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Azetidinones as Vasopressin V1a Antagonists

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Abstract

The azetidinone LY307174 (**1**) was identified as a screening lead for the vasopressin V1a receptor (IC₅₀ 45 nM at the human V1a receptor) based on molecular similarity to ketoconazole (**2**), a known antagonist of the luteinizing hormone releasing hormone receptor. Structure-activity relationships for the series were explored to optimize receptor affinity and pharmacokinetic properties, resulting in compounds with K_i values < 1 nM and brain levels after oral dosing ~100-fold higher than receptor affinities.

The neurohypophysial hormones vasopressin¹ and oxytocin exert a wide range of physiological effects through binding to specific membrane receptors belonging to the G protein-coupled receptor (GPCR) superfamily. To date, three vasopressin receptor subtypes and one oxytocin receptor have been pharmacologically and functionally described¹. V1a, V1b, and oxytocin receptors activate phospholipase C, resulting in the production of inositol 1,4,5-trisphosphate and diacylglycerol, mobilization of intracellular calcium, and activation of protein kinase C. V2 receptors stimulate adenylyl cyclase, resulting in the accumulation of cyclic AMP and activation of protein kinase A. All four receptor subtypes from several mammalian species have been recently cloned^{2–5}, as well as closely related receptors from bony fishes and invertebrates^{6, 7}. Although vasopressin is perhaps best-known for its role in the cardiovascular system, it also has actions in the central nervous system (CNS), and several CNS applications of vasopressin receptor antagonists have been suggested (reviewed in references⁸ and ⁹). A number of research groups have prepared antagonists directed at the vasopressin V1 receptor^{10–15}. While V1a antagonists have been made, none of these have been reported to penetrate the CNS efficiently.

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Our vasopressin antagonist program was initiated to identify a CNS-active V1a antagonist: one with potent affinity for the human V1a receptor ($IC_{50} < 10$ nM), good oral availability, and ability to penetrate the blood brain barrier - in short, a candidate for human clinical development targeting CNS disorders. The program began at Lilly in 1990 with the selection of a 1,500-compound "Neuropeptide Cassette" - a library intended to identify nonpeptide ligands for neuropeptide receptors. The library applied the concept of receptor crosstalk - previously well documented for the biogenic amine subfamily of GPCRs - to the neuropeptide receptors. The underlying concept is illustrated in Figure 1. Suppose there are two related receptors, A and B. Over the course of evolution, the two receptors have remained identical in part of the binding site (shown as the semicircular portion), while in the rest of the binding site the two receptors have drifted apart due to mutations (shown as the triangular portion in A and the rectangular portion in B). Furthermore, suppose that alpha, a high-affinity ligand for A, is already known, and we wish to find beta, a high-affinity ligand for B. One approach would be to screen all available analogs of alpha in the hope that a mutation in alpha would complement the mutation in A, resulting in a compound with high affinity for B, *i.e.*, beta^{16, 17}.

In the construction of the Neuropeptide Cassette, 26 known nonpeptide ligands for neuropeptide receptors (cholecystokinin, substance P, angiotensin II, opiate, leuteinizing hormone releasing hormone (LHRH), and motilin receptors) were used in the role of alpha, and molecular similarity searches in MACCS against the 26 alphas were used to identify "mutated" versions as candidates for beta. Similarity thresholds were adjusted to obtain ~300 matches for each of the 26 query structures, and the ~5,000 unique structures selected by this method were reduced to 1,500 using the leader clustering method.

Among the 26 query structures was ketoconazole, a marketed antifungal agent known to cause reproductive side-effects due to antagonism of the LHRH receptor¹⁸. The azetidinone LY307174 (**1**, Figure 2) was selected for screening based on 59% similarity to ketoconazole (**2**). Compound **1** was found to have an affinity of 45 nM for the cloned human V1a receptor. In agreement with the rationale in Figure 1, some features of **2** such as the dioxolane ring and terminal phenyl are conserved in **1**, while others are totally replaced.

Beyond the simple issue of affinity, **1** was considered an attractive lead for several reasons. LY307174 is a monocyclic beta-lactam (monobactam). Unlike fused-ring beta-lactams such as penicillins and cephalosporins, simple monobactams such as **1** are highly stable to chemical or enzymatic hydrolysis of the 4-membered azetidinone ring. The *cis* geometry of the rigid four-membered ring forces the three side-chains together into a fixed geometric configuration, presumably enabling complementarity with sub-pockets of the receptor. Although the molecular weight of **1** is relatively high (568.6), its compactness provided some hope that the series might show significant oral absorption.

After our lead compound was identified, we undertook a structure activity relationship (SAR) study utilizing the chiral Staudinger 2+2 cycloaddition reaction as depicted with a representative example in Scheme 1.

In the sequence in Scheme 1, *trans*-cinnamaldehyde is combined with glycine benzyl ester to form the imine **3**, which is then reacted with the chiral auxiliary acid chloride **4**, to form the 4-membered ring **5**. In the example given in Scheme 1, the *S*-chiral auxiliary induces formation of the *R-cis* configuration in the azetidinone product. The methylene group of the glycine constituent could be acylated, producing **6**, which displayed potent activity (V1a IC_{50} = 6.5 nM). The absolute stereochemistry of the chiral methine carbons of the azetidinone ring of **6**, depicted in Scheme 1, was assigned based on earlier reports on the absolute configuration of these azetidinone carbons produced through the Staudinger 2+2 reaction with the Evans' chiral

auxiliary^{19,20}. Confirming this assignment, the analog **7** was crystallized and the absolute configuration determined by X-ray analysis²¹ as shown in Figure 3.

The SAR strategy was based on the modification of four zones, A, B, C, and D, of the azetidinone molecule as depicted in Figure 4. A limited SAR explored effects of substitution in Zone A. For chemical accessibility, the dioxolane ring was replaced with isobutyl in many of the analogs (*i.e.*, L-leucine benzyl ester was used in the 2 + 2 reaction). The affinity of the parent isobutyl analog, **18**, was 39 nM (see Figure 6, below). Several alterations afforded a variety of azetidinones with a significant loss of V1a binding activity as represented in Figure 4. Similarly, a limited SAR at Zone B demonstrated that several modifications afforded derivatives with reduced V1a binding activity. Interestingly, replacing the phenyl with the 2-furyl substitution afforded a compound, **14**, with comparable activity, V1a IC₅₀ = 2 nM (Figure 5).

Zones C and D emerged as the most important points of structural modification. Removal of the Zone C substituent, as illustrated by the simple benzyl glycine derivative **5**, resulted in 10-fold loss of V1a activity, IC₅₀ = 400 nM. Interestingly, acylation of **5** with base and pivaloyl chloride afforded **6** with significantly enhanced potency (V1a IC₅₀ = 5 nM). Several Zone C modifications and attendant human IC₅₀ values are shown in Figure 6.

In **1**, Zone D is occupied by the benzyl ester. The ester functionality was considered a potential metabolic weak point, so the first priority was to ascertain whether a more stable group such as amide could substitute for the ester linkage. As an intermediate, the trimethylsilyl ester derivative, **19**, was prepared. Despite having equivalent bulk and hydrophobicity to the benzyl group, the trimethylsilyl substitution resulted in reduced activity (V1a IC₅₀ = 405 nM), indicating that the shape of the benzyl group was important. The trimethylsilyl ester, in turn, was transformed into the acid **20**, and subsequently coupled with benzylamine to afford the benzyl amide, **21** (V1a IC₅₀ = 43 nM). Thus, the ester functionality of **1** could be converted to the corresponding amide, **21**, without loss of binding affinity (V1a IC₅₀ = 45 nM vs. V1a IC₅₀ = 43 nM, respectively, see Figure 7).

At this point, the next step for this project was to find a lead structure that combined high receptor affinity (IC₅₀ < 10 nM) with useful brain levels (>10X the receptor affinity). Although **6** had good V1a binding affinity (IC₅₀ = 5 nM), it provided minimal brain penetration in dogs treated orally with 10 mg/kg (dog brain concentration of **6**, 10 nM at 4 hrs). In an effort to find an amide substitution that would enhance oral absorption and CNS penetration, the L-leucyl platform was constructed (Figure 8). Although not optimal for affinity, this platform was used as the basis for SAR exploration of Zone D because of its stability under conditions used for synthesis. The Zone D explorations had two objectives: to find the optimal substitution pattern for the benzylamide group, and to find other groups that would be compatible with high affinity, especially groups with charged functions that would improve water solubility. Rapid solution phase amide syntheses mediated by solid phase carbodiimide afforded over 50 amides²², some of which are illustrated in Figure 8.

A small substituent at the 3-position of the benzylamide (*e.g.*, Cl or CF₃) was found to provide optimal affinity. Among groups other than benzyl, piperidylpiperidine and piperidyl-ethyl-piperidine provided relatively good affinities. These groups would be positively charged at physiological pH, potentially leading to improved solubility. Interestingly, the piperidylpiperidine group was previously shown to provide optimal *in vivo* activity in an NK-1 clinical candidate²³.

The piperidyl-ethyl-piperidine **22** was evaluated in dogs dosed orally at 10 mg/kg. Its brain level at 4 hrs post administration was 100 nM, 10-fold better than **6** (see Figure 9). Because the isobutyl substitution in Zone C was known to be suboptimal for V1a affinity, the

corresponding compound with pivaloyl in Zone C was synthesized. Based on previous results, the pivaloyl group was expected to provide a roughly 8-fold boost in affinity, resulting in a predicted affinity ~10 nM for the combination. Instead, affinity was 85 nM (Figure 9).

In searching for an explanation of the non-additivity seen in **29**, it occurred to us that the binding mode of the molecule may have changed when benzyl was replaced with piperidyl-ethyl-piperidine. The two groups are after all completely different in shape and charge. With a proximally branched alkyl structure, the piperidyl-ethyl-piperidine group actually bears more resemblance to a typical Zone C substituent, such as methyldioxolane, pivaloyl, or isobutyl. We conjectured that the piperidyl-ethyl-piperidine bound into the Zone C pocket of the receptor, forcing the pivaloyl group into the free aqueous phase or into an unfavorable interaction with the Zone D pocket of the receptor. By this logic, reintroducing the benzylamide group in place of the pivaloyl should reoptimize binding in the Zone D pocket, creating a large boost in affinity. At this point, the vasopressin project was deprioritized, so confirmation of this hypothesis was put on hold pending completion of a successful out-licensing campaign.

After a four-year search for a partner, the program was reconstituted as a joint venture with Serenix Pharmaceuticals (later renamed Azevan), a start-up company created for this purpose. The initial strategy was to construct a scaffold with aminomalononic acid replacing L-leucine as depicted in Figure 10. If the piperidyl-ethyl-piperidine binds into Zone C according to the previous hypothesis, the binding pockets in Zones C and D should each tolerate a side-chain anchored by an amide coupling, allowing the piperidyl-ethyl-piperidinamide and benzylamide to be present simultaneously. With the correct stereochemistry, each group should be able to bind to its preferred pocket. The relevant aminomalonate platform was synthesized as illustrated in Scheme 2. Gratifyingly, **33** showed a dramatic enhancement of human V1a binding affinity (V1a K_i = 1.17 nM, Figure 11).

