH), 7.71 (2H, d, $^3J_{HH}$ = 8.0, *m*-CN ArH), 7.55 (2H, $^3J_{HH}$ = 8.0, *o*-CN ArH), 7.51 (1H, d, $^4J_{HH}$ = 3.0, C6 ArH), 7.13 (1H, dd, $^4J_{HH}$ = 3.0, $^3J_{HH}$ = 9.0, C8 ArH), 7.01 (1H, d, $^3J_{HH}$ = 9.0, C9 ArH), 5.17 (2H, s, **OCH$_2$** Ar), 4.29 (2H, s, CON**CH$_2$**C=S), 4.16 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.96 (2H, t, $^3J_{HH}$ = 7.0, N**CH$_2$**CH$_2$CO$_2$), 2.79 (2H, t, $^3J_{HH}$ = 7.0, **CH$_2$**CO$_2$,), 1.27 (3H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**); LRMS (FAB, M + H) 424.1.

4-(2-Carboxyethyl)-7-iodo-3,4-dihydro-1*H*-1,4-benzodiazepinedione-2-thione-5-one ethyl ester (23b). Thus 1.0 g (2.49 mmol) of **8b** yielded 0.95 g (91%) of **23b**. Mp 187–189 °C; TLC R_f = 0.44 (50% EtOAc/hexane, UV positive); ^{1}H NMR (CDCl$_3$, TMS) δ 8.21 (1H, d, $^4J_{HH}$ = 2.0, C6 ArH), 7.78 (1H, dd, $^4J_{HH}$ = 2.0, $^3J_{HH}$ = 9.0, C8 ArH), 6.88 (1H, d, $^3J_{HH}$ = 9.0, C9 ArH), 4.28 (2H, s, N**CH$_2$**C=S), 4.15 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.94 (2H, t, $^3J_{HH}$ = 7.0, N**CH$_2$**CH$_2$CO$_2$), 2.72 (2H, t, $^3J_{HH}$ = 7.0, **CH$_2$**CO$_2$Et), 1.27 (3H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**); ^{13}C NMR (CDCl$_3$) δ 198.8, 171.4, 165.3, 141.2, 140.4, 135.9, 128.9, 121.9, 90.4, 60.8, 58.0, 45.4, 32.8, 14.1.

4-(2-Carboxyethyl)-7-[4-(cyano)benzoyl]-amino)-3,4-dihydro-1*H*-1,4-benzodiazepine-2-thio-5-oxo ethyl ester (23c). Thus 1.5 g (3.6 mmol) of **8c** yielded 1.1 g (70%) of **23c** after the crude solid was collected via vacuum filtration, washed with cold THF, and dried in vacuo (10 mm Hg, 30 min). TLC R_f = 0.47 (67% EtOAc/hexane, UV positive); ^{1}H NMR (DMSO-d_6, TMS) δ 10.72 (1H, s, ArCONHAr), 8.24 (1H, br s, C6 ArH), 8.14 (2H, d, $^3J_{HH}$ = 7.8, *o*-NC ArH), 8.04 (2H, d, $^3J_{HH}$ = 7.8, *m*-NC ArH), 8.01 (1H, br d, $^3J_{HH}$ = 8.7, C8 ArH), 7.26 (1H, d, $^3J_{HH}$ = 8.7, C9 ArH), 4.25 (2H, s, N**CH$_2$**C=S), 4.06 (2H, q, $^3J_{HH}$ = 6.9, **OCH$_2$**), 3.80 (2H, t, $^3J_{HH}$ = 6.9, N**CH$_2$**CH$_2$CO$_2$Et), 2.71 (2H, t, $^3J_{HH}$ = 6.9, NCH$_2$**CH$_2$** CO$_2$Et), 1.18 (3H, t, $^3J_{HH}$ = 6.9, OCH$_2$**CH$_3$**); LRMS (FAB, M + H$^+$) 437.1; HRMS (FAB) *m/z* calcd for $C_{22}H_{21}N_4SO_4$, 437.1284; found: 437.1269.

Method A (Table 1). The general procedure is described for the preparation of **24a**. Compound **24d** was prepared similarly with the modifications described.

4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-cyanophenyl]methoxy]-1-methyl-6-oxo, ethyl ester (24a). (a) A vigorously stirred biphasic dichloromethane (10 mL) and water (10 mL) solution of **23a** (1.0 g, 2.36 mmol), methyl iodide (0.2 mL), and tetrabutylammonium hydrogen sulfate (catalytic amount 120 mg, 0.354 mmol, 0.15 equiv.) at room temperature was treated with 2 N sodium hydroxide (1.2 mL, 2.4 mmol, 1.01 equiv.). After stirring at room temperature for 16 h, the layers were separated and the aqueous layer extracted additionally with dichloromethane (2×60 mL). The combined organics were dried over sodium sulfate, decanted, and concentrated in vacuo to yield 7-[[4-cyanophenyl]methoxy]-4-propanoic acid, 3,4-dihydro-1,4-benzodiazepine-2-methyl mercaptyl-5-one, ethyl ester. The crude S–Me imidate of **23a** was characterized by ^{1}H NMR and LRMS. ^{1}H NMR (CDCl$_3$, TMS) δ 7.70 (2H, d, $^3J_{HH}$ = 8.0, *m*-NC ArH), 7.56 (2H, $^3J_{HH}$ = 8.0, *o*-NC ArH), 7.49 (1H, d, $^4J_{HH}$ = 3.0, C6 ArH), 7.18 (1H, d, $^3J_{HH}$ = 9.0, C9 ArH), 7.15 (1H, dd, $^4J_{HH}$ = 3.0, $^3J_{HH}$ = 9.0, C8 ArH), 5.18 (2H, s, O**CH$_2$**Ar), 4.16 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.94 (2H, t, $^3J_{HH}$ = 7.0, N**CH$_2$**CH$_2$CO$_2$), 3.91 (2H, s, N**CH$_2$**C=S), 2.78 (2H, t, $^3J_{HH}$ = 7.0, **CH$_2$**CO$_2$Et), 2.52 (3H, s, SMe), 1.24 (3H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**); LRMS (FAB, M + H) 438.1.

(b) The crude S–Me imidate of **23a** was dissolved in toluene (anhyd., 15 mL), treated with propargyl amine (four-fold excess, 0.64 mL) and pyridinum hydrochloride (0.23 g, 2.27 mmol, 0.96 equiv.). After heating to reflux and refluxing for 5 h the reaction mixture was cooled to room temperature and concentrated in vacuo.

The crude residue was purified by preparative column chromatography (100% EtOAc eluent) to yield 0.23 g (22%) of **24a**. TLC R_f=0.10 (100% EtOAc, UV positive); ^{1}H NMR ($CDCl_3$, TMS) δ 7.71 (2H, d, $^3J_{HH}$=8.0, *m*-NC ArH), 7.56 (2H, $^3J_{HH}$=8.0, *o*-NC ArH), 7.53 (1H, d, $^4J_{HH}$=3.0, C7 ArH), 7.24 (1H, d, $^3J_{HH}$=9.0, C10 ArH), 7.18 (1H, dd, $^4J_{HH}$=3.0, $^3J_{HH}$=9.0, C9 ArH), 6.80 (1H, br s, NC(CH_3)**CHN**), 5.21 (2H, ABq, J_{AB}=14.2, **OCH$_2$**Ar), 4.52 (1H, d, $^2J_{HH}$=16.0, **NCHHC**=N), 4.30 (1H, d, $^2J_{HH}$=16.0, **NCHHC**=N), 4.16 (2H, q, $^3J_{HH}$=7.0, **OCH$_2$**), 3.89 (2H, m, **NCH$_2$** CH_2CO_2), 2.66 (2H, m, **CH$_2$CO$_2$**Et), 2.29 (3H, s, NC(**CH$_3$**)CHN), 1.22 (3H, t, $^3J_{HH}$=7.0, OCH$_2$**CH$_3$**).

4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-iodo-1-methyl-6-oxo ethyl ester (24d). Prepared as described for **24a** with the modification that the S–Me imidate intermediate was not characterized. The thioamide **23b** (4.67 g, 11.1 mmol) was converted into the S–Me thiaimidate of **23b** in 82% yield (4.0 g) which was used directly in the reaction with propargyl amine (5.0 equiv.) and pyridinum hydrochloride (1.0 equiv.) in refluxing toluene (5 h). Thus **23b** (0.95 g, 2.27 mmol), was converted into **24d** in 66% yield (0.653 g) after preparative column chromatography (100% ethyl acetate): ^{1}H NMR ($CDCl_3$, TMS) δ 8.33 (1H, d, $^4J_{HH}$=2.0, C7 ArH), 7.86 (1H, dd, $^4J_{HH}$=2.0, $^3J_{HH}$=9.0, C9 ArH), 7.00 (1H, d, $^3J_{HH}$=9.0, C10 ArH), 6.82 (1H, bq, $^4J_{HH}$=1.0, NC(CH_3)**CHN**), 4.42 (1H, d, $^2J_{HH}$=15.0, **NCHHC**=N), 4.29(1H, d, $^2J_{HH}$=15.0, **NCHHC**=N), 4.14 (2H, m, **OCH$_2$**), 3.94 (2H, m, **NCH$_2$**CH_2CO_2), 2.66 (2H, m, **CH$_2$**CO_2 Et), 2.30 (H, d, $^4J_{HH}$=1.0 NC(**CH$_3$**) CHN), 1.23 (3H, t, $^3J_{HH}$=7.0, OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) δ 171.2, 165.5, 145.8, 140.7, 140.5, 140.4,132.7, 128.1,127.9, 124.6, 91.9, 60.7, 45.7, 45.1, 32.7, 14.1, 11.0.

Method B (Table 1). The general procedure is described for the preparation of **24b**. Compounds **24e** and **24f** were prepared similarly with the modifications to the length of the reaction time noted.

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-cyanophenyl]methoxy]-1-methyl-6-oxo, ethyl ester [24b]. Triazole **24b** was obtained using the procedure described for the preparation of **24a** with the modifications that the S–Me imidate intermediate was not characterized and was treated with acetic hydrazide (0.64 g, 8.6 mmol, 4.86 equiv.) instead of propargyl amine. The reflux time in toluene was five hours. Thus **23a** (0.75 g, 1.77 mmol) was converted to **24b** in 63% yield (0.5 g) after purification by preparative column chromatography (5/95 methanol/methylene chloride eluent): ^{1}H NMR ($CDCl_3$, TMS) δ 7.59 (2H, d, $^3J_{HH}$=8.0, *m*-NC ArH), 7.46 (2H, $^3J_{HH}$=8.0, *o*-NC ArH), 7.45 (1H, d, $^4J_{HH}$=3.0, C7 ArH), 7.17 (1H, d, $^3J_{HH}$=9.0, C10 ArH), 7.12 (1H, dd, $^4J_{HH}$=3.0, $^3J_{HH}$=9.0, C9 ArH), 5.12 (2H, dd, **OCH$_2$**Ar), 4.61 (1H, d, $^2J_{HH}$=16.0, **NCHHC**=N), 4.25 (1H, d, $^2J_{HH}$=16.0, NCHHC=N), 4.01 (2H, q, $^3J_{HH}$=7.0, **OCH$_2$**), 3.77 (2H, m, **NCH$_2$CH$_2$CO$_2$**), 2.57 (2H, m, **CH$_2$CO$_2$**Et), 2.46 (3H, s, NC(**CH$_3$**)N), 1.13 (3H, t, $^3J_{HH}$=7.0 OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) δ 171.1, 165.9, 157.6, 152.4, 150.5, 141.0, 132.4, 131.2, 127.5, 124.1, 119.4, 118.3, 116.8, 111.9, 69.3, 60.6, 45.2, 43.3, 32.4, 14.0, 12.1.

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-cyanophenyl]iodo]-1-methyl-6-oxo ethyl ester (24e). Prepared as described for **24b** with the modification that the S–Me imidate of **23b**, acetic hydrazide (5.0 equiv.), and pyridinum hydrochloride (1.0 equiv.) were refluxed in toluene for 16 h. Thus 1.0 g (0.23 mmol) of the S–Me imidate of **23b** yielded 0.925 g (81%) of triazole **24e** after purification by preparative column chromatography (7% EtOH/EtOAc eluent): TLC R_f=0.28 (7% EtOH/EtOAc, UV positive); ^{1}H NMR ($CDCl_3$, TMS) δ 8.27 (1H, d, $^4J_{HH}$=2.0, C7 ArH), 7.90 (1H, dd, $^3J_{HH}$=8.3, $^4J_{HH}$=2.0, C9 ArH), 7.02 (1H, d, $^3J_{HH}$=8.3, C10 ArH), 4.72 (1H, d, $^2J_{HH}$=16.0, **NCHHC**=N), 4.30 (1H, d, $^2J_{HH}$=16.0, NCHHC=N), 4.07 (2H, br q, $^3J_{HH}$=7.0, **OCH$_2$**, upon expansion appears as two quartets offset from each other by 1.5 Hz), 3.86 (1H, m, **NCHHCH$_2$CO$_2$**), 3.75 (1H, m, **NCHHCH$_2$CO$_2$**), 2.67 (1H, m, **CHHCO$_2$**Et), 2.56 (1H, m, **CHHCO$_2$**Et), 2.53 (s, 3H, N(**CH$_3$**)C=N), 1.80 (t, 3H, $^3J_{HH}$=7.0, CO_2CH_2**CH$_3$**).