Even though there was significant improvement in binding affinity in the aminomalonyl platform azetidinones (Figure 11), the compounds could only be obtained as diastereomeric mixtures, as a consequence of planarization of the malonate methine on dissociation of its acidic hydrogen. Consequently the L-aspartyl platform was prepared in the hope that this would retain the V1a binding affinity while obviating the issue of the labile methine hydrogen and the attendant diastereomeric mixture. These synthetic transformations are illustrated in Scheme 3. The most potent compound synthesized, **37**, had a V1a affinity comparable to that of **33** from the aminomalonyl platform (Figure 12).

Theorizing that the D-aspartyl platform would be more resistant to *in vivo* enzymatic hydrolysis, we prepared the platform with a D-aspartyl group in Zones C and D (see Scheme 4). Although in general the D-platforms tended to be less active than the L-platforms (see the D-glutamyl platform, below), this was less pronounced for the D-aspartyl platform. In the case of **41** (V1a K_i 1.49 nM, Figure 13), affinity of the D-aspartyl compound was actually slightly better than that of the corresponding L-aspartyl analog (**42**, V1a K_i = 1.89 nM). It is possible that the piperidylpiperidine side-chain may be able to adapt its conformation to match the receptor pocket in a unique way – a possibility that may also explain its superior pharmacokinetic properties in this series (*vide infra*) and the NK-1 series cited previously²³.

The same reasons that led to the investigation of the L- and D-aspartyl platforms also resulted in the preparation of a range of compounds derived from L- and D-glutamic acids. The syntheses of these L- and D-glutamyl platforms are also illustrated in Schemes 3, 4, and 5. The corresponding V1a binding affinities are shown (see Figures 14 and 15). For these two series, the most active compounds were found to be L-glutamate analogs, with the D-glutamate versions typically about 20-fold less active.

It is of interest that the highest affinity in the glutamyl platform was seen with the 4-cyclohexylpiperazine side-chain, which is identical to the piperidylpiperidine side-chain that gave optimal activity in the aspartyl platform, except that the basic amino group has been moved one bond closer, offsetting the additional methylene in the glutamyl moiety. In both of these examples, as well as in **37**, the positively charged group is 8 bonds away from the azetidine N. Thus it appears that optimal activity requires a positively charged group at a fixed distance from the azetidinone core. It is possible that the basic nitrogen binds to the same site as the guanidinium of arginine-vasopressin.

Five of the compounds with $K_i < 10 \text{ nM}$ ²⁴ were tested for plasma and brain levels in rats after oral dosing (20 mg/kg). Azetidinone **22** was used as a comparator. In this screening experiment, three compounds were dosed per animal, and plasma and brain levels were measured by LC/MS. Compound **41** showed the highest levels in both plasma and brain; however, several-fold lower levels were seen in follow-up experiments where **41** was dosed alone, suggesting that the initial higher levels were due in part to co-dosing with **22**. These results (plasma C_{max} 50–80 ng/ml in two separate experiments) were considered encouraging but fell somewhat short of expectations for a clinical candidate.

Theorizing that facile enzymatic cleavage of the benzylamide bond in Zone D reduced levels of **41**, we prepared **50** (SRX246) and **51** (SRX251) (see Figure 15 for structures and Scheme 4 for synthetic pathway). It was hoped that these molecules, the (*R*)- α -methylbenzyl and the N-methyl analogues of **41** respectively, would be more stable to enzymatic hydrolysis due to steric hindrance near the amide bond. Fortuitously, **50** and **51** proved to possess even higher *in vitro* V1a affinity than **41**. Compound **52**, the (*S*)- α -methylbenzyl diastereomer of **50**, was 600-fold less potent (V1a $K_i = 179 \text{ nM}$). Interestingly, **53**, the trifluoromethyl analog of **50**, had reduced V1a binding affinity (see Figure 15), suggesting that the α -methyl substitution shifted the binding mode of the adjacent phenyl ring. Azetidinone **54**, the N-methyl derivative of **50**, likewise had poor activity (V1a $K_i = 9.8 \text{ nM}$), indicating that the N-methyl and α -methyl modifications also were incompatible.

Plasma levels for **50** and **51** were roughly double those of **41**, to some degree confirming the original motivation for the construction of these two compounds. C_{max} values were 100 ng/ml and 160 ng/ml, respectively, compared to 50–80 ng/ml for **41** (Figure 17). Plasma T_{max} values for **50** and **51** were 4 hours. Plasma $T_{1/2}$ values for **50** and **51** were 2.5 hrs and 4 hrs, respectively. The brain levels of **50** and **51** exceeded their K_i values by approximately 100 fold; brain C_{max} values of **50** and **51** were 20 ng/ml and 48 ng/ml, respectively, equivalent to 28 nM and 62 nM. Brain $T_{1/2}$ values of ~6 hr for both compounds suggest the possibility of once-a-day dosing.

The binding affinity of all the reported compounds to other members of the vasopressin receptor family was also tested using cell lines that expressed human V1b and V2. The methods for human V1b and V2 binding assay are similar to that for V1a screening, namely the whole cell-based competitive binding assay, which is described in the section of Materials and Methods. The IC_{50} values of most Azevan compounds proved to be greater than 1 μM or 10 μM for both the V1b and V2 human receptor cell lines.

Conclusions

A novel series of vasopressin V1a antagonists has been designed from the unique monocyclic azetidinone platform. Subnanomolar affinities at the human V1a receptor have been achieved. On oral dosing, two members of the series, **50** (SRX246) and **51** (SRX251), reached brain levels ~100 times their *in vitro* receptor affinities. The candidate molecules are being further developed for human clinical evaluation.

Experimental

Chemistry:

The early experimental work that was initially performed at Eli Lilly (preparation and characterization of LY307174, *etc.*) is described in reference ²².

(4(S)-phenyloxazolidin-2-on-3-yl)acetyl chloride (**4**):

This material was not produced in one batch but rather prepared as required for each “chiral Staudinger 2+2 cycloaddition reaction”. Typically, a solution of 1 g (4.52 mmol) of (4(S)-phenyloxazolidin-2-on-3-yl)acetic acid (Evans, U.S. Patent No. 4,665,171) and 0.51 mL (5.88 mmol) of oxalyl chloride in 150 mL of dichloromethane was treated with a catalytic amount of anhydrous dimethylformamide (85 μ L/milliequivalent of acetic acid derivative) resulting in vigorous gas evolution. After 45 min, all gas evolution had ceased and the reaction mixture was concentrated under reduced pressure to provide the title compound as an off-white solid after drying for 2 h under vacuum. Compound **4** was used without purification for the “chiral Staudinger [2+2] cycloaddition reaction”.

General procedure for an amide formation from an activated ester derivative: *N*-Benzyloxycarbonyl-L-aspartic acid β -*t*-butyl ester α -(3-trifluoromethyl)benzylamide; (34a**, **34** with HNR_1R_2 = 3-(trifluoromethyl)benzylamine and n = 1):**

A solution of *N*-benzyloxycarbonyl-L-aspartic acid β -*t*-butyl ester α -*N*-hydroxysuccinimide ester (1.95 g, 4.64 mmol, Advanced ChemTech) in 20 mL of dry tetrahydrofuran was treated with 0.68 mL (4.74 mmol) of 3-(trifluoromethyl)benzylamine. Upon completion of the reaction as monitored by TLC (60:40 hexanes/ethyl acetate), the solvent was evaporated, and the resulting oil was partitioned between dichloromethane and a saturated aqueous solution of sodium bicarbonate. The organic layer was, dried over magnesium sulfate, filtered and evaporated to give 2.23 g (quantitative yield) of the title compound as a white solid; ¹H NMR (CDCl₃) δ 1.39 (s, 9H); 2.61 (dd, J =6.5 Hz, J =17.2 Hz, 1H); 2.98 (dd, J =3.7 Hz, J =17.0 Hz, 1H); 4.41 (dd, J =5.9 Hz, J =15.3 Hz, 1H); 4.50–4.57 (m, 2H); 5.15 (s, 2H); 5.96–5.99 (m, 1H); 6.95 (s, 1H); 7.29–7.34 (m, 5H); 7.39–7.43 (m, 2H); 7.48–7.52 (m, 2H).

The following compounds were obtained according to this procedure: *N*-Benzyloxycarbonyl-L-aspartic acid β -*t*-butyl ester α -[4-(2-phenylethyl)]piperazinamide; (34b**, **34** with HNR_1R_2 = 4-(phenylethyl)piperazine and n = 1):**

Use of *N*-benzyloxycarbonyl-L-aspartic acid β -*t*-butyl ester α -*N*-hydroxysuccinimide ester (5.0 g, 12 mmol, Advanced ChemTech) and 4-(phenylethyl)piperazine 2.27 mL (11.9 mmol) gave 5.89 g (quantitative yield) of **34b** as a white solid; ¹H NMR (CDCl₃) δ 1.40 (s, 9H); 2.45–2.80 (m, 10H); 3.50–3.80 (m, 4H); 4.87–4.91 (m, 1H); 5.08 (s, 2H); 5.62–5.66 (m, 1H); 7.17–7.33 (m, 10H).

***N*-Benzyloxycarbonyl-L-glutamic acid γ -*t*-butyl ester α -(3-trifluoromethyl)benzylamide; (**34c**, **34** with HNR_1R_2 = 3-(trifluoromethyl)benzylamine and n = 2):**

Use of *N*-benzyloxycarbonyl-L-glutamic acid β -*t*-butyl ester α -*N*-hydroxysuccinimide ester (4.83 g, 11.1 mmol, Advanced ChemTech) and 3-(trifluoromethyl)benzylamine 1.63 mL (11.4 mmol) gave 5.41 g (98%) of **34c** as a white solid; ¹H NMR (CDCl₃) δ 1.40 (s, 9H); 1.88–1.99 (m, 1H); 2.03–2.13 (m, 1H); 2.23–2.33 (m, 1H); 2.38–2.47 (m, 1H); 4.19–4.25 (s, 1H); 4.46–4.48 (m, 2H); 5.05–5.08 (m, 2H); 5.67–5.72 (m, 1H); 7.27–7.34 (m, 5H); 7.39–7.43 (m, 2H); 7.48–7.52 (m, 2H).

General procedure for amide formation from a carboxylic acid *N*-Benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -(3-trifluoromethyl)benzylamide; (38a**, **38** with $\text{HNR}_1\text{R}_2 = 3$ -(trifluoromethyl)benzylamine and $n = 1$):**

A solution of 1 g (2.93 mmol) of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate (Novabiochem) in 3–4 mL of dichloromethane was treated by sequential addition of 0.46 mL (3.21 mmol) of 3-(trifluoromethyl)benzylamine, 0.44 g (3.23 mmol) of 1-hydroxy-7-benzotriazole, and 0.62 g (3.23 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride. After at least 12 hours at ambient temperature or until complete as determined by thin layer chromatography (95:5 dichloromethane/methanol eluent), the reaction mixture was washed sequentially with a saturated aqueous sodium bicarbonate solution and with distilled water. The organic layer was dried over magnesium sulfate, filtered and evaporated to give 1.41 g (quantitative yield) of **38a** as a white solid; ^1H NMR (CDCl_3) δ 1.39 (s, 9H); 2.61 (dd, $J=6.5$ Hz, $J=17.2$ Hz, 1H); 2.98 (dd, $J=4.2$ Hz, $J=17.2$ Hz, 1H); 4.41 (dd, $J=5.9$ Hz, $J=15.3$ Hz, 1H); 4.50–4.57 (m, 2H); 5.10 (s, 2H); 5.96–6.01 (m, 1H); 6.91–7.00 (m, 1H); 7.30–7.36 (m, 5H); 7.39–7.43 (m, 2H); 7.48–7.52 (m, 2H).

The following compounds were obtained according to this procedure *N*-Benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -(2-fluoro-3-trifluoromethyl)benzylamide; (38b**, **38** with $\text{HNR}_1\text{R}_2 = (2\text{-fluoro-3-trifluoromethyl})\text{benzylamine}$ and $n = 1$):**

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate (Novabiochem) (0.25 g, 0.73 mmol) and 0.12 mL of (2-fluoro-3-trifluoromethyl)benzylamine gave 0.365 g (quantitative yield) of **38b** as a white solid; ^1H NMR (CDCl_3) δ 1.38 (s, 9H); 2.59 (dd, $J=6.5$ Hz, $J=17.0$ Hz, 1H); 2.95 (dd, $J=4.3$ Hz, $J=17.0$ Hz, 1H); 4.46–4.56 (m, 3H); 5.11 (s, 2H); 5.94–5.96 (m, 1H); 7.15 (t, $J=8.0$ Hz, 1H); 7.30–7.36 (m, 5H); 7.47–7.52 (m, 2H).

***N*-Benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -[(*S*)- α -methylbenzyl]amide; (**38c**, **38** with $\text{HNR}_1\text{R}_2 = (\text{S})\text{-}\alpha\text{-methylbenzylamine}$ and $n = 1$):**

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate (Novabiochem) (0.25 g, 0.73 mmol) and 0.094 mL of (*S*)- α -methylbenzylamine gave 0.281 g (90%) of **38c** as a white solid; ^1H NMR (CDCl_3) δ 1.41 (s, 9H); 1.44 (d, $J=7.0$ Hz, 3H); 2.61 (dd, $J=7.0$ Hz, $J=17.0$ Hz, 1H); 2.93 (dd, $J=4.0$ Hz, $J=17.5$ Hz, 1H); 4.50–4.54 (m, 1H); 5.04–5.14 (m, 3H); 5.94–5.96 (m, 1H); 6.76–6.80 (m, 1H); 7.21–7.37 (m, 10H).

***N*-Benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -[(*R*)- α -methylbenzyl]amide; (**38d**, **38** with $\text{HNR}_1\text{R}_2 = (\text{R})\text{-}\alpha\text{-methylbenzylamine}$ and $n = 1$):**

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate (Novabiochem) (0.25 g, 0.73 mmol) and 0.094 mL of (*R*)- α -methylbenzylamine gave 0.281 g (90%) of **38d** as a white solid; ^1H NMR (CDCl_3) δ 1.38 (s, 9H); 1.43 (d, $J=6.9$ Hz, 3H); 2.54 (dd, $J=7.3$ Hz, $J=17.2$ Hz, 1H); 2.87 (dd, $J=4.1$ Hz, $J=17.2$ Hz, 1H); 4.46–4.50 (m, 1H); 4.99–5.15 (m, 3H); 5.92–5.96 (m, 1H); 6.78–6.82 (m, 1H); 7.21–7.33 (m, 10H). ^{13}C NMR (CDCl_3) δ 21.99, 27.95, 37.58, 50.03, 50.92, 67.13, 81.87, 125.95, 126.72, 128.05, 128.21, 128.52, 128.58, 129.07, 136.08, 142.86, 155.93, 169.34, 171.31.

***N*-Benzyloxycarbonyl-D-aspartic acid γ -*t*-butyl ester α -[*N*-methyl-*N*-(3-trifluoromethylbenzyl)]amide; (**38e**, **38** with $\text{HNR}_1\text{R}_2 = \text{N-methyl-}N\text{-(3-trifluoromethylbenzyl)amine}$ and $n = 1$):**

Use of *N*-benzyloxycarbonyl-D-aspartic acid γ -*t*-butyl ester (0.303 g, 0.89 mmol, Novabiochem) and 0.168 g (0.89 mmol) of *N*-methyl-*N*-(3-trifluoromethylbenzyl)amine gave 0.287 g (65%) of **38e** as a white solid; ^1H NMR (CDCl_3) δ 1.40 (s, 9H); 2.55 (dd, $J=5.8$ Hz,

$J=15.8$ Hz, 1H); 2.81 (dd, $J=7.8$ Hz, $J=15.8$ Hz, 1H); 3.10 (s, 3H); 4.25 (d, $J=15.0$ Hz, 1H); 4.80 (d, $J=15.5$ Hz, 1H); 5.01–5.13 (m, 3H); 5.52–5.55 (m, 1H); 7.25–7.52 (m, 10H).

***N*-Benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -[(*S*)-1-(3-trifluoromethylphenyl)ethyl]amide; (**38f**, **38** with $\text{HNR}_1\text{R}_2 = (\text{S})$ -1-(3-trifluoromethylphenyl)ethylamine and $n = 1$):**

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate (Novabiochem) (84 mg, 0.25 mmol) and 47 mg of (*S*)-1-(3-trifluoromethylphenyl)ethylamine gave 122 mg (quantitative yield) of **38f** as a white solid; ^1H NMR (CDCl_3) δ 1.38 (s, 9H); 1.43 (d, $J=7.0$ Hz, 3H); 2.59 (dd, $J=6.7$ Hz, $J=17.3$ Hz, 1H); 2.93 (dd, $J=4.1$ Hz, $J=17.3$ Hz, 1H); 4.49–4.53 (m, 1H); 5.05–5.28 (m, 3H); 5.91–5.95 (m, 1H); 6.84–6.87 (m, 1H); 7.29–7.52 (m, 9H).

***N*-Benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -[(*R*)-1-(3-trifluoromethylphenyl)ethyl]amide; (**38g**, **38** with $\text{HNR}_1\text{R}_2 = (\text{R})$ -1-(3-trifluoromethylphenyl)ethylamine and $n = 1$):**

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate (Novabiochem) (150 mg, 0.44 mmol) and 83 mg of (*R*)-1-(3-trifluoromethylphenyl)ethylamine gave 217 mg (quantitative yield) of **38g** as a white solid; ^1H NMR (CDCl_3) δ 1.38 (s, 9H); 1.43 (d, $J=7.0$ Hz, 3H); 2.54 (dd, $J=7.4$ Hz, $J=17.3$ Hz, 1H); 2.87 (dd, $J=4.0$ Hz, $J=17.3$ Hz, 1H); 4.47–4.51 (m, 1H); 5.01–5.16 (m, 3H); 5.92–5.96 (m, 1H); 6.90–6.94 (m, 1H); 7.26–7.49 (m, 9H).

***N*-Benzyloxycarbonyl-D-glutamic acid γ -*t*-butyl ester α -(3-trifluoromethyl)benzylamide; (**38h**, **38** with $\text{HNR}_1\text{R}_2 = 3$ -(trifluoromethyl)benzylamine and $n = 2$):**

Use of *N*-benzyloxycarbonyl-D-glutamic acid γ -*t*-butyl ester (1.14 g, 3.37 mmol) and 0.53 mL (3.70 mmol, Novabiochem) of 3-(trifluoromethyl)benzylamine gave 1.67 g (quantitative yield) of **38h** as a white solid; ^1H NMR (CDCl_3) δ 1.40 (s, 9H), 1.89–1.97 (m, 1H); 2.04–2.12 (m, 1H); 2.24–2.31 (m, 1H); 2.39–2.46 (m, 1H); 4.19–4.24 (s, 1H); 4.45–4.48 (m, 2H); 5.03–5.09 (m, 2H); 5.71–5.74 (m, 1H); 6.83–6.88 (m, 1H); 7.26–7.34 (m, 5H); 7.40–7.43 (m, 2H); 7.49–7.52 (m, 2H). ^{13}C NMR (CDCl_3) δ 27.86, 27.99, 31.73, 54.51, 67.11, 81.19, 122.90, 124.22 (q, $^3J_{\text{C-F}}$, 4.0 Hz, 1C), 124.31, (q, $^3J_{\text{C-F}}$, 3.7 Hz, 1C), 125.06, 128.03, 128.22, 128.52, 129.17, 130.88, 130.98 (q, $^1J_{\text{C-F}}$, 32.3 Hz, CF_3), 136.07, 139.02, 171.43, 172.92.

***N*-Benzyloxycarbonyl-L-glutamic acid α -*t*-butyl ester γ -(4-cyclohexyl)piperazinamide; (**43a**, **43** with $\text{HNR}_1\text{R}_2 = 1$ -cyclohexylpiperazine and $n = 2$):**

Use of *N*-benzyloxycarbonyl-L-glutamic acid α -*t*-butyl ester (1.36 g, 4.03 mmol) and 0.746 g (4.43 mmol) of 1-cyclohexylpiperazine gave 1.93 g (98%) of **43a** as a white solid; ^1H NMR (CDCl_3) δ 1.02–1.12 (m, 5H); 1.43 (s, 9H), 1.60–1.64 (m, 1H); 1.80–1.93 (m, 5H); 2.18–2.52 (m, 8H); 3.38–3.60 (m, 4H); 4.20–4.24 (m, 1H); 5.03–5.13 (m, 2H); 5.53–5.57 (m, 1H); 7.28–7.34 (m, 5H).

General procedure for hydrogenation of a benzyloxycarbonyl amine L-aspartic acid β -*t*-butyl ester α -(3-trifluoromethyl)benzylamide; (35a**, **35** with $\text{HNR}_1\text{R}_2 = 3$ -(trifluoromethyl)benzylamine and $n = 1$):**

A suspension of 2.23 g (4.64 mmol) of *N*-benzyloxycarbonyl-L-aspartic acid β -*t*-butyl ester α -(3-trifluoromethyl)benzylamide **34a** and palladium (5% wt. on activated carbon, 0.642 g) in 30 mL of methanol was held under an atmosphere of hydrogen until complete conversion as determined by thin layer chromatography (95:5 dichloromethane/methanol eluent). The reaction was filtered to remove the palladium over carbon and the filtrate was evaporated to give 1.52 g (96%) of **7a** as an off-white solid; ^1H NMR (CDCl_3) δ 1.42 (s, 9H); 2.26 (brs, 2H); 2.63–2.71 (m, 1H); 2.82–2.87 (m, 1H); 3.75–3.77 (m, 1H); 4.47–4.50 (m, 2H); 7.41–7.52 (m, 4H); 7.90 (brs, 1H).