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-cyanophenyl]iodo]-1-*tert*-butyl-6-oxo ethyl ester (24f). Prepared as described for **24e** with the modification that *tert*-butyl hydrazide was used instead of acetic hydrazide. The S–Me thiaimidate of **23b** (1.0 g, 0.23) was converted into triazole **24f** in 81% yield after purification by preparative column chromatography (7% EtOH/EtOAc eluent); ^{1}H NMR ($CDCl_3$, TMS) δ 8.10 (1H, d, $^4J_{HH}$=2.2, C7 ArH), 7.82 (1H, dd, $^3J_{HH}$= 8.5, $^4J_{HH}$=2.2, C9 ArH), 7.17 (1H, d, $^3J_{HH}$ =8.5, C10 ArH), 4.56 (1H, br d, $^2J_{HH}$=16.0, **NCHHC**=N), 4.12 (1H, br d, $^2J_{HH}$=16.0, NCHHC=N), 3.98 (2H, br q, $^3J_{HH}$=7.0, **OCH$_2$**), 3.82 (1H, m, **NCHHCH$_2$CO$_2$**), 3.60 (1H, m, NCHHCH$_2$CO$_2$), 2.58 (1H, m, **CHHCO$_2$**Et), 2.48 (1H, m, **CHHCO$_2$**Et), 1.28 (9H, s, *t* Bu), 1.07 (3H, t, $^3J_{HH}$=7.0, OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) δ 170.8, 164.6, 161.4, 153.6, 139.7,139.5, 131.8, 131.5, 126.8, 93.7, 60.4, 44.5, 43.2, 33.0, 32.3, 29.9, 27.1, 13.8.

Method C (Table 1). The general procedure is described for the preparation of **24c**.

Quinazolino[3,2-*a*][1,4]benzodiazepine-6(5*H*)-propanoic acid, 8-[[4-cyanophenyl]methoxy]-7,13-dihydro-5,13-dioxo ethyl ester (24c). Compound **24c** was prepared from **23a** (0.75 g, 1.77 mmol) using the method described

for the preparation of **24b**, substituting methyl anthranilate (1.42 mL, 11.0 mmol, 6.2 equiv.) for acetic hydrazide. The reflux time in toluene was 5 h. Purification via preparative column chromatography (5/95, methanol/methylene chloride eluent) yielded 0.3 g of quinazoline **24c** (33%): ^{1}H NMR ($CDCl_3$, TMS) δ 8.33 (1H, d, $^3J_{HH}$ = 8.0, ArH), 7.78 (2H, m, ArH), 7.71 (2H, d, $^3J_{HH}$ = 8.0, *m*-NC ArH), 7.56 (4H, m, *o*-NC ArH, ArH), 7.43 (1H, d, $^4J_{HH}$ = 3.0 C7 ArH), 7.17 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 5.20 (2H, s, **OCH$_2$**Ar), 4.52 (1H, d, $^2J_{HH}$ = 16.0, **NCHHC**=N), 4.34 (1H, d, $^2J_{HH}$ = 16.0, NCH**HC**=N), 4.04 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.93 (2H, bt, **NCH$_2$**CH$_2$CO$_2$), 2.78 (1H, dt, $^2J_{HH}$ = 15.0, $^3J_{HH}$ = 6.0, **CH**HCO$_2$Et), 2.62 (1H, dt, $^2J_{HH}$ = 15.0, $^3J_{HH}$ = 6.0, **CH**HCO$_2$Et), 1.18 (3H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**).

Method D (Table 1). The general procedure is described for the preparation of **24g**.

4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-cyanobenzoyl]amino-1-methyl-6-oxo ethyl ester (24g). A slurry of **23c** (1.0 g, 2.3 mmol) in acetonitrile (anhyd., 60 mL) was treated with methyl iodide (0.65 g, 4.6 mmol, 2.0 equiv.) and heated to reflux. The reaction flask was charged with additional methyl iodide (0.65 g, 4.6 mmol) at 6, 7, and 8 h after the onset of reflux. Thirty minutes after the last charge with methyl iodide (t = 8 h) the reaction mixture became homogeneous. The solution was concentrated in vacuo and dried under vacuum (1.0 mm Hg) taking care to avoid exposure to moisture. The S–Me imidate was treated with a solution of propargyl amine (0.73 mL, 11.5 mmol, 5.0 equiv.) and pyridinum chloride (0.27 g, 2.3 mmol, 1.0 equiv.) in toluene (anhyd., 250 mL). The resulting slurry was heated to reflux for 6 h, cooled to approx. 50 °C, and the warm toluene solution decanted. The wet solids were dried under vacuum (1.0 mm Hg) and the crude product (350 mg) purified by preparative reverse phase HPLC to yield 240 mg (23%) of **24g**. RP HPLC (method **P4**) t_R (**24g**) = 60–70 min. ^{1}H NMR (DMSO-d_6, TMS) δ 10.89 (1H, s, ArC**ONH**Ar), 8.36 (1H, d, $^4J_{HH}$ = 2.4, C7 ArH), 8.22 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 2.4, C9 ArH), 8.16 (2H, d, $^3J_{HH}$ = 8.2, *o*-NC ArH), 8.08 (2H, d, $^3J_{HH}$ = 8.2, *m*-NC ArH), 7.74 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 7.30 (1H, s, NC(CH$_3$)**CH**N), 4.68 (1H, d, $^2J_{HH}$ = 16.0, NCHHC=N), 4.51 (1H, d, $^2J_{HH}$ = 16.0, NCHHC=N), 4.03 (2H, q, $^3J_{HH}$ = 6.9, **OCH$_2$**), 3.79 (2H, m, **NCH$_2$**CH$_2$CO$_2$Et), 2.62 (2H, t, $^3J_{HH}$ = 7.2, **CH$_2$**CO$_2$Et), 2.39 (3H, s, NC(**CH$_3$**)CHN), 1.14 (3H, t, $^3J_{HH}$ = 6.9, OCH$_2$**CH$_3$**). LRMS (FAB, M + H$^+$) 458.1; HRMS (FAB) *m/z* calcd for $C_{25}H_{24}N_5O_4$ 458.1828; found: 458.1819.

7-[[4-Cyanophenyl]ethynyl]-4-propanoic acid, 3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester [25]. The aryl iodide (934 mg, 2.3 mmol) and 4-ethynylbenzonitrile[42] (591 mg, 4.6 mmol, 2.0 equiv.) were dissolved in ethyl acetate (50 mL), degassed (3×evacuate/fill with N_2), treated with bis triphenylphosphine palladium dichloride (81.5 mg, 0.12 mmol, 0.05 equiv.) and degassed again (3×evacuate/fill with N_2). Copper iodide (44.2 mg, 0.23 equiv.) was added, the reaction mixture degassed treated with triethyl amine (1.189 g, 1.62 mL, 11.6 mmol, 5.0 equiv.) and stirred at room temperature. Within 10 min after addition of the triethylamine the yellow suspension turned dark brown. The reaction mixture stirred under argon at room temperature for 1.25 h at which time TLC (1.5% $CH_3OH/CHCl_3$, UV detection at 254 nM) indicated the reaction had gone to completion. The reaction mixture was diluted with ethyl acetate (50 mL) and transferred into a 250-mL separatory funnel. The ethyl acetate was washed with 10% citric acid (50 mL), satd $NaHCO_3$ (50 mL), brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by preparative column chromatography (82 g SiO_2, 1.5% $CH_3OH/CHCl_3$ eluent) to yield 746 mg (80%) of **25** as a light brown solid. mp 221–222 °C. ^{1}H NMR ($CDCl_3$, TMS) δ 9.18 (1H, s, Ar**NH**CO), 8.15 (1H, br s, C6 ArH), 7.60 (5H, 4H,2 d, J_{AB} = 6.0, NC ArH and 1H, dd, C8 ArH, coupling constants could not be determined), 7.05 (1H, d, $^3J_{HH}$ = 6.0, C9 ArH), 4.20 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 4.02 (2H, br s, **NCH$_2$**CONH), 3.90 (2H, t, $^3J_{HH}$ = 7.0, **NCH$_2$**CH$_2$CO$_2$), 2.77 (2H, t, $^3J_{HH}$ = 7.0, **CH$_2$**CO$_2$Et), 1.25 (t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**). LRMS (FAB, M + H$^+$) 402.14. HRMS (FAB) *m/z* calcd for $C_{23}H_{20}N_3O_4$ 402.1454, found: 402.1443.

4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-cyanophenyl]ethynyl]-1-methyl-6-oxo ethyl ester (26). Compound **26** was prepared using the procedure described for the preparation of **25**. It should be noted that **24d** (0.15 g, 0.34 mmol) was not completely soluble in ethyl acetate (4.0 mL) at room temperature (i.e. the reaction mixture was a slurry, not homogeneous) prior to the addition of the reagents and the reaction proceeding. The reaction was allowed to stir overnight then concentrated in vacuo and the residue purified by preparative column chromatography (100% ethyl acetate) to yield 0.157 g (78%) of **26**: ^{1}H NMR (DMSO-d_6, TMS) δ 8.01 (1H, d, $^4J_{HH}$ = 2.0, C7 ArH), 7.92 (2H, d, $^3J_{HH}$ = 8.0, *o*-NC ArH), 7.84 (1H, dd, $^4J_{HH}$ = 2.0, $^3J_{HH}$ = 9.0, C9 ArH), 7.78 (2H, d, $^3J_{HH}$ = 8.0, *m*-NC ArH), 7.61 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 6.82 (1H, br s, $^4J_{HH}$ = 1.0, NC(CH$_3$)**CH**N), 4.48 (1H, d, $^2J_{HH}$ = 15.0, **NCHHC**=N), 4.33 (1H, d, $^2J_{HH}$ = 15.0, **NCHHC**=N), 4.00 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.77 (2H, br t, **NCH$_2$**CH$_2$CO$_2$), 2.58 (2H, m, **CH$_2$**CO$_2$Et), 2.30 (3H, d, $^4J_{HH}$ = 1.0, NC(**CH$_3$**)CH), 1.13 (3H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) δ 171.3, 166.1, 135.5, 134.2, 133.1, 132.1, 128.1, 127.3, 121.6, 118.3, 111.9, 91.4, 89.5, 60.7, 45.7, 45.1, 32.7, 14.1, 11.1.

Method E (Table 2). This method is described for the preparation of **27a**. Compound **27c** was prepared similarly.

4*H*-[1,2,3,4]Tetrazolo[4,5*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[(4-cyanophenyl)methoxy]-6-oxo ethyl ester (27a). Compound **8a** (1.0 g, 2.5 mmol) was dissolved in THF (anhyd., 20 mL) and treated with DEAD (0.39 mL, 2.5 mmol, 1.0 equiv.), triphenylphosphine (0.65 g, 2.5 mmol, 1.0 equiv.), and trimethylsilylazide (0.33 mL, 2.5 mmol, 1.0 equiv.). The reaction mixture was magnetically stirred for 24 h at room temperature, treated with an additional 1.0 equiv. of DEAD, triphenylphosphine, and trimethylsilylazide and stirred for an additional 48 h. The reaction mixture was concentrated in vacuo and purified by sequential column chromatography (30% EtOAc/hexane then 100% EtOAc gradient elute for the first column, followed by a second chromatography with 5% acetone/CH_2Cl_2 then 10% acetone/CH_2Cl_2 gradient elute) to afford 0.79 g (72%) of **27a**: mp 64–67 °C; TLC R_f = 0.11 (50% EtOAc/hexane, UV positive); ^{1}H NMR ($CDCl_3$, TMS) δ 7.87 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 7.72 (2H, d, $^3J_{HH}$ = 8.0, NC Ar H), 7.66 (1H, d, $^4J_{HH}$ = 3.0, C7 ArH), 7.59 (2H, d, $^3J_{HH}$ = 8.0, NC ArH), 7.34 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 3.0 C9 ArH), 5.27 (2H, s, **OCH$_2$**Ar), 4.85 (2H, s, **NCH$_2$**C = N), 4.13 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.93 (2H, t, $^3J_{HH}$ = 6.0, **NCH$_2$**CH$_2$CO$_2$), 2.70 (2H, t, $^3J_{HH}$ = 6.0, **CH$_2$**CO$_2$Et), 1.24 (3H, s, OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) δ 171.4, 165.2, 158.7, 151.7, 141.0, 132.5, 128.5, 127.6, 121.0, 123.8, 120.7, 118.4, 117.0, 112.1, 69.4, 61.0, 46.2, 41.3, 33.0, 30.9, 14.1; HRMS (FAB) *m/z* calcd for $C_{22}H_{21}N_6O_4$ 433.1624, found: 433.1596.

4*H*-[1,2,3,4]Tetrazolo[4,5*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[(4-cyanophenyl)ethynyl]-6-oxo ethyl ester (27c). Thus **25** (1.0 g, 2.49 mmol) was converted into **27c** in 68% yield after sequential column chromatography first using 3% acetone/dichloromethane then 9% acetone/dichloromethane gradient elute followed by a second chromatography using 50% ethyl acetate/hexane then 80% ethyl acetate/hexane gradient elute: mp 167–169 °C; TLC R_f = 0.21 (50% EtOAc/hexane, UV positive); ^{1}H NMR ($CDCl_3$, TMS) δ 8.30 (1H, d, $^4J_{HH}$ = 2.0, C7 ArH), 7.97 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 7.86 (1H, dd, $^4J_{HH}$ = 2.0, $^3J_{HH}$ = 9.0, C9 ArH), 7.68 (2H, d, $^3J_{HH}$ = 8.0, NC ArH), 7.64 (2H, d, $^3J_{HH}$ = 8.0, NC ArH), 4.88 (2H, s, **NCH$_2$**C = N), 4.15 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.95 (2H, t, $^3J_{HH}$ = 6.0, **NCH$_2$**CH$_2$CO$_2$), 2.73 (2H, t, $^3J_{HH}$ = 6.0, **CH$_2$**CO$_2$Et), 1.25 (3H, s, OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) δ 171.5, 164.8, 152.1, 136.3, 135.8, 132.2, 129.7, 127.3, 126.9, 124.5, 122.3, 118.2, 112.4, 90.7, 64.1, 46.5, 41.3, 33.0, 14.2; HRMS (FAB) *m/z* calcd for $C_{23}H_{18}N_6O_3$: 427.1519, found: 427.1489.

Method F (Table 2). This method is described for the preparation of **27b**.