The following compounds were obtained according to this procedure L-aspartic acid β -*t*-butyl ester α -[4-(2-phenylethyl)]piperazinamide; (35b, 35 with $\text{HNR}_1\text{R}_2 = 4$ -(phenylethyl)piperazine and $n = 1$):

Use of *N*-benzyloxycarbonyl-L-aspartic acid β -*t*-butyl ester α -[4-(2-phenylethyl)]piperazinamide (5.89 g, 11.9 mmol) **34b** gave 4.24 g (98%) of **35b** as an off-white solid; ^1H NMR (CDCl_3): δ 1.42 (s, 9H); 2.61–2.95 (m, 10H); 3.60–3.90 (m, 4H); 4.35–4.45 (m, 1H); 7.17–7.29 (m, 5H).

L-glutamic acid γ -*t*-butyl ester α -(3-trifluoromethyl)benzylamide; (35c, 35 with $\text{HNR}_1\text{R}_2 = 3$ -(trifluoromethyl)benzylamine and $n = 2$):

Use of *N*-benzyloxycarbonyl-L-glutamic acid γ -*t*-butyl ester α -(3-trifluoromethyl)benzylamide (5.41 g, 10.9 mmol) **34c** gave 3.94 g (quantitative yield) of **35c** as an off-white solid; ^1H NMR (CDCl_3): δ 1.41 (s, 9H); 1.73–1.89 (m, 3H); 2.05–2.16 (m, 1H); 2.32–2.38 (m, 2H); 3.47 (dd, $J=5.0$ Hz, $J=7.5$ Hz, 1H); 4.47–4.49 (m, 2H); 7.36–7.54 (m, 4H); 7.69–7.77 (m, 1H).

D-aspartic acid β -*t*-butyl ester α -(3-trifluoromethyl)benzylamide; (39a, 39 with $\text{HNR}_1\text{R}_2 = 3$ -(trifluoromethyl)benzylamine and $n = 1$):

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -(3-trifluoromethyl)benzylamide (1.41 g, 2.93 mmol) **38a** gave 0.973 g (96%) of **39a** as an off-white solid; ^1H NMR (CDCl_3): δ 1.42 (s, 9H); 2.21 (brs, 2H); 2.67 (dd, $J=7.1$ Hz, $J=16.8$ Hz, 1H); 2.84 (dd, $J=3.6$ Hz, $J=16.7$ Hz, 1H); 3.73–3.77 (m, 1H); 4.47–4.50 (m, 2H); 7.41–7.52 (m, 4H); 7.83–7.87 (m, 1H).

D-aspartic acid β -*t*-butyl ester α -(2-fluoro-3-trifluoromethyl)benzylamide; (39b, 39 with $\text{HNR}_1\text{R}_2 = (2\text{-fluoro-3-trifluoromethyl})\text{benzylamine}$ and $n = 1$):

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -(2-fluoro-3-trifluoromethyl)benzylamide **38b** (0.36 g, 0.72 mmol) gave 0.256 g (92%) of **39b** as an off-white solid; ^1H NMR (CDCl_3) δ 1.39 (s, 9H); 2.50 (brs, 2H); 2.74 (dd, $J=7.0$ Hz, $J=16.5$ Hz, 1H); 2.86 (dd, $J=4.8$ Hz, $J=16.8$ Hz, 1H); 3.89 (brs, 2H); 4.47–4.57 (m, 2H); 7.16 (t, $J=7.8$ Hz, 1H); 7.48 (t, $J=7.3$ Hz, 1H); 7.56 (t, $J=7.3$ Hz, 1H); 7.97–8.02 (m, 1H).

D-aspartic acid β -*t*-butyl ester α -[(*S*)- α -methyl]benzylamide; (39c, 39 with $\text{HNR}_1\text{R}_2 = (\text{S})$ - α -methylbenzylamine and $n = 1$):

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -[(*S*)- α -methylbenzyl]amide (0.275 g, 0.65 mmol) **38c** gave 0.17 g (90%) of **39c** as an off-white solid; ^1H NMR (CDCl_3) δ 1.40 (s, 9H); 1.47 (d, $J=6.9$ Hz, 3H); 1.98 (brs, 2H); 2.49 (dd, $J=7.9$ Hz, $J=17.7$ Hz, 1H); 2.83 (dd, $J=3.6$ Hz, $J=16.7$ Hz, 1H); 3.69 (brs, 1H); 4.99–5.10 (m, 1H); 7.19–7.33 (m, 5H); 7.65–7.68 (m, 1H).

D-aspartic acid β -*t*-butyl ester α -[(*R*)- α -methylbenzyl]amide; (39d, 39 with $\text{HNR}_1\text{R}_2 = (\text{R})$ - α -methylbenzylamine and $n = 1$):

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -[(*R*)- α -methylbenzyl]amide (0.273 g, 0.64 mmol) **38d** gave 0.187 g (quantitative yield) of **39d** as an off-white solid; ^1H NMR (CD_3OD) δ 1.40 (s, 9H); 1.46 (d, $J=7.0$ Hz, 3H); 2.49 (dd, $J=7.0$ Hz, $J=16.5$ Hz, 1H); 2.61 (dd, $J=5.5$ Hz, $J=16.0$ Hz, 1H); 3.62 (dd, $J=6.0$ Hz, $J=7.0$ Hz, 1H); 4.98 (dd, $J=7.0$ Hz, $J=14.0$ Hz, 1H); 7.20–7.23 (m, 1H); 7.27–7.34 (m, 4H); ^{13}C NMR (CD_3OD) δ 22.45, 28.31, 41.38, 50.17, 52.92, 82.17, 127.13, 128.09, 129.53, 144.96, 172.14, 175.11.

D-aspartic acid β -*t*-butyl ester α -[N-methyl-N-(3-trifluoromethylbenzyl)]amide; (39e**, **39** with $\text{HNR}_1\text{R}_2 = \text{N-methyl-N-(3-trifluoromethylbenzyl)amine}$ and $n = 1$):**

Use of *N*-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -[N-methyl-N-(3-trifluoromethylbenzyl)]amide **38e** (0.282 g, 0.57 mmol) gave 0.195 g (95%) of **39e** as an off-white solid. **10e** exhibited a ^1H NMR spectrum consistent with the assigned structure.

D-aspartic acid β -*t*-butyl ester α -[(S)-1-(3-trifluoromethylphenyl)ethyl]amide; (39f**, **39** with $\text{HNR}_1\text{R}_2 = (\text{S})\text{-1-(3-trifluoromethylphenyl)ethylamine}$ and $n = 1$):**

Use of *N*-Benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -[(S)-1-(3-trifluoromethylphenyl)ethyl]amide **38f** (120 mg, 0.24 mmol) gave 80 mg (91%) of **39f** as an off-white solid; ^1H NMR (MeOH-d_4) δ 1.42 (s, 9H); 1.48 (d, $J=7.1$ Hz, 3H); 2.58 (dd, $J=7.0$ Hz, $J=16.8$ Hz, 1H); 2.69 (dd, $J=5.8$ Hz, $J=16.8$ Hz, 1H); 3.77–3.89 (m, 1H); 5.03–5.09 (m, 1H); 7.48–7.65 (m, 4H).

D-aspartic acid β -*t*-butyl ester α -[(R)-1-(3-trifluoromethylphenyl)ethyl]amide; (39g**, **39** with $\text{HNR}_1\text{R}_2 = (\text{R})\text{-1-(3-trifluoromethylphenyl)ethylamine}$ and $n = 1$):**

Use of *N*-Benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester α -[(R)-1-(3-trifluoromethylphenyl)ethyl]amide **38g** (217 mg, 0.44 mmol) gave 157 mg (quantitative yield) of **39g** as an off-white solid; ^1H NMR (MeOH-d_4) δ 1.39 (s, 9H); 1.49 (d, $J=7.1$ Hz, 3H); 2.55 (dd, $J=6.6$ Hz, $J=16.4$ Hz, 1H); 2.63 (dd, $J=5.7$ Hz, $J=16.4$ Hz, 1H); 3.60–3.64 (m, 1H); 5.00–5.08 (m, 1H); 7.47–7.67 (m, 4H).

D-glutamic acid γ -*t*-butyl ester α -(3-trifluoromethyl)benzylamide; (39h**, **39** with $\text{HNR}_1\text{R}_2 = 3\text{-(trifluoromethyl)benzylamine}$ and $n = 2$):**

Use of *N*-benzyloxycarbonyl-D-glutamic acid γ -*t*-butyl ester α -(3-trifluoromethyl)benzylamide (1.667 g, 3.37 mmol) **38h** gave 1.15 g (94%) of **39h** as an off-white solid; ^1H NMR (CDCl_3) δ 1.41 (s, 9H); 1.80–2.20 (m, 4H); 2.31–2.40 (m, 2H); 3.51–3.59 (m, 1H); 4.47–4.49 (m, 2H); 7.39–7.52 (m, 4H); 7.71–7.79 (m, 1H).

L-glutamic acid α -*t*-butyl ester γ -(4-cyclohexyl)piperazinamide; (44a**, **44** with $\text{HNR}_1\text{R}_2 = 1\text{-cyclohexylpiperazine}$ and $n = 2$):**

Use of *N*-Benzyloxycarbonyl-L-glutamic acid α -*t*-butyl ester γ -(4-cyclohexyl)piperazinamide (1.93 g, 3.96 mmol) **43a** gave 1.30 g (93%) of **44a** as an off-white solid; ^1H NMR (CDCl_3) δ 1.02–1.25 (m, 5H); 1.41 (s, 9H); 1.45–1.50 (m, 1H); 1.56–1.60 (m, 1H); 1.69–1.80 (m, 6H); 3.30 (dd, $J=4.8$ Hz, $J=8.5$ Hz, 1H); 3.44 (t, $J=9.9$ Hz, 2H); 3.56 (t, $J=9.9$ Hz, 2H).

General procedure for formation of a 2-azetidinone from an imine and an acetyl chloride:

Step 1: General procedure for formation of an imine from an amino acid derivative:

A solution of 1 equivalent of an α -amino acid ester or amide (such as **35**, **39** or **44**) in dichloromethane was treated sequentially with 1 equivalent of an appropriate aldehyde (such as *t*-cinnamaldehyde), and a dessicating agent, such as magnesium sulfate or silica gel, in the amount of about 2 grams of dessicating agent per gram of starting α -amino acid ester or amide. The reaction was stirred at ambient temperature until all of the reactants were consumed as measured by thin layer chromatography (CH_2Cl_2 95%/MeOH 5%). When complete, the reaction mixture was then filtered, the filter cake was washed with dichloromethane, and the filtrate concentrated under reduced pressure to provide the desired imine that was used as is in the subsequent step.

Step 2: General procedure for the [2+2] cycloaddition of an imine and an acetyl chloride:

A dichloromethane solution of the imine (such as **36**, **40**, or **45**) (10 mL dichloromethane/1 g imine) was cooled to 0°C. To this cooled solution was added 1.5 equivalents of an appropriate amine, typically triethylamine, followed by the dropwise addition of a dichloromethane solution of 1.1 equivalents of an appropriate acetyl chloride (such as **4**) (10 mL of dichloromethane/1 g appropriate acetyl chloride). The reaction mixture was allowed to warm to ambient temperature over 1 h and was then quenched by the addition of a saturated aqueous solution of ammonium chloride. The resulting mixture was partitioned between water and dichloromethane. The layers were separated and the organic layer was washed successively with 1N hydrochloric acid, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was either used directly for further reactions, or purified by chromatography or by crystallization from an appropriate solvent system before use.