4-*H*-[1,2,3,4]Tetrazolo[4,5*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[(4-cyanobenzoyl)amino]-6-oxo ethyl ester (27b). Benzodiazepinedione **8c** (0.312 g, 0.74 mmol) was dissolved in glyme (anhyd., 2.0 mL) and treated with triphenylphosphine (0.126 g, 1.48 mmol, 2.0 equiv.), trimethylsilylazide (0.20 mL, 1.48 mmol, 2.0 equiv.), and diethyl azodicarboxylate (258 mg, 0.233 mL, 1.48 mmol, 2.0 equiv.). The reaction mixture was stirred at room temperature for 16 h, concentrated in vacuo and the crude product purified first by silica column chromatography (50% EtOAc/hexane) and then via preparative RP HPLC to yield 170 mg (52%) of **27b**: mp 197–200 °C: TLC R_f = 0.30 (67% EtOAc/hexane, UV positive); RP HPLC (method **P6**) t_R = 50–56 min; ^{1}H NMR (DMSO-d_6, TMS) δ 10.94 (1H, s, ArCONHAr), 8.46 (1H, d, $^4J_{HH}$ = 2.4, C7 ArH), 8.30 (1H, dd, $^4J_{HH}$ = 2.4, $^3J_{HH}$ = 8.7, C9 ArH), 8.17 (2H, d, $^3J_{HH}$ = 8.1, *o*-NC ArH), 8.06 (2H, d, $^3J_{HH}$ = 8.1, *m*-NC ArH), 7.97 (1H, d, $^3J_{HH}$ = 8.7, C10 ArH), 4.97 (2H, s, **NCH$_2$**C = N), 3.95 (2H, q, $^3J_{HH}$ = 6.9, **OCH$_2$**), 3.86 (2H, t, $^3J_{HH}$ = 6.9, **NCH$_2$**CH$_2$CO$_2$), 2.59 (2H, t, $^3J_{HH}$ = 6.9, **CH$_2$**CO$_2$Et), 1.09 (3H, t, $^3J_{HH}$ = 6.9, OCH$_2$**CH$_3$**); ^{1}H NMR ($CDCl_3$, TMS) δ 8.60 (1H, br s, ArCONHAr), 8.39 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 2.4 C9 ArH), 8.20 (1H, d, $^4J_{HH}$ = 2.4, C7 ArH), 8.06 (2H, d, $^3J_{HH}$ = 8.0, *o*-NC ArH), 7.98 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 7.83 (2H, d, $^3J_{HH}$ = 8.0, *m*-NC ArH), 4.87 (2H, s, **NCH$_2$**C = N), 4.15 (2H, q, $^3J_{HH}$ = 7.2, **OCH$_2$**), 3.94 (2H, t, $^3J_{HH}$ = 6.3, **NCH$_2$**CH$_2$CO$_2$), 2.72 (2H, t, $^3J_{HH}$ = 6.3, **CH$_2$**CO$_2$Et), 1.26 (3H, t, $^3J_{HH}$ = 7.2 , OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) δ 171.4, 165.7, 164.5, 151.8, 139.8, 137.8, 132.3, 128.3, 127.2, 125.9, 125.0, 123.8, 122.9, 117.8, 115.5, 61.1, 46.3, 41.3, 32.9, 14.0; LRMS (FAB, M + H$^+$) 446.16; HRMS (FAB) *m/z* calcd for $C_{22}H_{20}N_7O_4$, 446.1577; found: 446.1569. Anal. calcd for $C_{22}H_{19}N_7O_4$(0.20 $C_4H_8O_2$): C, 59.14; H, 4.48; N, 21.17. Found: C, 59.43; H, 4.32; N, 20.86.

1-Ethyl-4-(2-carboxyethyl)-7-(4-cyano)benzamido-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (28). A small pressure tube (Kontes) equipped with a stir bar was charged with **8c** (100 mg, 0.24 mmol) dissolved in DMF (anhyd., 3.0 mL) and treated under an atmosphere of nitrogen with cesium carbonate (155 mg, 0.47 mmol, 1.96 equiv.) and ethyl bromide (26.1 mg, 17.9 mL, 0.24 mmol, 1.0 equiv.). The pressure tube was sealed, placed in an oil bath and heated to 60 °C. After 30 min at 60 °C, the pressure tube was removed from the oil bath, allowed to cool to room temperature and stirred for 2 h. The DMF solution was poured into a separatory funnel containing ethyl acetate (80 mL) and water (15 mL) and the pressure tube rinsed with additional ethyl acetate. The layers were separated and the ethyl acetate washed with water (2×15 mL), brine (1×15 mL), dried ($MgSO_4$), filtered and concentrated in vacuo to afford 77 mg (83%) of a clear syrup which was analyzed

by TLC (100% EtOAc eluent), analytical RP HPLC, and MS. All three analytical methods showed the presence of **8c** (30% by HPLC, $t_R = 4.29$ min) and a new product (70% by HPLC, $t_R = 4.38$ min). The new product was less polar on silica (R_f (new) = 0.66, 100% EtOAc, UV positive, R_f (**8c**) = 0.40, 100% EtOAc, UV positive). MS analysis of the crude reaction mixture showed the major component to have a mass of 449.2 consistent with a mono alkylated product and a minor component with a mass of 421.2 which is consistent with the mass for unreacted **8c**. Preparative chromatography (67% EtOAc/hexane, then 75% EtOAc/hexane to elute **28**, then 100% EtOAc to elute **8c**) afforded 45 mg (48%) of pure **28** and 13 mg of **8c**. The material prepared by alkylation of **8c** was identical in all respects to **28** prepared by the route described in Scheme 2 starting with *N*-ethyl isatoic anhydride indicating alkylation took place on the nitrogen of the N^1–C^2 amide and not on the amide in the linker: TLC $R_f = 0.66$ (100% EtOAc/hexane, UV positive); RP HPLC (method **A6**) t_R = 4.38 min; ^{1}H NMR (DMSO-d_6, TMS) δ 10.7 (1H, br s, ArCO**NH**Ar), 8.13 (3H, br d, $^3J_{HH} = 8.1$, NC ArH and C6 or C8 ArH), 8.04 (3H, br d, $^3J_{HH} = 8.1$, NC ArH and C6 or C8 ArH), 7.49 (1H, d, $^3J_{HH} = 9.3$, C9 ArH), 4.1–4.0 (4H, m, $^3J_{HH} = 6.9$, **OCH$_2$**CH$_3$ and **NCH$_2$**CH$_3$), 3.90–3.72 (4H, m, **NCH$_2$**C=O and **NCH$_2$** CH$_2$CO$_2$), 2.61 (2H, t, $^3J_{HH} = 6.9$, **CH$_2$**CO$_2$Et), 1.18 (3H, t, $^3J_{HH} = 7.0$, NCH$_2$**CH$_3$**), 1.04 (3H, t, $^3J_{HH} = 7.0$, OCH$_2$**CH$_3$**); LRMS (FAB, M + H$^+$) 449.2.

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)phenyl]ethynyl]-1-methyl-6-oxo ethyl ester acetate (29a). An oven dried 25-mL 14/20 round-bottom flask equipped with a stirring bar and a septum was cooled under argon and charged with **24e** (150 mg, 0.34 mmol). The aryl iodide was dissolved in dimethylacetamide (anhyd., 5.0 mL) and the DMA solution treated sequentially with 4-ethynylbenzamidine[42] (98 mg, 0.68 mmol, 2.0 equiv.), triethylamine (172 mg, 0.24 mL, 1.7 mmol, 5.0 equiv.), and copper iodide (6.5 mg, 0.0034 mmol, 0.01 equiv.) with degassing and purging of the reaction vessel with Ar after the addition of each reagent. A catalytic amount of $(PPh_3)_2PdCl_2$ (2.5 mg, 0.0036 mmol, 0.01 equiv.) was added, the reaction vessel degassed, purged with Ar and heated to 50 °C for 3 h. After cooling to room temperature the DMA was removed in vacuo and the crude residue purified by preparative chromatography (H_2O/HOAc/EtOH/CH_2Cl_2 eluent 10/10/20/60) to afford 100 mg (57%) of the amidino ester **29a**. TLC $R_f = 0.59$ (H_2O/HOAc/EtOH/CH_2Cl_2 10/10/20/60, UV positive). RP HPLC (method **A4**) $t_R = 8.85$ min (94% purity). RP HPLC (method **A7**) $t_R = 4.99$ min (86% purity). ^{1}H NMR (DMSO-d_6, TMS) δ 9.90 (2H, br s, **H$_2$NC=NH**, only two exchangeable protons observed), 8.04 (1H, s, $^4J_{HH} < 1.0$, C7 ArH), 7.92 (1H, d, $^3J_{HH} = 8.50$, $^4J_{HH} < 1.0$, C9 ArH), 7.88 (2H, d, $^3J_{HH} = 9.0$, H_2NC=NH ArH), 7.80 (2H, d, $^3J_{HH} = 8.0$, H_2NC=NH ArH), 7.76 (1H, d, $^3J_{HH} = 8.55$, C10 ArH), 4.76 (2H, d, $^2J_{HH} = 16.0$, NC**H**HC=N), 4.49 (2H, d, $^2J_{HH} = 16.0$, NCH**H**C=N), 4.02 (2H, q, $^3J_{HH} = 7.0$, **OCH$_2$**CH$_3$), 3.79 (2H, m, **NCH$_2$**CH$_2$CO$_2$), 2.59 (2H, m, **CH$_2$**CO$_2$Et), 2.56 (3H, s, N (**CH$_3$**)C=N), 1.15 (3H, t, $^3J_{HH} = 7.0$, OCH$_2$**CH$_3$**). LRMS (FAB, M + H) 457.16. HRMS (FAB) *m/z* calcd for $C_{25}H_{25}N_6O_3$ 457.1988, found: 457.1990.

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)phenyl]ethynyl]-1-*tert*-butyl-6-oxo ethyl ester trifluoroacetate (29b). Amidino ester **29b** was prepared using the same experimental procedure described for the preparation of **29a** with the modification that the material obtained from the preparative chromatography was subjected to an additional purification via preparative RP HPLC (method **P10**). Thus, 150 mg of aryl iodide **24f** (0.31 mmol) was converted to 76.7 mg of amidino ester **29b** (40% yield) after sequential chromatography on silica gel (H_2O/HOAc/EtOH/CH_2Cl_2 10/10/20/60) followed by RP HPLC. TLC $R_f = 0.97$ (H_2O/HOAc/EtOH/CH_2Cl_2 10/10/20/60, UV positive). RP HPLC (method **P10**) t_R = 35–41 min RP HPLC (method **A4**) $t_R = 9.67$ min (87% purity). RP HPLC (method **A7**) $t_R = 5.92$ min. (83.5% purity). ^{1}H NMR (DMSO-d_6, TMS) δ 9.40 (1H, br s, H_2NC=**NH**), 9.25 (2H, br s, **H$_2$NC**=NH), 7.97 (1H, d, $^4J_{HH} = 2.0$, C7 ArH), 7.92 (1H, dd, $^3J_{HH} = 8.3$, $^4J_{HH} = 2.0$, C9 ArH), 7.89 (2H, d, $^3J_{HH} = 9.0$, H_2NC=NH ArH), 7.85 (2H, d, $^3J_{HH} = 9.0$, H_2NC=NH ArH), 7.75 (1H, d, $^3J_{HH} = 8.3$, C10 ArH), 4.72 (1H, d, $^2J_{HH} = 16.0$, NC**H**HC=N), 4.33 (1H, d, $^2J_{HH} = 16.0$, NCH**H**C=N), 4.02 (2H, q, $^3J_{HH} = 7.0$, **OCH$_2$**CH$_3$), 3.75 (2H, t, $^3J_{HH} = 7.0$, **NCH$_2$**CH$_2$CO$_2$), 2.56 (2H, t, $^2J_{HH} = {}^3J_{HH} = 7.0$, $^3J_{HH} = 2.0$, **CH$_2$**CO$_2$Et), 1.16 (3H, t, $^3J_{HH} = 7.0$, OCH$_2$**CH$_3$**). LRMS (FAB, M + H) 499.24. HRMS (FAB) *m/z* calcd for $C_{28}H_{31}N_6O_3$ 499.2457, found: 499.2438.

Method G (Table 3). The general procedure is detailed for the preparation of **29d**. Compounds **29e**, **29g**, **29h**, and **29j** were also prepared using **Method G** with the modifications noted.

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)phenyl]methoxy]-1-methyl-6-oxo ethyl ester acetate (29d). A solution of **24b** (0.50 g, 1.12 mmol) was dissolved in pyridine (3 mL)/diethylamine (3 mL) and saturated with hydrogen sulfide gas (bubbled into the solution via a pipette) at room temperature. The reaction was monitored by TLC (100% EtOAc) and determined to be complete after 3 h. The mixture was concentrated in vacuo, dissolved in 3 mL of methylene chloride, treated with

methyl iodide (2.0 mL) and heated to 50 °C for 30 min. The volatiles were removed in vacuo, the residue dissolved in ethanol (5.0 mL) and the ethanolic solution treated with ammonium acetate (0.5 g). The reaction mixture was heated to 50 °C for 30 min, cooled to room temperature and the crude amidino ester purified by preparative RP HPLC to afford 0.150 g of **29d** (26%). RP HPLC (method **P3**) t_R = 36.1 min; RP HPLC (method **A4**) t_R = 9.09 min (93% purity). RP HPLC (method **A7**) t_R = 4.46 min (≥99% purity). ^{1}H NMR (D_2O, HOD) δ 7.60 (2H, d, $^3J_{HH}$ = 8.0, *m*-H_2NC=NH ArH), 7.46 (2H, $^3J_{HH}$ = 8.0, *o*-H_2NC=NH ArH), 7.33 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 7.23 (1H, d, $^4J_{HH}$ = 3.0, C7 ArH), 7.18 (1H, dd, $^4J_{HH}$ = 3.0, $^3J_{HH}$ = 9.0, C9 ArH), 5.05 (2H, s, **OCH$_2$**Ar), 4.48 (1H, d, $^2J_{HH}$ = 16.0, **NCH**HC=N), 4.27 (1H, d, $^2J_{HH}$ = 16.0, **NCH**HC=N), 3.93 (1H, m, **NCH**HCH$_2$CO$_2$), 3.74 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.48 (1H, dt, $^2J_{HH}$ = 14.0, $^3J_{HH}$ = 7.0, **NCH**HCH$_2$CO$_2$), 2.55 (1H, m, **CH**HCO$_2$Et), 2.40 (1H, dt, $^2J_{HH}$ = 15.0, $^3J_{HH}$ = 6.0, **CH**HCO$_2$Et), 2.33 (3H, s, NC(**CH$_3$**)N), 0.85 (3H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**). LRMS (FAB, M+H) 463.21 HRMS (FAB) *m/z* calcd for $C_{24}H_{27}N_6O_4$ 463.2094, found: 463.2088.

Quinazolino[3,2-*a*][1,4]benzodiazepine-6(5*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)phenyl]methoxy]-7,13-dihydro-5,13-dioxo ethyl ester (29e). Subjecting compound **24c** (0.3 g, 0.6 mmol) to hydrogen sulfide (mp thioamide 240–242 °C), MeI/CH_2Cl_2, and ammonium acetate in ethanol resulted a product that precipitated from the reaction mixture. Isolation via vacuum filtration followed by sequential washing of the white powder with 1/1 ethanol/ether, then ether afforded 0.22 g (71%) of an inseparable 80/20 mixture of **29e**/**24c**. mp (80/20 mixture) 235 238 °C. RP HPLC (method **A4**) t_R (**29e**/**24c**) = 9.49 min/9.82 min. ^{1}H NMR (DMSO-d_6, TMS, 80/20 mixture) δ 8.21 (2H, br s, **H$_2$NC**=**NH**, only 2 exchangeable protons detected), 8.16 (1H, d, $^3J_{HH}$ = 7.90, quinazoline ArH, 29e), 7.88 (1H, apparent t, $^3J_{HH}$ = 8.6, $^3J_{HH}$ = 8.1, quinazoline ArH, **29e**), 7.81 (2H, d, $^3J_{HH}$ = 8.12 *o*-H_2NC=NH ArH, **29e**), 7.71 (2H, d, $^3J_{HH}$ = 8.33, *m*-H_2NC=NH ArH, **29e**), 7.68 (1H, d, $^3J_{HH}$ = 8.33, quinazoline ArH, **29e**), 7.59 (1H, apparent t, $^3J_{HH}$ = 7.3, quinazoline ArH, **29e**), 7.56 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH, **29e**), 7.32 (1H, d, $^4J_{HH}$ = 3.0, C7 ArH, **29e**), 7.29 (1H, dd, $^4J_{HH}$ = 3.0, $^3J_{HH}$ = 9.0, C9 ArH, **29e**), 5.35 (2H, br s, **OCH$_2$**Ar, **29e**), 4.47 (1H, d, $^3J_{HH}$ = 15.40, **NCH**HC=N, **29e**), 4.33 (1H, d, $^3J_{HH}$ = 15.40, **NCH**HC=N, **29e**), 3.98 (1H, m, **NCH**HCH$_2$CO$_2$, **29e**), 3.79 (1H, q for **OCH$_2$** of **29e**, $^3J_{HH}$ = 7.0, overlapping with a multiplet from **NCH**HCH$_2$CO$_2$ of **24c**), 3.73 (1H, q for **OCH$_2$** of **29e**, $^3J_{HH}$ = 7.0, overlapping with a multiplet from **NCH**HCH$_2$CO$_2$ of **24c**), 3.64 (1H, m, **NCH**HCH$_2$CO$_2$, **29e**), 2.71 (1H, m, **CH**HCO$_2$Et, **29e**), 2.53 (1H, m, partially obscured by DMSO-d_6 peak, **CH**HCO$_2$Et, **29e**), 1.04 (0.6H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**, **24c**), 0.96 (2.4H, t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**, **29e**). LRMS (ES, M+H) 509.2 (24c), LRMS (ES, M+H) 526.4 (**29e**). LRMS (FAB, M+H$^+$) 526.21 (**29e**). HRMS (FAB) *m/z* calcd for $C_{29}H_{28}N_5O_5$ 526.2090, found: 526.2089 (**29e**).

4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(aminoiminomethyl)phenyl]ethynyl]-1-methyl-6-oxo ethyl ester acetate (29g). Heterocycle **26** (0.15 g) was converted into amidino ester **29g** with the following modification to Method G (Table 3): the thioamide was purified by column chromatography (100% ethyl acetate) prior to treatment with MeI. The crude amidino ester was purified by preparative RP HPLC to yield 25 mg (16%) of **29g**: RP HPLC (method **P1**) t_R = 27.6 min; ^{1}H NMR (D_2O, HOD) δ 7.56 (1H, d, $^3J_{HH}$ = 9.6, C10 ArH), 7.54 (2H, d, $^3J_{HH}$ = 11.3, *o*-H_2NC=NH ArH), 7.40 (1H, br d, $^3J_{HH}$ = 9.6, C9 ArH), 7.29 (1H, br s, C7 ArH), 7.23 (2H, d, $^3J_{HH}$ = 11.3, *m*-H_2NC=NH ArH), 6.58 (1H, br s, NC(CH$_3$)**CH**N), 4.25 (1H, d, $^2J_{HH}$ = 15.0, **NCH**HC=N), 4.14 (1H, d, $^2J_{HH}$ = 15.0, **NCH**HC=N), 3.83 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.60 (2H, m, **NCH$_2$**CH$_2$CO$_2$), 2.41 (2H, br t, **CH$_2$** CO$_2$Et), 2.10 (3H, br s, NC (**CH$_3$**)CHN), 0.93 (3H,t, $^3J_{HH}$ = 7.0, OCH$_2$**CH$_3$**). LRMS (FAB, M + H) 456.1.

4*H*-[1,2,3,4]Tetrazolo[4,5*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[(4-(iminoaminomethyl)phenyl)methoxy]-6-oxo ethyl ester acetate (29h). Amidino ester **29h** was prepared from **27c** (0.5 g, 1.16 mmol) in 66% yield (343 mg) after preparative RP HPLC purification (method **P3**): mp 213–216 °C; RP HPLC (method **A1**) t_R = 5.15 min (85% purity); RP HPLC (method **A4**) t_R = 8.36 min (80% purity); ^{1}H NMR (CD$_3$OD, TMS) δ 7.88 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 7.84 (2H, d, $^3J_{HH}$ = 8.0, H_2NC=NH ArH) 7.73 (2H, d, $^3J_{HH}$ = 8.0, H_2NC=NH ArH), 7.62 (1H, d, $^4J_{HH}$ = 3.0, C7 ArH), 7.47 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 3.0, C9 ArH), 5.36 (2H, s, **OCH$_2$**Ar), 4.90 (2H, s, **NCH$_2$**C=N), 4.01 (2H, q, $^3J_{HH}$ = 7.0, **OCH$_2$**), 3.93 (2H, t, $^3J_{HH}$ = 6.0, **NCH$_2$** CH$_2$CO$_2$), 2.64 (2H, t, $^3J_{HH}$ = 6.0, **CH$_2$**CO$_2$Et), 1.14 (3H, t, $^3J_{HH}$ = 7.0 OCH$_2$**CH$_3$**); ^{13}C NMR (CDCl$_3$) δ 172.9, 168.2, 167.3, 160.4, 154.0, 144.4, 129.9, 129.2, 125.5, 125.1, 121.6, 118.3, 70.6, 61.9, 47.1, 41.7, 33.9, 22.6, 14.5. HRMS (FAB) *m/z* calcd for $C_{22}H_{21}N_7O_4$ 450.1890, found: 450.1869.

4*H*-[1,2,3,4]Tetrazolo[4,5*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-iminoaminomethyl)phenyl]ethynyl]-6-oxo ethyl ester acetate (29j). Compound **29j** was prepared from **27c** (0.5 g, 1.17 mmol) in 34% yield (175 mg) after preparative reverse phase purification (method **P1**). RP HPLC (method **A4**) t_R (**29j**) = 8.38 min (97% purity). RP HPLC (method **A7**) t_R = 4.16 min (93% purity). ^{1}H NMR (CD$_3$OD, TMS) δ 8.23 (1H, d, $^4J_{HH}$ = 2.0, C7 ArH), 8.03 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH),

7.97 (1H, dd, $^4J_{HH}=2.0$, $^3J_{HH}=9.0$, C9 ArH), 7.84 (2H, d, $^3J_{HH}=9.0$, $H_2NC=NH$ ArH), 7.74 (2H, d, $^3J_{HH}=9.0$, $H_2NC=NH$ ArH), 4.96 (2H, s, $NCH_2C=N$), 4.04 (2H, q, $^3J_{HH}=7.0$, OCH_2), 3.97 (2H, t, $^3J_{HH}=6.0$, $NCH_2CH_2CO_2$), 2.67 (2H, t, $^3J_{HH}=6.0$, CH_2CO_2Et), 1.15 (3H, s, OCH_2CH_3). LRMS (FAB, $M+H^+$) 444.18. HRMS (FAB) *m/z* calcd. for $C_{23}H_{22}N_7O_3$ 444.1784, found: 444.1773.

Method H (Table 3). The general procedure is detailed for the preparation of **29f**.

4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(aminoiminomethyl) benzoyl] amino]-1-methyl-6-oxo, ethyl ester, trifluoroacetate (29f). (a) Preparation of 4*H*-imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-thioamidobenzoyl] amino-1-methyl-6-oxo, ethyl ester. Imidazo compound **24g** (80 mg, 0.17 mmol) was dissolved in pyridine/triethylamine (anhyd., 2 mL/Fluka purissima, 1 mL), transferred to a pressure tube (Kontes, no. 15, 2.5 cm (OD)×9.5 cm) equipped with a stir bar, and the pyridine/Et_3N solution saturated with hydrogen sulfide gas via a pipette. The pressure tube was sealed, heated to 65 °C and stirred at 65 °C for 30 min. The reaction mixture was cooled to room temperature and the solution poured into a separatory funnel containing ethyl acetate (30 mL)/water (30 mL). After thorough mixing, the aqueous phase was removed and the organic phase was washed with water (20 mL), brine (30 mL), dried ($MgSO_4$), filtered and concentrated in vacuo to give 75 mg (90%) of the thioamide. ^{1}H NMR ($CDCl_3$, TMS) δ 9.47 (1H, s, ArCONHAr), 8.46 (1H, dd, $^3J_{HH}=8.7$, $^4J_{HH}=2.4$, C9 ArH), 8.23 (1H, br s, **NH**), 8.11 (1H, d, $^4J_{HH}=2.7$, C7 ArH), 8.03 (1H, br s, **NH**), 7.71 (2H, d, $^3J_{HH}=8.0$, *o*-$H_2NC=S$ ArH), 7.67 (1H, m, C10 ArH), 7.30 (2H, m, *m*-$H_2NC=S$ ArH), 6.88 (1H, s, $NC(CH_3)CHN$), 4.45 (1H, d, $^2J_{HH}=15.6$, $NCHHC=N$), 4.33 (1H, d, $^2J_{HH}=15.6$, $NCHHC=N$), 4.13 (2H, q, $^3J_{HH}=7.0$, OCH_2), 3.68 (2H, m, $NCH_2CH_2CO_2Et$), 2.57 (2H, t, $^3J_{HH}=6.9$, CH_2CO_2Et), 2.33 (3H, s, $NC(CH_3)CHN$), 1.25 (3H, t, $^3J_{HH}=7.0$, OCH_2CH_3). LRMS (FAB $M+H^+$) 492. Exact mass (FAB) *m/z* calcd for $C_{25}H_{26}N_5SO_4$ 492.1706, found: 492.1715.

(b) 4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-thioamidobenzoyl]amino-1-methyl-6-oxo, ethyl ester (75 mg, 0.16 mmol) was suspended in anhydrous acetonitrile (3 mL) in a pressure tube (Kontes, no. 15, 2.5 cm (OD)×9.5 cm) and the slurry was treated with methyl iodide (15 mL, 250 mmol, xs). The pressure tube was sealed and the heterogeneous solution heated to 85 °C (oil bath) and stirred at 85 °C for 1 h after which time the reaction mixture was a homogeneous solution. The solution was transferred to an oven dried 24/40 round-bottomed flask (which had been cooled under nitrogen) and the pressure tube rinsed with anhydrous acetonitrile (3.0 mL). The volatiles were removed under high vacuum, taking care to avoid exposure to moisture and the crude product was dissolved in absolute ethanol (3 mL). Under a blanket of nitrogen the ethanolic solution was treated with ammonium acetate (52 mg, 0.68 mmol, 4.25 equiv.), the round-bottomed flask capped with a rubber septum which was punctured with an 18 gauge needle to allow the methyl mercaptan to escape, and the reaction mixture stirred overnight at room temperature. The solution was concentrated in vacuo and the crude product purified by preparative RP HPLC to yield 24.5 mg (24%) of **29f** (TFA salt form), after lyophilization. RP HPLC (method **P4**) $t_R=33$–40 min. RP HPLC (method **A4**) $t_R=8.02$ min (82% purity). RP HPLC (method **A7**) $t_R=4.26$ min (86% purity). ^{1}H NMR (DMSO-d_6, TMS) δ 10.8 (1H, s, ArCONHAr), 9.45 (1H, br s, **NH**), 9.12 (2H, br s, **NH**), 8.33 (1H, d, $^4J_{HH}=2.7$, C7 ArH), 8.21 (1H, dd, $^3J_{HH}=11.7$, $^4J_{HH}=2.7$, C9 ArH), 8.20 (2H, d, $^3J_{HH}=8.4$, *o*-$H_2NC=NH$ ArH), 7.97 (2H, d, $^3J_{HH}=8.4$, *m*-$H_2NC=NH$ ArH), 7.66 (1H, d, $^3J_{HH}=11.7$, C10 ArH), 7.06 (1H, s, $NC(CH_3)CHN$), 4.58 (2H, d, $^2J_{HH}=15.9$, $NCHHC=N$), 4.41 (2H, d, $^2J_{HH}=15.9$, $NCHHC=N$), 4.03 (2H, q, $^3J_{HH}=7.2$, OCH_2CH_3), 3.77 (2H, t, $^3J_{HH}=7.5$, $NCH_2CH_2CO_2Et$), 2.59 (2H, t, $^3J_{HH}=7.5$, CH_2CO_2Et), 2.35 (3H, s, $NC(CH_3)CHN$), 1.15 (3H, t, $^3J_{HH}=7.2$, OCH_2CH_3). LRMS (FAB, $M+H^+$) 475.2. HRMS (FAB) *m/z* calcd for $C_{25}H_{27}N_6O_4$ 475.2090, found: 475.2096.

Method I (Table 3). The general procedure is detailed for the preparation of **29i**.