The following compounds were prepared according to this sequence of procedures: *tert*-Butyl [3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate (**55**):

The imine prepared from 4.53 g (34.5 mmol) of glycine *tert*-butyl ester and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 5.5 g (30%) of **55** as colorless crystals (recrystallized, *n*-chlorobutane); mp 194–195 °C. ¹H NMR (CDCl₃) δ 1.39 (s, 9H); 3.43 (d, J=18.0 Hz, 1H); 4.15 (dd, J=7.5 Hz, J=8.7Hz, 1H); 4.24 (d, J=18.0Hz, 1H); 4.55–4.63 (m, 3H); 4.80 (dd, J=7.7 Hz, J=8.5 Hz, 1H); 6.05–6.13 (m, 1H); 6.64 (d, J=16.0 Hz, 1H); 7.28–7.42 (m, 10H).

2(S)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid *N*-(3-trifluoromethylbenzyl)amide (**56**):

Imine **36a** prepared from 1.52 g (4.39 mmol) of L-aspartic acid β-*t*-butyl ester α-(3-trifluoromethyl)benzylamide (**35a**) and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 2.94 g of an orange-brown oil that gave, after flash column chromatography purification (70:30 hexanes/ethyl acetate), 2.06 g (70%) of **56** as a white solid; ¹H NMR (CDCl₃) δ 1.39 (s, 9H); 2.46 (dd, J=11.1 Hz, J=16.3 Hz, 1H); 3.18 (dd, J=3.8 Hz, J=16.4 Hz, 1H); 4.12–4.17 (m, 1H); 4.26 (d, J=5.0 Hz, 1H); 4.45 (dd, J=6.0 Hz, J=14.9 Hz, 1H); 4.54 (dd, J=5.3 Hz, J=9.8 Hz, 1H); 4.58–4.66 (m, 3H); 4.69–4.75 (m, 1H); 4.81 (dd, J=3.8 Hz, J=11.1 Hz, 1H); 6.25 (dd, J=9.6 Hz, J=15.8 Hz, 1H); 6.70 (d, J=15.8 Hz, 1H); 7.14–7.17 (m, 2H); 7.28–7.46 (m, 11H); 7.62 (s, 1H); 8.27–8.32 (m, 1H).

2(S)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid *N*-[4-(2-phenylethyl)]piperazinamide (**57**):

Imine **36b** prepared from 4.20 g (11.6 mmol) of L-aspartic acid β -*t*-butyl ester α -[4-(2-phenylethyl)]piperazinamide (**35b**) and *t*-cinnamaldehyde was combined with 2-(4(*S*)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 4.37 g (55%) of **57** as a white solid after flash column chromatography purification (50:50 hexanes/ethyl acetate); ^1H NMR (CDCl_3) δ 1.34 (s, 9H); 2.26–2.32 (m, 1H); 2.46–2.63 (m, 4H); 2.75–2.89 (m, 4H); 3.24–3.32 (m, 1H); 3.49–3.76 (m, 3H); 4.07–4.13 (m, 1H); 4.30 (d, $J=4.6$ Hz, 1H); 4.22–4.48 (m, 1H); 4.55–4.61 (m, 1H); 4.69–4.75 (m, 1H); 5.04–5.09 (m, 1H); 6.15 (dd, $J=9.3$ Hz, $J=15.9$ Hz, 1H); 6.63 (d, $J=15.8$ Hz, 1H); 7.18–7.42 (m, 15H).

(*S*)-(tert-Butoxycarbonyl)ethyl-2-[3(*S*)-(4(*S*)-phenyloxazolidin-2-on-3-yl)-4(*R*)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide (**58**):

Imine **36c** prepared from 3.94 g (10.93 mmol) of L-glutamic acid γ -*t*-butyl ester α -(3-trifluoromethyl)benzylamide (**35c**) and *t*-cinnamaldehyde was combined with 2-(4(*S*)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 5.53 g (75%) of **58** as a white solid after flash column chromatography purification (70:30 hexanes/ethyl acetate); ^1H NMR (CDCl_3) δ 1.36 (s, 9H); 1.85–1.96 (m, 1H); 2.18–2.49 (m, 3H); 4.14–4.19 (m, 1H); 4.30 (d, $J=4.9$ Hz, 2H); 4.44 (dd, $J=6.1$ Hz, $J=14.9$ Hz, 1H); 4.56–4.67 (m, 4H); 4.71–4.75 (m, 1H); 6.26 (dd, $J=9.6$ Hz, $J=15.8$ Hz, 1H); 6.71 (d, $J=15.8$ Hz, 1H); 7.16–7.18 (m, 2H); 7.27–7.49 (m, 11H); 7.60 (s, 1H); 8.08–8.12 (m, 1H).

2(*R*)-(tert-Butoxycarbonylmethyl)-2-[3(*S*)-(4(*S*)-phenyloxazolidin-2-on-3-yl)-4(*R*)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide (**59**):

Imine **40a** prepared from 0.973 g (2.81 mmol) of D-aspartic acid β -*t*-butyl ester α -(3-trifluoromethyl)benzylamide (**39a**) and *t*-cinnamaldehyde was combined with 2-(4(*S*)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 1.53 g (82%) of **59** as a white solid after flash column chromatography purification (70:30 hexanes/ethyl acetate); ^1H NMR (CDCl_3) δ 1.37 (s, 9H); 3.10 (dd, $J=3.7$ Hz, $J=17.8$ Hz, 1H); 3.20 (dd, $J=10.7$ Hz, $J=17.8$ Hz, 1H); 4.02 (dd, $J=3.6$ Hz, $J=10.6$ Hz, 1H); 4.11–4.17 (m, 1H); 4.24 (d, $J=4.9$ Hz, 1H); 4.46 (dd,

$J=5.8$ Hz, $J=15.1$ Hz, 1H); 4.58–4.67 (m, 3H); 4.70–4.76 (m, 1H); 6.27 (dd, $J=9.5$ Hz, $J=15.8$ Hz, 1H); 6.79 (d, $J=15.8$ Hz, 1H); 7.25–7.50 (m, 13H); 7.63 (s, 1H); 8.50–8.54 (m, 1H).

2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(2-fluoro-3-trifluoromethylbenzyl)amide (**60**):

Imine **40b** prepared from 0.256 g (0.70 mmol) of D-aspartic acid β -*t*-butyl ester α -(2-fluoro-3-trifluoromethyl)benzylamide (**39b**) and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl (**4**) to give 0.287 g (60%) of **60** as a white solid after flash column chromatography purification (70:30 hexanes/ethyl acetate); ^1H NMR (CDCl_3) δ 1.38 (s, 9H); 3.12 (dd, $J=4.0$ Hz, $J=17.8$ Hz, 1H); 3.20 (dd, $J=10.4$ Hz, $J=17.8$ Hz, 1H); 4.05 (dd, $J=3.9$ Hz, $J=10.4$ Hz, 1H); 4.14 (dd, $J=J'=8.2$ Hz, 1H); 4.25 (d, $J=4.9$ Hz, 1H); 4.59–4.67 (m, 4H); 4.74 (t, $J=8.3$ Hz, 1H); 6.36 (dd, $J=9.6$ Hz, $J=15.8$ Hz, 1H); 6.83 (d, $J=15.8$ Hz, 1H); 7.02–7.07 (m, 1H); 7.28–7.55 (m, 12H); 8.44–8.48 (m, 1H).

2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)- α -methylbenzyl]amide (**61**):

Imine **40c** prepared from 0.167 g (0.57 mmol) of D-aspartic acid β -*t*-butyl ester [(S)- α -methylbenzyl]amide (**39c**) and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 0.219 g (63%) of **61** as a white solid after flash column chromatography purification (70:30 hexanes/ethyl acetate); ^1H NMR (CDCl_3) δ 1.35 (s, 9H); 1.56 (d, $J=7.0$ Hz, 3H); 2.97 (dd, $J=3.5$ Hz, $J=18.0$ Hz, 1H); 3.15 (dd, $J=11.0$ Hz, $J=17.5$ Hz, 1H); 4.01 (dd, $J=3.0$ Hz, $J=11.0$ Hz, 1H); 4.14 (t, $J=8.5$ Hz, 1H); 4.24 (d, $J=5.0$ Hz, 1H); 4.57 (dd, $J=5.0$ Hz, $J=9.5$ Hz, 1H); 4.64 (t, $J=8.8$ Hz, 1H); 5.07 (t, $J=8.5$ Hz, 1H); 5.03–5.09 (m, 1H); 6.43 (dd, $J=9.5$ Hz, $J=16.0$ Hz, 1H); 6.83 (d, $J=16.0$ Hz, 1H); 7.16–7.20 (m, 1H); 7.27–7.49 (m, 14H); 8.07–8.10 (m, 1H).

2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)-α-methylbenzyl]amide (**62**):

Imine **40d** prepared from 0.187 g (0.46 mmol) of D-aspartic acid β-*t*-butyl ester [(R)-α-methylbenzyl]amide (**39d**) and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 0.25 g (64%) of **62** as a white solid after flash column chromatography purification (70:30 hexanes/ethyl acetate); ¹H NMR (CDCl₃) δ 1.36 (s, 9H); 1.59 (d, J=7.1 Hz, 3H); 3.10 (dd, J=3.5 Hz, J=17.8 Hz, 1H); 3.22 (dd, J=10.9 Hz, J=17.8 Hz, 1H); 3.93 (dd, J=3.5 Hz, J=10.8 Hz, 1H); 4.14 (t, J=8.1 Hz, 1H); 4.24 (d, J=5.0 Hz, 1H); 4.58 (dd, J=5.0 Hz, J=9.5 Hz, 1H); 4.65 (t, J=8.7 Hz, 1H); 4.74 (t, J=8.2 Hz, 1H); 5.06–5.14 (m, 1H); 6.32 (dd, J=9.5 Hz, J=15.8 Hz, 1H); 6.74 (d, J=15.8 Hz, 1H); 7.19–7.43 (m, 15H); 8.15–8.18 (m, 1H). ¹³C NMR (CDCl₃) δ 21.84, 27.98, 35.44, 49.56, 54.81, 61.00, 61.98, 63.60, 71.00, 81.15, 121.25, 126.33, 126.80, 126.87, 127.18, 128.41, 128.75, 128.78, 129.69, 135.27, 135.91, 139.03, 143.99, 157.97, 162.99, 167.26, 170.94.

2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-methyl-N-(3-trifluoromethylbenzyl)amide (**63**):

Imine **40e** prepared from 0.195 g (0.41 mmol) of D-aspartic acid β-*t*-butyl ester α-[N-methyl-N-(3-trifluoromethylbenzyl)]amide (**39e**) and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 0.253 g (69%) of **63** as a white solid after flash column chromatography purification (70:30 hexanes/ethyl acetate); ¹H NMR (CDCl₃) δ 1.36 (s, 9H); 2.53 (dd, J=4.0 Hz, J=17.0 Hz, 1H); 3.06 (dd, J=10.8 Hz, J=16.8 Hz, 1H); 3.13 (s, 3H); 4.12 (dd, J=8.0 Hz, J=9.0 Hz, 1H); 4.26 (d, J=5.0 Hz, 1H); 4.38 (d, J=15.0 Hz, 1H); 4.46 (dd, J=5.0 Hz, J=9.5 Hz, 1H); 4.56 (t, J=6.8 Hz, 1H); 4.70–4.79 (m, 2H); 5.27 (dd, J=4.0 Hz, J=11.0 Hz, 1H); 6.22 (dd, J=9.3 Hz, J=15.8 Hz, 1H); 6.73 (d, J=15.8 Hz, 1H); 7.33–7.45 (m, 14H).

2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)-1-(3-trifluoromethylphenyl)ethyl]amide (**64**):

Imine **40f** prepared from 0.08 g (0.22 mmol) of D-aspartic acid β -*t*-butyl ester [(S)- α -methylbenzyl]amide (**39f**) and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 53 mg (35%) of **64** as a white solid after flash column chromatography purification (70:30 hexanes/ethyl acetate); ^1H NMR (CDCl_3) δ 1.36 (s, 9H); 1.59 (d, $J=7.0$ Hz, 3H); 3.00 (dd, $J=3.4$ Hz, $J=17.8$ Hz, 1H); 3.19 (dd, $J=11.0$ Hz, $J=17.8$ Hz, 1H); 4.01 (dd, $J=3.4$ Hz, $J=11.0$ Hz, 1H); 4.07–4.19 (m, 2H); 4.25 (d, $J=5.0$ Hz, 1H); 4.58–4.78 (m, 3H); 5.09–5.16 (m, 1H); 6.44 (dd, $J=9.5$ Hz, $J=15.8$ Hz, 1H); 6.83 (d, $J=15.8$ Hz, 1H); 7.19–7.47 (m, 13H); 7.63 (s, 1H), 8.26–8.29 (m, 1H).

2(R)-(tert-Butoxycarbonylmethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)-1-(3-trifluoromethylphenyl)ethyl]amide (**65**):

Imine **40g** prepared from 0.16 g (0.44 mmol) of D-aspartic acid β -*t*-butyl ester [(R)- α -methylbenzyl]amide (**39g**) and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 0.166 g (55%) of **65** as a white solid after flash column chromatography purification (70:30 hexanes/ethyl acetate); ^1H NMR (CDCl_3) δ 1.36 (s, 9H); 1.60 (d, $J=7.1$ Hz, 3H); 3.10 (dd, $J=3.7$ Hz, $J=17.8$ Hz, 1H); 3.19 (dd, $J=10.6$ Hz, $J=17.8$ Hz, 1H); 3.93 (dd, $J=3.7$ Hz, $J=10.6$ Hz, 1H); 4.16 (t, $J=8.1$ Hz, 1H); 4.25 (d, $J=5.0$ Hz, 1H); 4.58 (dd, $J=5.0$ Hz, $J=9.5$ Hz, 1H); 4.67 (t, $J=8.6$ Hz, 1H); 4.74 (t, $J=8.2$ Hz, 1H); 5.10–5.19 (m, 1H); 6.27 (dd, $J=9.5$ Hz, $J=15.8$ Hz, 1H); 6.74 (d, $J=15.8$ Hz, 1H); 7.17–7.59 (m, 13H); 7.75 (s, 1H), 8.25–8.27 (m, 1H).

2(R)-(tert-Butoxycarbonyl)ethyl-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide (**66**):

Imine **40h** prepared from 1.15 g (3.20 mmol) of D-glutamic acid γ -*t*-butyl ester α -(3-trifluoromethyl)benzylamide (**39h**) and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 1.84 g (85%) of **66** as a white solid after flash column chromatography purification (70:30 hexanes/ethyl acetate); ^1H NMR (CDCl_3) δ 1.37 (s, 9H); 2.23–2.39 (m, 4H); 3.71–3.75 (m, 1H); 4.13–4.18 (m, 1H); 4.31 (d, $J=4.9$ Hz, 1H); 4.44–4.51 (m, 2H); 4.56–4.68 (m, 2H); 4.71–4.76 (m, 1H); 6.26 (dd, $J=9.5$ Hz, $J=15.8$ Hz, 1H); 6.71 (d, $J=15.8$ Hz, 1H); 7.25–7.52 (m, 13H); 7.63 (s, 1H); 8.25–8.30 (m, 1H).

tert-Butyl 2(S)-(2-(4-cyclohexylpiperazin-1-ylcarbonyl)ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate (**67**)

Imine **45a** prepared from 1.282 g (3.63 mmol) of L-glutamic acid α -*t*-butyl ester γ -(4-cyclohexyl)piperazinamide (**44a**) and *t*-cinnamaldehyde was combined with 2-(4(S)-phenyloxazolidin-2-on-3-yl) acetyl chloride (**4**) to give 1.946 g (80%) of **67** as a white solid after flash column chromatography purification (50:50 hexanes/ethyl acetate); ^1H NMR (CDCl_3) δ 1.15–1.26 (m, 6H); 1.39 (s, 9H); 1.55–1.64 (m, 2H); 1.77–1.83 (m, 3H); 2.22–2.35 (m, 2H); 2.40–2.50 (m, 6H); 2.75–2.79 (m, 1H); 3.43–3.48 (m, 1H); 3.56–3.60 (m, 2H); 3.75–3.79 (m, 1H); 4.10 (t, $J=8.3$ Hz, 1H); 4.31–4.35 (m, 2H); 4.58 (t, $J=8.8$ Hz, 1H); 4.73 (t, $J=8.4$ Hz, 1H); 6.17 (dd, $J=8.6$ Hz, $J=16.0$ Hz, 1H); 6.65 (d, $J=16.0$ Hz, 1H); 7.27–7.42 (m, 10H).

General procedure for acylation of an (azetidin-2-on-1-yl)acetate:

A solution of (azetidin-2-on-1-yl)acetate in tetrahydrofuran (0.22 M in azetidinone) is cooled to -78°C and treated with lithium bis(trimethylsilyl)amide (2.2 equivalents). The resulting anion is treated with an appropriate acyl halide (1.1 equivalents). Upon complete conversion of the azetidinone, the reaction is quenched with saturated aqueous ammonium chloride and partitioned between ethyl acetate and water. The organic phase is washed sequentially with 1N hydrochloric acid, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The resulting organic layer is dried (magnesium sulfate) and evaporated. The residue is purified by silica gel chromatography with an appropriate eluent, such as 60:40 hexanes/ethyl acetate.

The following compound was prepared according to this procedure: 2,2,2-Trichloroethyl 2 (RS)-(tert-butoxycarbonyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetate (**30**):

Azetidinone **55**, 9.0 g (20 mmol), was acylated with 4.2 g (20 mmol) of trichloroethylchloroformate to give 7.0 g (56%) of **30** as a white solid; mp $176\text{--}178^{\circ}\text{C}$. ^1H NMR (CDCl_3) δ 1.31–1.34 and 1.43–1.46 (m and m', 9H); 4.12–4.18 (m, 1H); 4.53–4.90 (m; 6H); 5.18–5.24 (m, 1H); 6.10–6.18 (m, 1H); 6.61–6.66 (m, 1H); 7.28–7.41 (m, 10H).

2(RS)-(tert-Butoxycarbonyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide (**31**):

A solution of 0.20 g (0.32 mmol) of **30** and 52 μ L (0.36 mmol) of (3-trifluoromethylbenzyl) amine in THF was heated at reflux. Upon complete conversion (TLC), the solvent was evaporated and the residue was crystallized (chloroform/hexane) to give 0.17 g (82%) of **31** as a white solid; mp 182–184 $^{\circ}$ C. 1 H NMR (CDCl_3) δ 1.36–1.44 (m, 9H); 4.13–4.14–4.91 (m; 8H); 6.23–6.31 (m, 1H); 6.65–6.78 (m, 1H); 7.20–7.60 (m, 14H); 7.88–7.92 and 8.08–8.12 (m and m', 1H). Anal. Calcd for $\text{C}_{35}\text{H}_{34}\text{F}_3\text{N}_3\text{O}_6$: C, 64.71; H, 5.28; N, 6.47. Found: C, 64.78; H, 5.31; N, 6.36.

General procedure for hydrolysis of a *tert*-butyl ester:

A solution of *tert*-butyl ester derivative in formic acid, typically 1 g in 10 mL, is stirred at ambient temperature until no more ester is detected by thin layer chromatography (dichloromethane 95%/methanol 5%), a typical reaction time being around 3 hours. The formic acid is then evaporated under reduced pressure to yield the corresponding carboxylic acid.

The following compounds were prepared according to this procedure: 2(R,S)-(Carboxy)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide (32):

Compound **31** (0.30 g, 0.46 mmol) was hydrolyzed to give 0.27 g (quantitative yield) of **32** as an off-white solid; ¹H NMR (CDCl₃) δ 4.17–5.28 (m, 9H); 6.21–6.29 (m, 1H), 6.68–6.82 (m, 1H); 7.05–7.75 (m, 13H); 9.12–9.18 (m, 1H).

2(S)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide (68):

Compound **56** (1.72 g, 2.59 mmol) was hydrolyzed to give 1.57 g (quantitative yield) of **68** as an off-white solid; ^1H NMR (CDCl_3) δ 2.61 (dd, $J=9.3$ Hz, $J=16.6$ Hz, 1H); 3.09–3.14 (m, 1H); 4.10–4.13 (m, 1H); 4.30 (d, $J=4.5$ Hz, 1H); 4.39–4.85 (m, 6H); 6.20 (dd, $J=9.6$ Hz, $J=15.7$

Hz, 1H); 6.69 (d, $J=15.8$ Hz, 1H); 7.12–7.15 (m, 2H); 7.26–7.50 (m, 11H); 7.61 (s, 1H); 8.41–8.45 (m, 1H).

2(S)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[4-(2-phenylethyl)]piperazinamide (**69**):

Compound **57** (1.88 g, 2.78 mmol) was hydrolyzed to give 1.02 g (60%) of **69** as an off-white solid; ^1H NMR (CDCl_3) δ 2.63 (dd, $J=6.0$ Hz, $J=16.5$ Hz, 1H); 2.75–2.85 (m, 1H); 3.00 (dd, $J=8.2$ Hz, $J=16.6$ Hz, 1H); 3.13–3.26 (m, 4H); 3.37–3.56 (m, 4H); 3.86–4.00 (m, 1H); 4.05–4.11 (m, 1H); 4.24 (d, $J=5.0$ Hz, 1H); 4.46–4.66 (m, 1H); 4.65–4.70 (m, 1H); 5.10–5.15 (m, 1H); 6.14 (dd, $J=9.3$ Hz, $J=15.9$ Hz, 1H); 6.71 (d, $J=15.9$ Hz, 1H); 7.22–7.41 (m, 15H); 12.02 (s, 1H).