4-*H*-[1,2,3,4]Tetrazolo[4,5-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)benzoyl]amino]-6-oxo, ethyl ester, trifluoroacetate (29i). (a) 4-*H*-[1,2,3,4] tetrazolo[4,5-a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-thioamidobenzoyl]amino]-6-oxo, ethyl ester was prepared from the benzonitrile **27b** (93 mg, 0.17 mmol) via the procedure described in the preparation of compound **29f** with the modification that in preparing the thioamide the sealed pressure tube was heated to 50 °C and stirred at 50 °C for 90 min. The reaction was worked up as described in part (a) of the experimental for compound **29f** to yield 60 mg of 4-*H*-[1,2,3,4]tetrazolo[4,5a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-thioamidobenzoyl] amino]-6-oxo, ethyl ester (76%). RP HPLC (method **A2**) t_R (thioamide) = 4.27 min. ^{1}H NMR (CD_3OD, TMS) δ 8.46 (1H, d, $^4J_{HH}=2.4$, C7 ArH), 8.26 (1H, dd, $^4J_{HH}=2.4$, $^3J_{HH}=9.0$, C9 ArH), 8.00 (2H, s, *o*-$H_2NC=S$ ArH), 7.99 (2H, s, *m*-$H_2NC=S$ ArH), 7.96 (1H, d, $^3J_{HH}=9.0$, C10 ArH), 4.94 (2H, s, $NCH_2C=N$), 4.05 (2H, q, $^3J_{HH}=7.2$, OCH_2), 3.97 (2H, t, $^3J_{HH}=6.3$, $NCH_2CH_2CO_2Et$), 2.69 (2H, t, $^3J_{HH}=6.3$, CH_2CO_2Et), 1.09 (2H,

t, $^3J_{HH}$ = 7.2, OCH_2CH_3). LRMS (FAB, M + H$^+$) 480 HRMS (FAB, M + H$^+$) *m/z* calcd for $C_{22}H_{22}N_7SO_4$ 480.1454, found: 480.1450.

(b) 4-*H*-[1,2,3,4]Tetrazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-thioamidobenzoyl]amino]-6-oxo, ethyl ester (0.21 g, 0.47 mmol) was suspended in methyl iodide (20 mL), and addition of *N*-methyl pyrrolidinone (2 mL) was required to achieve a homogeneous solution (the thioamide was sparingly soluble in acetonitrile). The reaction mixture was stirred overnight at room temperature and concentrated in vacuo to a volume of 2 mL, taking care to avoid exposure to moisture. Dry chloroform (5 mL) was added and the solution concentrated in vacuo to remove traces of methyl iodide. The resulting crude residue was placed under high vacuum for 15 min, dissolved in absolute ethanol (3 mL) and treated with 'anhydrous ammonium acetate' in excess (≥20 equiv.). 'Anhydrous' ammonium acetate was prepared by stirring ammonium acetate (1.2 g, 15 mmol) in anhydrous ethanol (9 mL) with freshly activated 3 A sieves for 16 h under argon at room temperature. The ethanol was decanted and the 3 A sieves plus solid ammonium acetate were placed under vacuum (1.0 mm Hg) for 2 h. Anhydrous ammonium acetate was added as a solid in one portion to the ethanolic solution of the S–Me imidate, the flask stoppered with a rubber septum that had been punctured with an 18 gauge needle (to vent) and stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and the crude product purified by preparative RP HPLC to yield 24.5 mg (24%) of **29i**. For the preparation of **29i**, the yield was significantly lower if ammonium acetate was used without removal of excess water. RP HPLC (method **P7**) t_R = 35–40 min. RP HPLC (method **A3**) t_R = 6.71 min (97% purity). RP HPLC (method **A7**) t_R = 6.25 min (96% purity). ^{1}H NMR (DMSO-d_6, TMS) δ 11.0 (1H, br s, ArCO**NH**Ar), 9.44 (1.5 H, br s, **H_2NC**=**NH**), 9.17 (1.5 H, br s, **H_2NC**=**NH**), 8.46 (1H, d, $^4J_{HH}$ = 2.4, C7 ArH), 8.31 (1H, dd, $^3J_{HH}$ = 8.7, $^4J_{HH}$ = 2.4, C9 ArH), 8.19 (2H, d, $^3J_{HH}$ = 8.4, *o*-H_2NC=NH ArH), 7.95 (3H, 2 overlapping d's, $^3J_{HH}$ = 8.7, $^3J_{HH}$ = 8.4, C10 ArH and *m*-H_2NC=NH ArH), 4.95 (2H, br s, N**CH_2**C=N), 3.93 (2H, q, $^3J_{HH}$ = 7.0, **OCH_2**), 3.85 (2H, t, $^3J_{HH}$ = 7.0, N**$CH_2CH_2CO_2$**Et), 2.57 (2H, t, $^3J_{HH}$ = 7.5, **CH_2CO_2**Et), 1.07 (3H, t, $^3J_{HH}$ = 7.0, OCH_2**CH_3**). LRMS (FAB, M + H$^+$) 463.18. HRMS (FAB) *m/z* calcd for $C_{22}H_{23}N_8O_4$ 463.1842, found 463.1835.

Method J (Table 3). The general procedure is illustrated for preparation and isolation of **30a**. Compounds **30b** and **30d** were similarly prepared using ethanol as the solvent for the saponification, sodium hydroxide as the base (5.0 equiv.), and acetic acid to quench. After concentration to dryness, the crude amidino acids were purified by preparative RP HPLC. The reaction for the conversion of **29d** to **30d** was complete in 45 min. Amidino acids **30c**, **30g**, **30h**, and **30j** were prepared as described for **30a** with the modification that methanol was used as the solvent for the saponification.

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)phenyl]ethynyl]-1-methyl-6-oxo, trifluoroacetate (30a). Amidino ester **29a** (20 mg, 0.04 mmol) was dissolved in absolute ethanol (2 mL), treated with aqueous 2 N sodium hydroxide (0.1 mL, 0.20 mmol, 5.0 equiv.) and stirred at room temperature for 1 h with the conversion of **29a** to **30a** monitored by RP analytical HPLC. The reaction mixture was quenched by addition of acetic acid (0.5 mL), concentrated in vacuo and purified by preparative RP HPLC (method **P10**) to afford 15.5 mg of **30a** (82%) as a white powder after lyophilization from CH_3CN/H_2O w/0.1% TFA. RP HPLC (method **A4**) t_R = 8.0 min (>99% purity). RP HPLC (method **A7**) t_R = 4.38 min (>99% purity). ^{1}H NMR (CD_3OD, TMS) δ 8.12 (1H, d, $^4J_{HH}$ = 2.0, C7 ArH), 7.94 (1H, dd, $^4J_{HH}$ = 2.0, $^3J_{HH}$ = 8.55, C9 ArH), 7.84 (2H, d, $^3J_{HH}$ = 8.8, H_2NC=NH ArH), 7.78 (2H,d, $^3J_{HH}$ = 8.8, H_2NC=NH ArH), 7.69 (1H, d, $^3J_{HH}$ = 8.30, C10 ArH), two doublets at approx. 4.8 and 4.4 ppm for N**CH_2**C=N are obscured by the HOD peak and coupling constants could not be determined, 3.90 (2H, m, N**$CH_2CH_2CO_2$**H), 2.73 (2H, m, **CH_2CO_2**H), 2.68 (3H, s, N(**CH_3**)C=N). LRMS (FAB, M + H$^+$) *m/z* 429.17. HRMS (FAB) *m/z* calcd for $C_{23}H_{21}N_6O_3$: 429.1675, found: 429.1695.

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)phenyl]ethynyl]-1-*tert*-butyl-6-oxo, trifluoroacetate (30b). Amidino ester **29b** (25 mg, 0.05 mmol) was converted via **Method J** (Table 3) to amidino acid **30b** which was purified by preparative RP HPLC purification and lyophilized from CH_3CN/H_2O w/0.1% TFA to yield 21 mg of **30b** (89%) a white powder. RP HPLC (method **A4**) t_R = 8.54 min (>99% purity). RP HPLC (method **A7**) t_R = 4.66 min (96% purity). ^{1}H NMR (CD_3OD, TMS) δ 8.05 (1H, d, $^4J_{HH}$ = 2.0, C7 ArH), 7.90 (1H, dd, $^4J_{HH}$ = 2.0, $^3J_{HH}$ = 8.4, C9 ArH), 7.83 (2H, d, $^3J_{HH}$ = 8.4, H_2NC=NH ArH), 7.78 (2H, d, $^3J_{HH}$ = 8.4, H_2NC=NH ArH), 7.76 (1H, d, $^3J_{HH}$ = 8.4, C10 ArH), 4.76 (1H, d, $^2J_{HH}$ = 16.4, N**C**HHC=N), 4.43 (1H, d, $^2J_{HH}$ = 16.4, NCHHC=N), 3.85 (2H, m, N**$CH_2CH_2CO_2$**H), 2.62 (2H, m, **CH_2CO_2**H), 1.40 (9H, s, N (**CH_3**)$_3$C=N). LRMS (FAB, M + H$^+$) 471.22. HRMS (FAB) *m/z* calcd for $C_{26}H_{26}N_6O_3$: 471.2145, found: 471.2165.

4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(aminoiminomethyl)phenyl]methoxy]-1-methyl-6-oxo, acetate (30c). (a) 4*H*-imidazo[1,2-*a*][1,4]benzo-

diazepine-5(6*H*)-propanoic acid, 8-[[4-cyanophenyl]-methoxy]-1-methyl-6-oxo, ethyl ester (0.1 g, 0.22 mmol) was converted into the amidino ester **29c** via **Method G** (Table 3) with the following modifications: Triethylamine (2 mL) was substituted for diethylamine in the reaction with hydrogen sulfide. In the conversion of the benzonitrile **24a** into the amidino ester, methanol was substituted for ethanol as the solvent for the reaction with ammonium acetate (0.25 g). The crude amidino ester **29c** was purified by preparative RP HPLC (method **P2**) and the fractions containing the major component concentrated in vacuo. The HPLC purified amidino ester was saponified in methanol and isolated as described for **30a** to yield 5 mg (5% overall from **24a**) of pure **30c** (acetate salt form). RP HPLC (method **A4**) $t_R = 8.44$ min. (97% purity). RP HPLC (method **A7**) $t_R = 5.10$ min. (89% purity). ^{1}H NMR (D_2O, HOD) δ 7.63 (2H, d, $^3J_{HH} = 8.0$, *m*-$H_2NC{=}NH$ ArH), 7.53 (2H, $^3J_{HH} = 8.0$, *o*-$H_2NC{=}NH$ ArH), 7.44 (1H, d, $^3J_{HH} = 9.0$, C10 ArH), 7.36 (1H, d, $^4J_{HH} = 3.0$, C7 ArH), 7.26 (1H, dd, $^4J_{HH} = 3.0$, $^3J_{HH} = 9.0$, C9 ArH), 7.17 (1H, br s, $^4J_{HH}$ ca. 1.0, NC(CH_3)**CH**N), 5.21 (2H, dd, **OCH$_2$**Ar), 3.79 (1H, dt, N**CH**HCH$_2$ CO_2), 3.78 (1H, d, $^2J_{HH} = 16.0$, N**CH**HC=N), 3.63 (2H, 1H, dt, NCH**H**CH$_2CO_2$ and 1H, d, $^2J_{HH} = 16.0$, NCH**H**C=N), 2.54 (2H, m, **CH$_2$CO$_2$**H), 2.22 (3H, s, N (**CH$_3$**)C=CH). LRMS (ES, M+H$^+$) 434.2. HRMS (FAB) *m/z* calcd for $C_{23}H_{24}N_5O_4$: 434.1828, found: 434.1794.

4*H*-[1,2,4]Triazolo[4,3-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(iminoaminomethyl) phenyl] methoxy]-1-methyl-6-oxo, acetate (30d). Compound **29d** (75 mg, 0.144 mmol) was saponified using the procedure described for the preparation of **30a**. Purification via by preparative RP HPLC (method **P3**) and lyophilization from CH_3CN/H_2O w/0.5% HOAc yielded 55.5 mg of **30d** (10% overall from **24b**) RP HPLC (method **P3**) $t_R = 33.2$ min. RP HPLC (method **A4**) $t_R = 8.58$ min (95% purity). RP HPLC (method **A7**) $t_R = 3.77$ min (95% purity). ^{1}H NMR (DMSO-d_6, TMS) δ 9.33 (1H, br s, $H_2NC{=}$**NH** or **CO$_2$H**), 9.0 (1H, br s, $H_2NC{=}$**NH** or **CO$_2$H**), 8.05 (2H, br s, **H$_2$NC**=NH), 7.83 (2H, d, $^3J_{HH} = 8.1$, $H_2NC{=}NH$ ArH), 7.70 (2H, d, $^3J_{HH} = 8.4$, $H_2NC{=}NH$ ArH), 7.62 (1H, d, $^3J_{HH} = 8.8$, C10 ArH), 7.44 (1H, d, $^4J_{HH} = 3.0$, C7 ArH), 7.37 (1H, dd, $^4J_{HH} = 3.0$, $^3J_{HH} = 8.8$, C9 ArH), 5.35 (2H, s, **OCH$_2$**Ar), 4.72 (1H, d, $^2J_{HH} = 16.1$, N**CH**HC=N), 4.37 (1H, d, $^2J_{HH} = 16.1$, NCH**H**C=N), 3.69 (2H, m, **NCH$_2$**CH$_2$ CO_2), 2.53 (3H, s, N (**CH$_3$**)C=N), 2.48 (2H, m, coincident with DMSO-d_6 peak, CH$_2$**CH$_2$**CO$_2$). LRMS (ES, M+H$^+$) 435.2. HRMS (FAB) *m/z* calcd for $C_{22}H_{23}N_6O_4$: 435.1781, found: 435.1744.