2(S)-(Carboxyethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide (**70**):

Compound **58** (4.97 g, 7.34 mmol) was hydrolyzed to give 4.43 g (97%) of **70** as an off-white solid; ^1H NMR (CDCl_3) δ 1.92–2.03 (m, 1H); 2.37–2.51 (m, 3H); 4.13–4.19 (m, 1H); 3.32 (d, $J=4.9$ Hz, 1H); 4.35–4.39 (m, 1H); 4.44 (dd, $J=5.9$ Hz, $J=14.9$ Hz, 1H); 4.50–4.57 (m, 2H); 4.61–4.67 (m, 1H); 4.70–4.76 (m, 1H); 6.24 (dd, $J=9.6$ Hz, $J=15.8$ Hz, 1H); 6.70 (d, $J=15.8$ Hz, 1H); 7.18–7.47 (m, 14H).

2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide (**71**):

Compound **59** (1.51 g, 2.27 mmol) was hydrolyzed to give 1.38 g (quantitative yield) of **71** as an off-white solid; ^1H NMR (CD_3OD) δ 2.93 (dd, $J=5.5$ Hz, $J=17.0$ Hz, 1H); 2.99 (dd, $J=9.0$ Hz, $J=17.0$ Hz, 1H); 4.17 (dd, $J=7.0$ Hz, $J=9.0$ Hz, 1H); 4.36 (dd, $J=5.5$ Hz, $J=9.0$ Hz, 1H); 4.40–4.45 (m, 2H) 4.55 (d, $J=15.5$ Hz, 1H); 4.65 (dd, $J=5.0$ Hz, $J=9.0$ Hz, 1H); 4.70 (dd, $J=J'=9.0$ Hz, 1H); 4.89 (dd, $J=7.0$ Hz, $J=9.0$ Hz, 1H); 6.26 (dd, $J=9.0$ Hz, $J=16.0$ Hz, 1H); 6.83 (d, $J=16.0$ Hz, 1H); 7.24–7.32 (m, 5H); 7.38–7.48 (m, 6H); 7.51–7.57 (m, 2H); 7.64 (s, 1H). ^{13}C NMR (CD_3OD) δ 43.82, 55.92, 62.01, 63.34, 64.52, 72.58, 123.03, 124.97 (q, $^3J_{\text{C-F}}$, 3.8Hz, 1C), 125.54, (q, $^3J_{\text{C-F}}$, 3.8Hz, 1C), 127.82, 128.55, 129.66, 129.81, 130.34, 130.44, 130.54, 131.77 (q, $^1J_{\text{C-F}}$, 32.3Hz, CF_3), 132.3–132.4 (m, 1C), 137.23, 138.42, 139.71, 141.19, 160.28, 166.12, 171.23.

2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(2-fluoro-3-trifluoromethylbenzyl)carboxamide (**72**):

Compound **60** (0.26 g, 0.38 mmol) was hydrolyzed to give 0.238 g (quantitative yield) of **72** as an off-white solid; ^1H NMR (CDCl_3) δ 3.27 (d, $J=7.2$ Hz, 1H); 4.06 (t, $J=7.2$ Hz, 1H); 4.15 (t, $J=8.1$ Hz, 1H); 4.27 (d, $J=4.8$ Hz, 1H); 4.56–4.76 (m, 5H); 6.34 (dd, $J=9.5$ Hz, $J=15.7$ Hz, 1H); 6.80 (d, $J=15.7$ Hz, 1H); 7.06 (t, $J=7.7$ Hz, 1H); 7.31–7.54 (m, 12H); 8.58 (t, $J=5.9$ Hz, 1H).

2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)-α-methylbenzyl]amide (**73**):

Compound **61** (0.215 g, 0.35 mmol) was hydrolyzed to give 0.195 g (quantitative yield) of **73** as an off-white solid; ^1H NMR (CDCl_3) δ 1.56 (d, $J=7.0$ Hz, 1H); 3.10 (dd, $J=4.5$ Hz, $J=17.9$ Hz, 1H); 3.18 (dd, $J=9.8$ Hz, $J=17.9$ Hz, 1H); 4.00 (dd, $J=4.5$ Hz, $J=9.7$ Hz, 1H); 4.14 (t, $J=8.2$ Hz, 1H); 4.26 (d, $J=4.7$ Hz, 1H); 5.02–5.09 (m, 1H); 6.41 (dd, $J=9.4$ Hz, $J=15.8$ Hz, 1H); 6.78 (d, $J=15.8$ Hz, 1H); 7.18 (t, $J=7.3$ Hz, 1H); 7.26–7.43 (m, 14H); 8.29 (d, $J=8.2$ Hz, 1H).

2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)-α-methylbenzyl]amide (**74**):

Compound **62** (0.22 g, 0.35 mmol) was hydrolyzed to give 0.20 g (quantitative yield) of **74** as an off-white solid; ^1H 1.59 (d, $J=7.0$ Hz, 1H); 3.25 (d, $J=7.0$ Hz, 2H); 3.92 H NMR (CDCl_3) δ (t, $J=7.3$ Hz, 1H); 4.15 (t, $J=8.3$ Hz, 1H); 4.26 (d, $J=5.0$ Hz, 1H); 4.52 (dd, $J=4.8$ Hz, $J=9.3$ Hz, 1H); 4.65 (t, $J=8.8$ Hz, 1H); 4.72 (t, $J=8.3$ Hz, 1H); 5.07–5.28 (m, 1H); 6.29 (dd, $J=9.5$ Hz, $J=15.6$ Hz, 1H); 6.71 (d, $J=16.0$ Hz, 1H); 7.20–7.43 (m, 15H); 8.31 (d, $J=8.0$ Hz, 1H). ^{13}C NMR (CDCl_3) δ 21.73, 34.23, 49.83, 54.37, 61.02, 62.04, 63.64, 71.10, 120.71, 126.36, 126.91, 126.96, 127.20, 128.49, 128.78, 128.89, 129.74, 135.16, 135.84, 139.43, 143.68, 158.06, 163.37, 167.38, 174.53.

2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-methyl-N-(3-trifluoromethylbenzyl)amide (75):

Compound **63** (0.253 g, 0.37 mmol) was hydrolyzed to give 0.232 g (quantitative yield) of **75** as an off-white solid; ^1H NMR (CDCl_3) δ 3.07–3.15 (m, 4H); 4.13 (t, $J=8.2$ Hz, 1H); 4.30 (d, $J=4.9$ Hz, 1H); 4.46–4.78 (m, 5H); 5.23 (dd, $J=4.6$ Hz, $J=9.7$ Hz, 1H); 6.20 (dd, $J=9.4$ Hz, $J=15.9$ Hz, 1H); 6.73 (d, $J=15.9$ Hz, 1H); 7.25–7.43 (m, 14H).

2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)-1-(3-trifluoromethylphenyl)ethyl]amide (76):

Compound **64** (38.5 mg, 0.06 mmol) was hydrolyzed to give 35 mg (quantitative yield) of **76** as an off-white solid. ^1H NMR (CDCl_3) δ 1.58 (d, $J=7.0$ Hz, 3H); 3.07–3.25 (m, 2H); 4.02 (dd, $J=4.8$ Hz, $J=9.5$ Hz, 1H); 4.15 (t, $J=8.2$ Hz, 1H); 4.27 (d, $J=4.8$ Hz, 1H); 4.57 (dd, $J=4.8$ Hz, $J=9.4$ Hz, 1H); 4.61–4.77 (m, 2H); 5.07–5.29 (m, 1H); 6.41 (dd, $J=9.4$ Hz, $J=15.8$ Hz, 1H); 6.79 (d, $J=15.8$ Hz, 1H); 7.26–7.45 (m, 13H); 7.63 (s, 1H); 8.41 (d, $J=8.1$ Hz, 1H).

2(R)-(Carboxymethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)-1-(3-trifluoromethylphenyl)ethyl]amide (**77**):

Compound **65** (166 mg, 0.24 mmol) was hydrolyzed to give 152 mg (quantitative yield) of **77** as an off-white solid. ^1H NMR (CDCl_3) δ 1.60 (d, $J=7.0$ Hz, 3H); 3.08–3.26 (m 2H); 3.93 (dd, $J=4.7$ Hz, $J=9.5$ Hz, 1H); 4.17 (t, $J=8.1$ Hz, 1H); 4.26 (d, $J=4.7$ Hz, 1H); 4.54 (dd, $J=4.7$ Hz, $J=9.4$ Hz, 1H); 4.62–4.76 (m, 2H); 5.06–5.28 (m, 1H); 6.24 (dd, $J=9.4$ Hz, $J=15.8$ Hz, 1H); 6.73 (d, $J=15.8$ Hz, 1H); 7.28–7.58 (m, 13H); 7.74 (s, 1H); 8.40 (d, $J=7.4$ Hz, 1H).

2(R)-(Carboxyethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide (**78**):

Compound **66** (0.604 g, 0.89 mmol) was hydrolyzed to give 0.554 g (quantitative yield) of **78** as an off-white solid; ^1H NMR (CDCl_3) δ 2.27–2.34 (m, 4H); 4.10–4.30 (m, 2H); 4.30 (d, $J=4.3$ Hz, 1H); 4.40–4.64 (m, 4H); 4.47 (t, $J=8.1$ Hz, 1H); 6.22 (dd, $J=9.4$ Hz, $J=15.7$ Hz, 1H); 6.70 (d, $J=15.7$ Hz, 1H); 7.25–7.57 (m, 14H); 8.32–8.36 (m, 1H).

2(S)-(2-(1-ylcarbonyl)ethyl)-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetid-2-on-1-yl]acetic acid (**79**):

Compound **67** (0.787 g, 1.28 mmol) was hydrolyzed to give 0.665 g (92%) of **79** as an off-white solid; ^1H NMR (CDCl_3) δ 1.05–1.13 (m, 1H); 1.20–1.40 (m, 5H); 1.60–1.64 (m, 1H); 1.79–1.83 (m, 2H); 2.00–2.05 (m, 2H); 2.22–2.44 (m, 3H); 2.67–2.71 (m, 1H); 2.93–3.01 (m, 4H); 3.14–3.18 (m, 1H); 3.38–3.42 (m, 1H); 3.48–3.52 (m, 1H); 3.64–3.69 (m, 1H); 4.06–4.14 (m, 2H); 4.34–4.43 (m, 2H); 4.56 (t, $J=8.8$ Hz, 1H); 4.73 (t, $J=8.4$ Hz, 1H); 6.15 (dd, $J=9.1$ Hz, $J=16.0$ Hz, 1H); 6.65 (d, $J=16.0$ Hz, 1H); 7.25–7.42 (m, 10H).

The compounds shown in Table 1/Figure 18, were prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **32**, and 3-(trifluoromethyl)benzylamine was replaced with the appropriate amine HNR_1R_2 ; all compounds exhibited an ^1H NMR spectrum consistent with the assigned structure.

ASPARTIC ACID DERIVATIVES

The compounds shown in Table 2/Figure 19, were prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **68**, and 3-(trifluoromethyl)benzylamine was replaced with the appropriate amine HNR_1R_2 ; all compounds exhibited an ^1H NMR spectrum consistent with the assigned structure.