4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(aminoiminomethyl)phenyl]ethynyl]-1-methyl-6-oxo, acetate (30g). Amidino ester **29g** (25 mg, 0.055 mmol) was saponified in methanol using the procedure described for the preparation of **30a** to yield 20 mg (85%) of **30g** after preparative RP HPLC purification and lyophilization from CH_3CN/H_2O w/0.5% HOAc. RP HPLC (method **P1**) $t_R = 23.5$ min. RP HPLC (method **A4**) $t_R = 7.78$ min (97% purity). RP HPLC (method **A7**) $t_R = 4.35$ min (94% purity). ^{1}H NMR (DMSO-d_6, TMS) δ 9.55 (1H, br s, **H$_2$NC=NH**), 9.44 (1H, br s, **H$_2$NC=NH**, only two exchangeable protons observed), 8.07 (1H, d, $^4J_{HH} = 1.5$, C7 ArH), 7.92 (3H, d, $^3J_{HH} = 8.4$, $H_2NC{=}NH$ ArH and C9 ArH, $^4J_{HH}$ coupling constant could not be determined), 7.85 (2H, d, $^3J_{HH} = 8.1$, $H_2NC{=}NH$ ArH), 7.76 (1H, d, $^3J_{HH} = 8.4$, C10 ArH), 7.20 (1H, br s, N(CH_3)C=**CH**), 4.74 (1H, d, $^2J_{HH} = 16.9$, N**CH**HC=N), 4.48 (1H, d, $^2J_{HH} = 16.1$, NCH**H**C=N), 3.73 (2H, m, **NCH$_2$** CH_2CO_2), 2.55 (2H, m, NCH$_2$**CH$_2$**CO$_2$), 2.53 (3H, s, N(**CH$_3$**)C=CH), LRMS (ES, M+H$^+$) 428.5. HRMS (FAB) *m/z* calcd for $C_{24}H_{25}N_5O_3$: 428.1723, found: 428.1705.

4*H*-[1,2,3,4]Tetrazolo[4,5a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[(4-(iminoaminomethyl)phenyl)methoxy]-6-oxo, acetate (30h). Compound **29h** (50 mg, 0.1 mmol) was saponified in methanol using the procedure described for the preparation of **30a** to afford **30h** (30 mg) in 83% yield after preparative RP HPLC purification (method **P9**) and lyophilization from CH_3CN/H_2O w/0.5% HOAc. mp 238–240 °C dec.; RP HPLC (method **P9**) $t_R = 18.8$ min; RP HPLC (method **A4**) $t_R = 7.71$ min (>99% purity). RP HPLC (method **A7**) $t_R = 3.97$ min (91% purity). ^{1}H NMR (DMSO-d_6, TMS) δ 9.32 (1H, br s, **H$_2$NC=NH**), 9.06 (1H, br s, **H$_2$NC=NH**, only two exchangeable protons are observed), 7.87 (1H, d, $^3J_{HH} = 9.0$, C10 ArH), 7.84 (2H, d, $^3J_{HH} = 8.0$, **H$_2$NC=NH** ArH), 7.71 (2H, d, $^3J_{HH} = 8.0$, **H$_2$NC=NH** ArH), 7.58 (1H, d, $^4J_{HH} = 3.0$, C7 ArH), 7.50 (1H, dd, $^3J_{HH} = 9.0$, $^4J_{HH} = 3.0$, C9 ArH), 5.40 (2H, s, **NCH$_2$**C=N), 4.90 (2H, s, **OCH$_2$**Ar), 3.78 (2H, t, $^3J_{HH} = 7.0$, **NCH$_2$**CH$_2CO_2$H), 2.49 (2H, m, NCH$_2$ **CH$_2$**CO$_2$H, coincident with reference peak for DMSO-d_6). LRMS (FAB, M+H$^+$) 422.16. HRMS (FAB) *m/z* calcd for $C_{20}H_{20}N_7O_4$ 422.1577, found: 422.1583.

4*H*-[1,2,3,4]Tetrazolo[4,5a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-iminoaminomethyl)phenyl]ethynyl]-6-oxo acetate (30j). Saponification of **29j** (50 mg, 0.9 mmol) in methanol using the procedure described for the preparation of **30a** yielded 47 mg (95%) of **30j** after purification by RP HPLC (method **P9**) and lyophilization from CH_3CN/H_2O w/0.5% HOAc. RP HPLC (method **P9**) $t_R = 20.4$ min. RP HPLC (method **A4**) $t_R = 7.52$ min (>99% purity). RP HPLC (method **A7**) $t_R = 3.97$ min (>99% purity). ^{1}H NMR (DMSO-d_6 with 10 μL F_3CCO_2D, TMS) δ 9.39 (1H, br s, **H$_2$NC=NH**), 9.22 (1H, br s, **H$_2$NC=NH**, only two exchangeable

protons observed), 8.19 (1H, br s, C7 ArH), 8.10 (2H, apparent t, $^3J_{HH}$ = 8.30, C9 and C10 ArH), 7.88 (4H, br s, **H_2NC=NH** ArH), 4.99 (2H, s, N**CH_2**C=N), 3.80 (2H, t, $^3J_{HH}$ = 6.8, N**$CH_2CH_2CO_2$**), 2.54 (2H, t, $^3J_{HH}$ = 6.8 **CH_2CO_2**); HRMS (FAB) *m/z* calcd for $C_{21}H_{18}N_7O_3$: 416.1471, found: 416.1448.

Method K (Table 3). The general procedure is detailed for the preparation of **30e**.

Method L (Table 3). The general procedure is detailed for the preparation of **30e**.

Quinazolino[3,2-*a*][1,4]benzodiazepine-6(5*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)phenyl]methoxy]-7,13-dihydro-5,13-dioxo, acetate (30e). Amidino ester **29e** (50 mg, 0.085 mmol) was dissolved in THF (4 mL)/H_2O (2 mL) and the room temperature solution treated sequentially with 30% hydrogen peroxide (0.77 mL, 0.765 mmol, 9.0 equiv.) and lithium hydroxide (60 mg, 0.256 mmol, 3.0 equiv.). The reaction mixture was stirred for 2 h at room temperature and quenched by addition of a saturated solution of sodium bisulfite followed by acetic acid (0.25 mL). Concentration in vacuo and purification via preparative RP HPLC yielded 5.0 mg of **30e** (10%). It should be noted that the lithium hydroperoxide could also be preformed by dissolution of the lithium hydroxide (60 mg) in THF/H_2O (2 mL/1 mL) and treating with hydrogen peroxide (0.77 mL). The preformed reagent could then be added to a THF/H_2O (2 mL/1 mL) solution of **29e**. With the preformed reagent, **30e** was isolated in 39% yield. Alternatively, **30e** could be prepared via hydrolysis of amidino ester **29e** in 50% TFA/H_2O at 60 °C for 3 h. Concentration and purification of the crude reaction mixture via RP HPLC yielded **30e** as the TFA salt in 46% yield. RP HPLC (method **P7**) t_R = 40–45 min. RP HPLC (method **A4**) t_R = 5.95 min (>99% purity) RP HPLC (method **A7**) t_R = 8.75 min (95% purity) ^{1}H NMR (DMSO-d_6, TMS) δ 8.16 (1H, d, $^3J_{HH}$ = 8.1, quinazoline ArH), 7.88 (1H, apparent t, $^3J_{HH}$ = 7.3, $^3J_{HH}$ = 8.1, quinazoline ArH), 7.79 (2H, d, $^3J_{HH}$ = 8.1 *o*-H_2NC=NH ArH), 7.75 (2H, d, $^3J_{HH}$ = 8.1 *m*-H_2NC=NH ArH), 7.64 (1H, d, $^3J_{HH}$ = 8.43, quinazoline ArH), 7.58 (1H, apparent t, $^3J_{HH}$ = 7.3, quinazoline ArH), 7.54 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 7.30 (1H, d, $^4J_{HH}$ = 3.0, C7 ArH), 7.28 (1H, dd, $^4J_{HH}$ = 3.0, $^3J_{HH}$ = 9.0, C9 ArH), 5.37 (1H, d, $^2J_{HH}$ = 15.0, O**CH_2**Ar), 5.32 (1H, d, $^2J_{HH}$ = 15.0, O**CH_2**Ar), 4.46 (1H, d, $^3J_{HH}$ = 15.0, NCHHC=N), 4.33 (1H, d, $^3J_{HH}$ = 15.40, NCHHC=N), 3.83 (1H, m, NCHHCH_2CO_2), 3.52 (1H, m, NCHHCH_2CO_2), 2.29 (2H, m, **CH_2CO_2**H). LRMS (FAB, M+H$^+$) 498.18. HRMS (FAB) *m/z* calcd for $C_{27}H_{24}N_5O_5$ 498.1777, found: 498.1800.

Method M (Table 3). The general procedure is detailed for the preparation of **30f**.

4*H*-Imidazo[1,2-*a*][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(aminoiminomethyl) benzoyl] amino] 1-methyl-6-oxo, trifluoroacetate (30f). Amidino ester **29f** (23 mg, 0.40 mmol) was dissolved in 3/2/1 tetrahydrofuran/methanol/water (total volume = 0.6 mL), treated with 1 N sodium hydroxide solution (1.25 mL, 3.0 equiv.) and stirred at room temperature overnight. The reaction mixture was concentrated in vacuo and the crude product purified by preparative RP HPLC to afford 24.5 mg (24%) of **30f** (trifluoroacetate salt form) after lyophilization from CH_3CN/H_2O w/0.1% TFA. RP HPLC (method P5) t_R = 50–52.5 min. RP HPLC (method **A4**) t_R = 8.02 min (91% purity). RP HPLC (method **A7**) t_R = 8.75 min (85% purity). ^{1}H NMR (DMSO-d_6, TMS) δ 10.8 (1H, s, ArCONHAr), 9.45 (1H, br s, H_2NC=NH), 9.11 (2H, br s, **H_2NC**=NH), 8.33 (1H, d, $^4J_{HH}$ = 2.4, C7 ArH), 8.20 (3H, 2H, d, $^3J_{HH}$ = 8.4, *o*-H_2NC=NH ArH, upon expansion br d, 1H, *J* values could not be determined C9 ArH), 7.97 (2H, d, $^3J_{HH}$ = 8.4, *m*-H_2NC=NH ArH), 7.66 (1H, d, $^3J_{HH}$ = 11.7, C10 ArH), 7.03 (1H, s, NC(CH_3)CHN)), 4.58 (2H, d, $^2J_{HH}$ = 16.2, NCHHC=N), 4.41 (2H, d, $^2J_{HH}$ = 16.2, NCHHC=N), ~3.7 hidden partially by water peak, (2H, m, N**$CH_2CH_2CO_2$**H), ~2.5 hidden under DMSO, (2H, m, **CH_2CO_2**H), 2.35 (3H, s, NC(**CH_3**)CHN). LRMS (ES, M+H$^+$) 447.2. HRMS (FAB) *m/z* calcd for $C_{23}H_{23}N_6O_4$ 447.1781, found: 447.1764.

Method N (Table 3). The general procedure is detailed for the preparation of **30i**.

4-*H*-[1,2,3,4]Tetrazolo[4,5a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)benzoyl]amino]-6-oxo, trifluoroacetate (30i). Amidino ester **29i** (45.0 mg, 0.078 mmol) was dissolved in THF/H_2O (3/1, 3.0 mL/1.0 mL) and treated with 1.0 M LiOH (0.195 mL, 0.195 mmol, 2.5 equiv.). The reaction mixture was stirred at room temperature for 40 h, quenched by the addition of citric acid and purified by preparative RP HPLC to give 28 mg (65%) of **30i**. RP HPLC (method **P7**) t_R = 32–36 min. RP HPLC (method **A4**) t_R = 7.29 min (>98.5% purity). RP HPLC (method **A7**) t_R = 4.60 min (99% purity). ^{1}H NMR (D_2O, TMS) δ 8.23 (.67H, d, $^4J_{HH}$ = 2.1, C7 ArH), 8.10 (0.33H, d, $^4J_{HH}$ = 2.1, C7 ArH), 8.09 (1H, d, $^3J_{HH}$ = 8.7, C10 ArH), 8.05 (2H, d $^3J_{HH}$ = 8.4, *o*-H_2NC=NH ArH), 7.97 (1H, dd, $^3J_{HH}$ = 8.7, $^4J_{HH}$ = 2.0, C9 ArH), 7.85 (2H, d, $^3J_{HH}$ = 8.10, *m*-H_2NC=NH ArH), 4.98 (2H, br s, N**CH_2**C=N), 3.97 (2H, t, $^3J_{HH}$ = 6.0, N**CH_2** CH_2CO_2H), 2.71 (2H, t, $^3J_{HH}$ = 6.0, **CH_2CO_2**H). ^{1}H NMR (DMSO-d_6, TMS) δ 10.91 (1H, s, ArCONHAr), 9.44 (1.5H, br s, **H_2NC**=NH), 9.15 (1.5 H, br s, **H_2NC**=NH), 8.46 (1H, d, $^4J_{HH}$ = 2.4, C7 ArH), 8.30 (1H, dd, $^3J_{HH}$ = 8.7, $^4J_{HH}$ = 2.4, C9 ArH), 8.19 (2H, d, $^3J_{HH}$ = 8.7, *o*-H_2NC=NH ArH), 7.95 (3H, 2

overlapping d's, $^3J_{HH}=8.7$, $^3J_{HH}=8.1$, C10 ArH and *m*-$H_2NC=NH$ ArH), 4.95 (2H, br s, $NCH_2C=N$), 3.80 (2H, t, $^3J_{HH}=7.0$, $NCH_2CH_2CO_2Et$), 2.53 (2H, t, $^3J_{HH}=7.5$, CH_2CO, partially obscured by DMSO-d_6 solvent peak). LRMS (FAB, $M+H^+$) 435.15. HRMS (FAB) *m/z* calcd for $C_{20}H_{19}N_8O_4$ 435.1529, found: 435.1528.

***N*-(2-Amino-5-nitrobenzoyl)-β-alanine ethyl ester (31).** 5-nitro isatoic anhydride (10.0 g, 48 mmol) was suspended in dichloromethane (anhyd., 200 mL), treated with β alanine ethyl ester hydrochloride (9.23 g, 60 mmol, 1.25 equiv.), triethyl amine (12.2 g, 16.8 mL, 120 mmol, 2.5 equiv.), and DMAP (0.61 g, 4.8 mmol, 0.1 equiv.) under an atmosphere of nitrogen. The reaction mixture was stirred under nitrogen for 16 h at room temperature, diluted with additional dichloromethane (100 mL) and transferred to a 500 mL separatory funnel. The dichloromethane was washed with water (2×100 mL), then brine (1×100 mL), and the aqueous washes extracted additionally with dichloromethane (2×100 mL). The dichloromethane extracts were combined, dried (Na_2SO_4), filtered, concentrated in vacuo, and the resulting dark oil purified by column chromatography (75% EtOAc/hexane) to yield 5.33 g (39%) of **31** as a yellow solid. mp 135–137 °C; TLC $R_f=0.86$ (50% EtOAc/hexane, UV positive). ^{1}H NMR ($CDCl_3$, TMS) δ 8.31 (1H, d, $^4J_{HH}=2.7$ C6 ArH), 8.08 (1H, dd, $^3J_{HH}=9.0$, $^4J_{HH}=2.7$, C4 ArH), 6.88 (1H, br t, **CONH** CH_2), 6.64 (1H, d, $^3J_{HH}=9.0$ C3 ArH), 6.49 (2H, br s, Ar**NH$_2$**), 4.20 (2H, q, $^3J_{HH}=7.2$ **OCH$_2$**), 3.70 (2H, apparent q, $^3J_{HH}=6.0$ **NCH$_2$**CH_2CO_2), 2.65 (2H, t, $^3J_{HH}=6.0$ **CH$_2$**CO_2Et), 1.29 (3H, t, $^3J_{HH}=7.2$ OCH_2 **CH$_3$**). LRMS (FAB, $M+H^+$) 282.1. HRMS (FAB) *m/z* calcd for $C_{12}H_{16}N_3O_5$ 282.1090; found: 282.1098. Anal. calcd for $C_{12}H_{15}N_3O_5$: C, 51.24; H, 5.38; N, 14.94; Found: C, 51.41; H, 5.36; N, 14.92.

***N*-[2-[(Diphenyl)methylamino]-5-nitrobenzoyl]-β-alanine ethyl ester (32).** A 250-mL 24/40 round-bottomed flask equipped with a stir bar was charged with **31**, (3.95 g, 14.05 mmol, 1.0 equiv.), dichloroethane (anhyd., 70.3 mL), 2,6 lutidine (3.01 g, 3.27 mL, 28.1 mmol, 2.0 equiv.), finely powdered potassium carbonate (3.88 g, 28.1 mmol, 2.0 equiv.), and benzhydryl bromide (3.82 g, 15.5 mmol, 1.1 equiv.). The flask was equipped with a reflux condenser and the reaction mixture heated to reflux under an atmosphere of nitrogen for 6 h after which time TLC (75% EtOAc/hexane) indicated that **31** had been consumed. The reaction was cooled to room temperature, transferred to a 250 mL separatory funnel and the dichloroethane washed with water (1×50 mL), and brine (1×50 mL). The aqueous washes were re-extracted with additional dichloroethane, the dichloroethane extracts combined, dried ($MgSO_4$), and concentrated in vacuo to give a yellow oil. The product was purified by sequential preparative column chromatography (the first column using 50% EtOAc/hexane as the eluent and the second chromatography using 25% EtOAc/hexane to separate **32** from traces of the higher R_f benzhydryl bromide) to yield 5.73 g (91%) of **32** as a yellow oil. The yellow oil slowly solidified upon standing at room temperature. mp 113–114 °C; ^{1}H NMR ($CDCl_3$, TMS) δ 9.45 (1H, d, $^3J_{HH}=6.0$, Ar**NH**-$CH(Ar)_2$), 8.33 (1H, d, $^4J_{HH}=2.4$, C6 ArH), 8.0 (1H, dd, $^3J_{HH}=9.3$, $^4J_{HH}=2.4$, C4 ArH), 7.31 (5H, s, **ArCHAr**), 7.30–7.24 (5H, m, **ArCHAr**), 6.97 (1H, br t, **CONHCH$_2$**), 6.51 (1H, d, $^3J_{HH}=9.3$ C3 ArH), 5.66 (1H, d, $^3J_{HH}=6.0$, Ar**NHCH**$(Ar)_2$), 4.17 (2H, q, $^3J_{HH}=7.1$, **OCH$_2$**), 3.67 (2H, apparent q, $^3J_{HH}=6.0$, **NCH$_2$**CH_2CO_2), 2.62 (2H, t, $^3J_{HH}=6.0$, **CH$_2$**CO_2), 1.26 (3H, t, $^3J_{HH}=7.1$, OCH_2**CH$_3$**). ^{13}C NMR ($CDCl_3$) δ 172.39, 167.97, 153.02, 141.05, 136.03, 129.04, 128.43, 127.81, 127.11, 124.37, 113.75, 112.28, 62.01, 60.97, 35.34, 33.84, 14.1. LRMS (FAB, $M+H^+$) 448.19. HRMS (FAB) *m/z* calcd for $C_{25}H_{26}N_3O_5$ 448.1872; found: 448.1851. Anal. calcd for $C_{25}H_{25}N_3O_5$: C, 67.10; H, 5.63; N, 9.39. Found: C, 66.77; H, 5.46; N, 9.33.

***N*-[2-[(Diphenyl)methylamino]-5-aminobenzoyl]-β-alanine ethyl ester (33).** A dry, 50-mL two-necked 14/20 round-bottomed flask equipped with a reflux condenser and a stir bar was charged with nitro aniline **32** (500 mg, 1.12 mmol) which had been dissolved in acetonitrile (anhyd., 15 mL). The acetonitrile solution was treated with triethylamine (482 mg, 0.66 mL, 4.76 mmol, 4.25 equiv.), formic acid (219 mg, 0.18 mL, 4.76 mmol, 4.25 equiv.), 5% palladium on carbon catalyst (20 mg, 4% by wt) and refluxed for 18 h. TLC (100% EtOAc) indicated the presence of a new product which could be visualized by treatment with ninhydrin spray and charring (**32** did not stain positive when treated similarly). The reaction mixture was cooled to room temperature, filtered through a pad of Celite, and concentrated in vacuo to give a dark oil. The crude product was purified by preparative column chromatography (100% EtOAc) to yield 384 mg (83%) of **33** as an oil which slowly solidified upon standing at room temperature. mp 102–103 °C. ^{1}H NMR ($CDCl_3$, TMS) δ 7.37–7.20 (10H, m, **ArCHAr**), 6.75 (1H, br s, C6 ArH), 6.73 (1H, br t, **CONH** CH_2), 6.62 (1H, br d, $^3J_{HH}=9.0$ C4 ArH), 6.44 (1H, d, $^3J_{HH}=9.0$, C3 ArH), 5.52 (1H, br s, Ar**NHCH**$(Ar)_2$), 4.16 (2H, q, $^3J_{HH}=7.0$, **OCH$_2$**), 3.64 (2H, apparent q, $^3J_{HH}=6.0$ **NCH$_2$**CH_2CO_2), 2.59 (2H, t, $^3J_{HH}=6.0$, **CH$_2$**CO_2), 1.27 (3H, t, $^3J_{HH}=7.2$, OCH_2**CH$_3$**). ^{13}C NMR ($CDCl_3$) δ 172.77, 172.33, 169.32, 167.94, 152.97, 143.13, 142.13, 140.99, 135.95, 135.50, 128.99, 128.57, 128.35, 127.75, 127.22, 127.04, 126.97, 124.39, 121.18, 116.52, 114.83, 114.33, 113.68, 112.22, 62.56, 61.95, 60.90, 60.69, 35.30, 34.91, 33.99, 33.79, 14.10. LRMS (FAB, M^+) 417.20. HRMS (FAB, M^+) *m/z* calcd for $C_{25}H_{27}N_3O_3$ 417.2052; found: 417.2044.

***N*-[2-[(Diphenyl)methylamino]-5-*N*-*tert*-butoxycarbonyl aminobenzoyl]-β-alanine ethyl ester (34).** A dry 50-mL 24/40 round-bottomed flask equipped with a stir bar was charged with a THF solution (anhyd., 25 mL) of **33** (3.52 g, 8.43 mmol), BOC-ON (2.08 g, 8.43 mmol, 1.0 equiv.) and DMAP (1.03 g, 8.43 mmol, 1.0 equiv.). The uncapped reaction flask was placed in an oil bath and heated to 85 °C for 4 h during which time the THF evaporated. The reaction mixture was cooled to room temperature and allowed to stand for 16 h. The residue was dissolved in the minimal amount of dichloromethane, absorbed onto silica gel and purified by preparative column chromatography (25% EtOAc/hexane eluent) to yield 3.20 g of as a pink foam mp 148–150 °C. ^{1}H NMR ($CDCl_3$, TMS) δ 8.20 (1H, br s, Ar**NH**CH(Ar)$_2$), 7.48 (1H, br s, C6 ArH), 7.37–7.21 (10H, m, **ArCHAr**), 6.96 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 2.0, C4 ArH), 6.80 (1H, br t, CO**NH**CH$_2$), 6.47 (1H, d, $^3J_{HH}$ = 9.0 C3 ArH), 6.20 (1H, br s, Ar**NH**COOtBu), 5.54 (1H, s, ArNH**CH**(Ar)$_2$), 4.17 (2H, q, $^3J_{HH}$ = 7.2 **OCH$_2$**), 3.65 (2H, apparent q, $^3J_{HH}$ = 6.0 **NCH$_2$**CH$_2$CO$_2$), 2.61 (2H, t, $^3J_{HH}$ = 6.0 **CH$_2$**CO$_2$), 1.48 (9H, s, ArNHCOO**tBu**), 1.27 (3H, t, $^3J_{HH}$ = 7.2, OCH$_2$**CH$_3$**). LRMS (FAB, M$^+$) 517.26. HRMS (FAB) *m/z* calcd for $C_{30}H_{36}N_3O_5$ 517.2577; found: 517.2585. Anal. calcd for $C_{30}H_{35}N_3O_5$ (0.50 H_2O) C, 68.42; H, 6.89; N, 7.97. Found: C, 68.53; H, 6.91; N, 7.97.

1-(Diphenylmethyl)-4-(2-carboxyethyl)-7-[*N*-*tert*-butoxycarbonyl amino]-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (35). N-Boc anilino compound **34** (3.01 g, 5.81 mmol) was dissolved in dichloromethane (150 mL, 0.039 M), transferred to a 24/40 500-mL round-bottomed flask equipped with a stir bar and treated with potassium phosphate buffer (150 mL, 1.0 M KPhos, pH 7.0). Bromoacetylbromide (2.35 g, 1.10 mL, 11.62 mmol, 2.0 equiv.) was added dropwise slowly and the reaction vigorously stirred for 3 h at room temperature. The reaction mixture was transferred into a 500-mL separatory funnel and the layers separated. The aqueous layer was extracted additionally with dichloromethane (2×75 mL), the organic extracts combined, washed with water (1×), brine(1×), dried ($MgSO_4$), and filtered into a 24/40 500 mL round-bottomed flask equipped with a stir bar. The 500-mL round-bottomed flask was flushed with nitrogen and under an atmosphere of nitrogen treated with DBU (1.24 g, 1.21 mL, 8.14 mmol, 1.44 equiv.). The reaction was stirred for 16 h at room temperature, transferred to a 500-mL separatory funnel, washed with water (1×), 10% HCl (2×), brine (1×), dried ($MgSO_4$), filtered and concentrated in vacuo to a viscous, pink syrup. The crude residue was purified by preparative column chromatography (33% EtOAc/hexane eluent) to yield 2.29 g (71%) of **35** as a pink foam: mp 97–100 °C; TLC R_f = 0.63 (50 % EtOAc/hexane, UV positive); ^{1}H NMR ($CDCl_3$, TMS) δ 7.55 (1H, d, $^4J_{HH}$ = 3.0, C6 ArH), 7.42–7.0 (11H, m, **ArCHAr** and C8 ArH), 6.98 (1H, d, $^3J_{HH}$ = 8.7, C9 ArH), 6.68 (1H, s, **NCH**(Ar)$_2$), 6.62 (1H, br s, Ar**NH**COOtBu), 4.18 (1H, d, $^2J_{HH}$ = 14.5, **NCH**HC = O), 4.12 (2H, q, $^3J_{HH}$ = 7.2, **OCH$_2$**), 3.96 (1H, m,**NCH**HCH$_2$CO$_2$), 3.79 (1H, d, $^2J_{HH}$ = 14.5, NCHHC = O), 3.78 (1H, m, NCHHCH$_2$CO$_2$), 2.60 (2H, m, **CH$_2$**CO$_2$Et), 1.48 (9H, s, ArNHCOO**tBu**), 1.25 (3H, t, $^3J_{HH}$ = 7.2 , OCH$_2$**CH$_3$**); ^{13}C NMR ($CDCl_3$) δ 172.15, 168.67, 166.60, 152.25, 139.78, 139.39, 137.06, 136.57, 131.69, 131.54, 131.20, 130.85, 128.15, 127.95, 127.83, 127.02, 119.96, 116.80, 81.13, 63.78, 60.71, 43.42, 35.24, 33.61, 28.17, 14.13; LRMS (FAB, M + H$^+$) 558.26. HRMS (FAB) *m/z* calcd for $C_{32}H_{36}N_3O_7$ 558.2604; found: 558.2609. Anal. calcd for $C_{32}H_{35}N_3O_6$ (0.75 H_2O) C, 67.17; H, 6.61; N, 7.34. Found: C, 67.15; H, 6.53; N, 7.01.

4-(2-Carboxyethyl)-7-[*N*-*tert*-butoxycarbonyl amino]-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione ethyl ester (36). A 125-mL Paar hydrogenation bottle was charged with **35** (1.61 g, 2.89 mmol), 50 mL of glacial acetic acid, and Pearlman's catalyst (160 mg, 10% by wt). The Paar bottle was wrapped with a heating jacket, evacuated and filled with nitrogen (2×), then evacuated and filled with hydrogen (3×). The Paar flask and its contents were hydrogenated at 50 psi (55 °C) for 24 h, cooled to room temperature and the excess hydrogen evacuated. The solution was filtered through a pad of Celite and the Celite pad rinsed with additional acetic acid. The acetic acid was removed in vacuo and the residue dissolved in water/ethyl acetate, transferred to a separatory funnel and the layers separated. The aqueous layer was extracted additionally with ethyl acetate, the ethyl acetate extracts combined, washed with water (1×), brine (1×), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by preparative column chromatography (75% EtOAc/hexane elute) to afford 1.09 g (73%) of **36** as a white foam mp 70–80 °C (shrinks to a goo). TLC R_f = 0.36 (67% EtOAc/hexane, UV positive). ^{1}H NMR ($CDCl_3$, TMS) δ 8.24 (1H, s, Ar**NH**CO CH$_2$N), 7.72 (2H, br s w/a shoulder, C6 ArH and C8 ArH), 6.92 (1H, d, $^3J_{HH}$ = 9.0, C9 ArH), 6.76 (1H, s, Ar **NH**COOtBu), 4.14 (2H, q, $^3J_{HH}$ = 7.2, **OCH$_2$**), 3.95 (2H, s, **NCH$_2$**C = O), 3.93 (2H, t, $^3J_{HH}$ = 6.6, **NCH$_2$**CH$_2$ CO$_2$), 2.73 (2H, t, $^3J_{HH}$ = 6.6, **CH$_2$**CO$_2$Et), 1.48 (9H, s, ArNHCOO**tBu**), 1.25 (3H, t, $^3J_{HH}$ = 7.2, OCH$_2$**CH$_3$**). LRMS (FAB, M + H$^+$) 392.18. HRMS (FAB) *m/z* calcd for $C_{19}H_{26}N_3O_6$ 392.1822; found: 392.1820. Anal. calcd for $C_{19}H_{25}N_3O_6$: C, 58.30; H, 6.44; N, 10.74. Found: C, 58.06; H, 6.46; N, 10.39.

4-*H*-[1,2,3,4]Tetrazolo[4,5a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[*N*-*tert*-butoxycarbonyl amino]-6-oxo, ethyl ester (37). The N-Boc tetrazole **37** was prepared from **36** using the procedure described for the preparation of **27c**. Treatment of **36** (3.039 g, 7.77 mmol) with

triphenylphosphine (2.04 g, 7.77 mmol, 1.0 equiv.), DEAD (1.35 g, 1.2 mL, 7.77 mmol, 1.0 equiv.), and trimethylsilylazide (895 mg, 1.0 mL, 7.77 mmol, 1.0 equiv.) in THF (anhyd., 45 mL) and stirring at room temperature for 24 h, followed by a second charge of the reagents and stirring for 48 h yielded the crude tetrazole **37**. The reaction was quenched by the addition of aqueous ammonium ceric(IV) nitrate (385 mL of a 0.1 M soln = 5.5% by wt [21.20 g $(NH_4)_2$ $Ce(NO_3)_6$ in 385 mL H_2O], 38.5 mmol, 5.0 equiv.) to decompose any excess trimethylsilylazide. Dichloromethane was added (300 mL), the reaction mixture stirred vigorously for 45 min and transferred into a 1-L separatory funnel. The layers were separated and the aqueous layer extracted additionally with dichloromethane. The dichloromethane extracts were combined, washed with water (1×), brine (1×), dried ($MgSO_4$), filtered, and concentrated in vacuo to an oil. The residue was purified by preparative column chromatography (67% EtOAc/hexane eluent) to yield a viscous syrup. Trituration of the syrup with ether resulted in the formation of a pale yellow solid which was collected by vacuum filtration in a fritted funnel (course, 30 mL ASTM 40–60) and air dried to yield 1.84 g of the N-Boc tetrazole ethyl ester **37** (57%) mp 207–208 °C. TLC R_f = 0.64 (67% EtOAc/hexane, UV positive). TLC R_f = 0.43 (20% acetone/dichloromethane, UV positive). ^{1}H NMR ($CDCl_3$, TMS) δ 7.99 (1H, d, $^4J_{HH}$ = 2.0, C7 ArH), 7.90 (1H, dd, $^3J_{HH}$ = 9.0, $^4J_{HH}$ = 2.0, C9 ArH), 7.85 (1H, d, $^3J_{HH}$ = 9.0, C10 ArH), 6.88 (1H, br s, Ar**NH**COOtBu), 4.82 (2H, s, N**CH_2**C=N), 4.15 (2H, q, $^3J_{HH}$ = 7.2, O**CH_2**), 3.92 (2H, t, $^3J_{HH}$ = 6.6, N**$CH_2CH_2CO_2$**), 2.71 (2H, t, $^3J_{HH}$ = 6.6, **CH_2CO_2**Et), 1.53 (9H, s, ArNHCOO**tBu**), 1.24 (3H, t, $^3J_{HH}$ = 7.2, OCH_2**CH_3**). ^{13}C NMR ($CDCl_3$) δ 171.57, 165.44, 152.27, 151.76, 140.23, 133.13, 133.0, 127.68, 124.80, 123.00, 122.78, 121.5, 87.72, 61.05, 46.33, 41.46, 33.07, 28.22, 14.10; LRMS (FAB, M+H$^+$) 417.19; HRMS (FAB) *m/z* calcd for $C_{19}H_{25}N_6O_5$, 417.1886; found: 392.1876.

4-*H*-[1,2,3,4]Tetrazolo[4,5a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-amino-6-oxo, ethyl ester (38). N-Boc tetrazole ethyl ester **37** (857 mg, 2.06 mmol) was dissolved in dichloromethane (anhyd., 40 mL), treated under an atmosphere of nitrogen with trifluoroacetic acid (2.96 g, 2.0 mL, 20.6 mmol, 10 equiv.) and stirred 18 h at room temperature. The reaction mixture was concentrated in vacuo, the crude trifluoroacetate salt dissolved in dichloromethane (30 mL), treated with saturated potassium carbonate (30 mL) and stirred vigorously for 5 min. The biphasic solution was transferred to a separatory funnel, the layers separated, and the aqueous layer washed with additional dichloromethane. The dichloromethane layers were combined, washed with water (1×) and brine (1×). All of the aqueous layers were combined and extracted additionally with dichloromethane. The dichloromethane extracts were combined, dried ($MgSO_4$), filtered and concentrated in vacuo to give the crude aniline **38**. Purification by preparative column chromatography (20% acetone/dichloromethane eluent) afforded 436 mg (74%) of **38** as a pale yellow solid: mp 105–108 °C; TLC R_f = 0.23 (20% acetone/dichloromethane, UV positive); ^{1}H NMR ($CDCl_3$, TMS) δ 7.69 (1H, d, $^3J_{HH}$ = 8.7, C10 ArH), 7.30 (1H, d, $^4J_{HH}$ = 2.5 C7 ArH), 6.96 (1H, dd, $^3J_{HH}$ = 8.7, $^4J_{HH}$ = 2.5, C9 ArH), 4.78 (2H, s, N**CH_2**C=N), 4.14 (2H, q, $^3J_{HH}$ = 7.2, O**CH_2**), 3.91 (2H, t, $^3J_{HH}$ = 6.6, 6.3, N**$CH_2CH_2CO_2$**), 2.69 (2H, t, $^3J_{HH}$ = 6.6, 6.3, **CH_2** CO_2 Et), 1.24 (3H, t, $^3J_{HH}$ = 7.2, OCH_2**CH_3**); ^{13}C NMR ($CDCl_3$) 171.37, 165.80, 151.31, 148.15, 128.04, 123.34, 120.80, 118.74, 116.62, 60.88, 45.99, 41.19, 32.96, 13.98; LRMS (FAB, M+H$^+$) 317.14; HRMS (FAB) *m/z* calcd for $C_{14}H_{17}N_6O_3$, 317.1362; found: 317.1373; Anal. calcd for $C_{14}H_{16}N_6O_3$: C, 53.16; H, 5.10; N, 26.57. Found: C, 52.95; H, 5.26; N, 26.24.

4-*H*-[1,2,3,4]Tetrazolo[4,5a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-cyanobenzoyl] amino]-6-oxo, ethyl ester (27b). Anilino tetrazole ethyl ester **38** (200 mg, 0.63 mmol) was dissolved in dioxane (anhyd., 10 mL), treated under an atmosphere of nitrogen with finely powdered potassium carbonate (350 mg, 2.53 mmol, 4.0 equiv.) and *p*-cyanobenzoyl chloride (419 mg, 2.53 mmol, 4.0 equiv.). The reaction mixture was heated to 70 °C, stirred at 70 °C for 2 h and cooled to room temperature. The reaction was quenched by the addition of water and partitioned between EtOAc and water. The ethyl acetate layer was washed with water (1×), brine (1×), dried (Na_2SO_4), filtered and concentrated in vacuo to give 275 mg (98%) of a white foam, mp 197–200 °C. Compound **27b** prepared by acylation of **38** with *p*-cyano benzoyl chloride could be used without further purification and was identical in all respects with **27b** that was prepared from **8c**.

4-*H*-[1,2,3,4]Tetrazolo[4,5a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(hydroxyiminoaminomethyl)-benzoyl] amino]-6-oxo, ethyl ester (39). A dry 14/20 50-mL round-bottomed flask equipped with a stir bar was charged with hydroxylamine hydrochloride (134 mg, 1.93 mmol, 5.0 equiv.), absolute ethanol (anhyd., 200 proof, 10 mL) and heated to 50 °C under an atmosphere of nitrogen. After a constant 50 °C temperature was maintained for 5 min, triethylamine (156 mg, 0.21 mL, 1.54 mmol, 4.0 equiv.) was added and the reaction mixture stirred at 50 °C for 10 min. A second dry 14/20 round-bottom flask was charged with **27b** (172 mg, 0.385 mmol) to which was added the 50 °C ethanolic hydroxylamine solution (the flask containing the hydroxylamine solution was rinsed with 5 mL of additional absolute ethanol, total volume abs EtOH 15 mL). The reaction was stirred at 50 °C for 18 h under an

atmosphere of nitrogen, cooled to room temperature, and the ethanol removed in vacuo to give a white solid. The white solid was removed from the round-bottomed flask by scraping with a spatula, and placed in a sintered glass funnel (30 mL, ASTM 40–60 C) The white solid was washed with water (1×15 mL), 50% EtOAc/hexane (1×15 mL), and 100% EtOAc (2×15 mL). Drying under vacuum (20 mm Hg) for 18 h gave 146 mg of the desired hydroxyamidine **39** (79%) as a white powder mp 176–180 °C dec.; ^{1}H NMR (DMSO-d_6, TMS) δ 10.73 (1H, s, HONHC=**NH**), 10.0 (1H, br s, **HONHC**=NH), 8.48 (1H, d, $^4J_{HH}$=2.0, C7 ArH), 8.23 (1H, dd, $^3J_{HH}$=8.7, $^4J_{HH}$=2.0, C9 ArH), 8.02 (2H, d, $^3J_{HH}$=8.4, *o*-HONHC=NH ArH),7.95 (1H, d, $^3J_{HH}$=8.7, C10 ArH), 7.84 (2H, d, $^3J_{HH}$=8.4, *m*-HONHC=NH ArH), 6.30 (2H, br s, **HONHC**=NH and ArCO**NH**Ar), 4.95 (2H, s, N**CH$_2$**C=N), 3.93 (2H, q, $^3J_{HH}$=7.2 , O**CH$_2$**), 3.84 (2H, t, $^3J_{HH}$=6.9, N**CH$_2$**CH$_2$CO$_2$), 2.57 (2H, t, $^3J_{HH}$=6.9, **CH$_2$**CO$_2$Et), 1.07 (3H, t, $^3J_{HH}$=7.2, OCH$_2$ **CH$_3$**); LRMS (FAB, M+H$^+$) 479.18; HRMS (FAB) *m/z* calcd for $C_{22}H_{23}N_8O_5$ 479.1791; found: 479.1786.

4-*H*-[1,2,3,4]Tetrazolo[4,5a][1,4]benzodiazepine-5(6*H*)-propanoic acid, 8-[[4-(iminoaminomethyl)benzoyl]amino]-6-oxo, ethyl ester, trifluoroacetate (29i). This compound was prepared from **38** using the procedure described by Eldred et al.[32] A dry 10-mL 14/20 round-bottom flask equipped with a stir bar was charged with **39** (148 mg, 0.309 mmol), glacial acetic acid (2.0 mL), acetic anhydride (51 mg, 47 mL) and flushed with nitrogen. The palladium on carbon catalyst (5% Pd–C, 9 mg, 6.75% catalyst by wt) was added, the flask flushed with nitrogen and equipped with a three way gas adapter to which was attached a balloon filled with hydrogen. The flask was evacuated/filled with hydrogen (3×) and hydrogenated under approximately 1 atmosphere of hydrogen at room temperature for 63 h. After removal of excess hydrogen the reaction mixture was filtered through a pad of Celite, the reaction vessel rinsed with additional acetic acid (5 mL) which was used to rinse the Celite pad. The filtrate was concentrated in vacuo and the residue purified by preparative RP HPLC (method **P8**) to afford 88 mg (55%) of the amidino ester **29i** which was identical in all respects to the material prepared from **27b**. Amidino ester **29i** was saponified (LiOH, THF/H$_2$O 3/1, room temperature) to give amidino acid **30i** in 65% yield.

Acknowledgements

The authors are grateful to Hope Steinmetz and Sherry Bullens of the Cardiovascular Department for conducting the platelet aggregation assays; to Craig Muir, Chip Alee, Ann Wawrukiewicz, and Andrea Hebert of the Bioanalytical Technology Department for conducting the ELISA assay; to Kathy O'Connell and Jim Bourell for providing the mass spectral analysis; to Sheldon Mullins and Dr. Thomas R. Gadek for their assistance in obtaining the ^{1}H NMR spectra on the 400 MHz instrument; and to Dr. Michael C. Venuti and Dr. John Burnier for their support of the GPIIb/IIIa program at Genentech.

References and Notes

1. (a) The majority of the analogues, whose preparation and in vitro evaluation are described in this study, are novel tricyclic GPIIb/IIIa antagonists. However, the preparation and in vitro evaluation of one novel tetracyclic GPIIb/IIIa antagonist is also included. (b) A portion of the work described herein, as well as SAR of additional tricyclic IIbIIIa antagonists containing a nonoptimized arginine surrogate, was presented at the 209th National ACS Convention, Anaheim, CA.; Abstract 229, MEDI.

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