The compounds shown in Table 3/Figure 20, were prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **69**, and 3-(trifluoromethyl)benzylamine was replaced with the appropriate amine HNR_1R_2 ; all compounds exhibited an ^1H NMR spectrum consistent with the assigned structure.

The compounds shown in Table 4/Figure 21, were prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **71**, and 3-(trifluoromethyl)benzylamine was replaced with the appropriate amine HNR_1R_2 ; all compounds exhibited an ^1H NMR spectrum consistent with the assigned structure.

A typical example being **41**: 2(R)-[[4-(Piperidin-1-yl)piperidin-1-yl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(3-trifluoromethylbenzyl)amide:

Compound **41** was obtained as an off-white solid after flash silica gel column chromatography (CH_2Cl_2 99% and down to 90%/MeOH 1% and up to 10%, NH_4OH < 1%). ^1H NMR (CDCl_3) δ 1.34–1.54 (m, 4H); 1.60–1.74 (m, 4H); 1.84–1.95 (m, 2H); 2.42–2.66 (m, 6H); 2.87–2.95 (m, 1H); 3.13–3.19 (m, 1H); 3.30–3.37 (m, 1H); 3.77–3.84 (m, 1H); 4.09–4.18 (m, 2H); 4.22 (dd, $J=4.5$ Hz, 1H); 4.43–4.56 (m, 2H); 4.58–4.66 (m, 2H); 4.70–4.74 (m, 2H); 6.25–6.32 (m, 1H); 6.80–6.85 (m, 1H); 7.25–7.53 (m, 13H); 7.64 (s, 1H); 8.62–8.69 (m, 1H). ^{13}C NMR (CDCl_3) δ 24.02, 25.30, 27.10, 29.63, 33.72/33.75, 41.18/41.37, 43.15, 44.56/44.67, 49.90/49.98, 54.79/54.92, 61.16, 61.94/61.98, 62.50/62.62, 63.30/63.34, 71.06, 120.69, 123.05, 123.77 (q, $^3J_{\text{C-F}}$, 3.5 Hz, 1C), 124.52 (q, $^3J_{\text{C-F}}$, 3.8 Hz, 1C), 125.21, 126.78, 127.27, 128.77, 128.85, 128.92, 129.65, 129.74, 130.55, 130.76/130.79, 135.27, 135.68/135.72, 139.43, 139.48/139.53, 158.30, 163.18, 168.07/168.34, 169.41. HRMS (FAB) calcd for $\text{C}_{42}\text{H}_{47}\text{F}_3\text{N}_5\text{O}_5$ 758.3529, found 758.3510 ($\text{M}+\text{H}$) $^+$.

2(R)-[[4-(Piperidin-1-yl)piperidin-1-yl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-(2-fluoro-3-trifluoromethylbenzyl) amide (**102**):

Compound **102** was prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **72** and 3-(trifluoromethyl)benzylamine was replaced with 4-

(1-piperidinyl)piperidine. **102** exhibited a ^1H NMR spectrum consistent with the assigned structure. HRMS (FAB) calcd for $\text{C}_{42}\text{H}_{46}\text{F}_4\text{N}_5\text{O}_5$ 776.3435, found 776.3463 ($\text{M}+\text{H}$) $^+$.

2(R)-[[4-(Piperidin-1-yl)piperidin-1-yl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)- α -methylbenzyl]amide (**52**):

Compound **52** was prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **73** and 3-(trifluoromethyl)benzylamine was replaced with 4-(1-piperidinyl)piperidine. **52** exhibited a ^1H NMR spectrum consistent with the assigned structure. HRMS (FAB) calcd for $\text{C}_{42}\text{H}_{50}\text{N}_5\text{O}_5$ 704.3812, found 704.3806 ($\text{M}+\text{H}$) $^+$.

2(R)-[[4-[2-(1-Piperidinyl)ethyl]piperidin-1-yl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)- α -methylbenzyl]amide (**103**):

Compound **103** was prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester

monohydrate was replaced with **73** and 3-(trifluoromethyl)benzylamine was replaced with 4-[2-(1-piperidinyl)ethyl]piperidine. **103** exhibited a ^1H NMR spectrum consistent with the assigned structure. HRMS (FAB) calcd for $\text{C}_{44}\text{H}_{54}\text{N}_5\text{O}_5$ 732.4125, 732.4108 ($\text{M}+\text{H}$) $^+$.

2(R)-[[4-(Piperidin-1-yl)piperidin-1-yl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)- α -methylbenzyl]amide (**50**):

Compound **50** was prepared using the “General procedure for amide formation from a carboxylic acid”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **74** and 3-(trifluoromethyl)benzylamine was replaced with 4-(1-piperidinyl)piperidine. **50** was obtained as an off-white solid after flash silica gel column chromatography (CH_2Cl_2 99% and down to 90%/MeOH 1% and up to 10%, NH_4OH < 1%). ^1H NMR (CDCl_3) δ 1.30–1.52 (m, 4H); 1.56–1.72 (m, 7H); 1.81–1.90 (m, 2H); 2.40–2.60 (m, 6H); 2.88–2.96 (m, 1H); 3.14–3.20 (m, 1H); 3.36–3.44 (m, 1H); 3.79–3.86 (m, 1H); 3.99–4.04 (m, 1H); 4.15 (t, $J=8.3$ Hz, 1H); 4.21 (dd, $J=5.0$ Hz, $J=8.0$ Hz, 1H); 4.44–4.55 (m, 1H); 4.60–4.75 (m, 3H); 5.07–5.14 (m, 1H); 6.29–6.36 (m, 1H); 6.74–6.79 (m, 1H); 7.19–7.46 (m, 15H); 8.23–8.28 (m, 1H). ^{13}C NMR (CDCl_3) δ 21.87/21.90, 23.84, 24.90, 26.81, 33.56/33.59, 41.11/41.23, 44.52/44.57, 49.59, 49.67/49.75, 54.97, 55.16, 61.03, 61.77/61.81, 62.43/62.52, 63.37, 70.95, 121.16, 126.30, 126.79, 126.83, 127.25, 128.40, 128.70, 129.63, 129.67, 135.29/135.31, 135.80/135.83, 139.13, 143.93/143.96, 157.96, 163.29, 167.94/167.96, 168.23, 168.51. HRMS (FAB) calcd for $\text{C}_{42}\text{H}_{50}\text{N}_5\text{O}_5$ 704.3812, found 704.3801 ($\text{M}+\text{H}$) $^+$.

2(R)-[[4-[2-(1-Piperidinyl)ethyl]piperidin-1-yl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)-α-methylbenzyl]amide (104):

Compound **104** was prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β-*t*-butyl ester monohydrate was replaced with **74** and 3-(trifluoromethyl)benzylamine was replaced with 4-[2-(1-piperidinyl)ethyl]piperidine. **104** exhibited a ^1H NMR spectrum consistent with the assigned structure. HRMS (FAB) calcd for $\text{C}_{44}\text{H}_{54}\text{N}_5\text{O}_5$ 732.4125, 732.4127 ($\text{M}+\text{H}$) $^+$.

2(R)-[[4-(Piperidin-1-yl)piperidin-1-yl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-methyl-N-(3-trifluoromethylbenzyl)amide (51):

Compound **51** was prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β-*t*-butyl ester monohydrate was replaced with **75** and 3-(trifluoromethyl)benzylamine was replaced with 4-(1-piperidinyl)piperidine. **51** was obtained as an off-white solid after flash silica gel column chromatography (CH_2Cl_2 99% and down to 90%/MeOH 1% and up to 10%, NH_4OH < 1%).

51 exhibited a ^1H NMR spectrum consistent with the assigned structure. HRMS (FAB) calcd for $\text{C}_{43}\text{H}_{49}\text{F}_3\text{N}_5\text{O}_5$ 772.3686, found 772.3674 ($\text{M}+\text{H}$) $^+$.

2(R)-[[4-(Piperidin-1-yl)piperidin-1-yl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(R)- α -methyl-3-trifluoromethylbenzyl]amide (**53**):

Compound **53** was prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **77** and 3-(trifluoromethyl)benzylamine was replaced with 4-(1-piperidinyl)piperidine. **53** was obtained as an off-white solid after flash silica gel column chromatography (CH_2Cl_2 99% and down to 90%/MeOH 1% and up to 10%, NH_4OH < 1%). **53** exhibited a ^1H NMR spectrum consistent with the assigned structure. HRMS (FAB) calcd for $\text{C}_{43}\text{H}_{49}\text{F}_3\text{N}_5\text{O}_5$ 772.3686, found 772.3703 ($\text{M}+\text{H}$) $^+$.

2(R)-[[4-(Piperidin-1-yl)piperidin-1-yl]carbonylmethyl]-2-[3(S)-(4(S)-phenyloxazolidin-2-on-3-yl)-4(R)-(2-styryl)azetidin-2-on-1-yl]acetic acid N-[(S)- α -methyl-3-trifluoromethylbenzyl]amide (**105**):

Compound **105** was prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **76** and 3-(trifluoromethyl)benzylamine was replaced with 4-(1-piperidinyl)piperidine. **105** was obtained as an off-white solid after flash silica gel column chromatography (CH_2Cl_2 99% and down to 90%/MeOH 1% and up to 10%, NH_4OH < 1%). **105** exhibited a ^1H NMR spectrum consistent with the assigned structure. HRMS (FAB) calcd for $\text{C}_{43}\text{H}_{49}\text{F}_3\text{N}_5\text{O}_5$ 772.3686, found 772.3666 ($\text{M}+\text{H}$) $^+$.

GLUTAMIC ACID DERIVATIVES

The compounds shown in Table 6/Figure 22, were prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **70**, and 3-(trifluoromethyl)benzylamine was replaced with the appropriate amine HNR_1R_2 ; all compounds exhibited an ^1H NMR spectrum consistent with the assigned structure.

The compounds shown in Table 7/Figure 23, were prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **79**, and 3-(trifluoromethyl)benzylamine was replaced with the appropriate amine HNR_1R_2 ; all compounds exhibited an ^1H NMR spectrum consistent with the assigned structure.

The compounds shown in Table 8/Figure 24, were prepared using the “**General procedure for amide formation from a carboxylic acid**”, except that N-benzyloxycarbonyl-D-aspartic acid β -*t*-butyl ester monohydrate was replaced with **78**, and 3-(trifluoromethyl)benzylamine was replaced with the appropriate amine HNR_1R_2 ; all compounds exhibited an ^1H NMR spectrum consistent with the assigned structure.

Biological evaluation:

V1a Receptor Binding Assay (Azevan Pharmaceuticals Inc.):

A cell line expressing the human V1a receptor in CHO cells (henceforth referred to as the hV1a cell line) was obtained from Dr. Michael Brownstein, NIMH, Bethesda, MD, USA. The hV1a cell culture and cell-based hV1a receptor binding assay were performed according to the methods described by Thibonnier et al.²⁶ with modifications. The hV1a cell line was grown in alpha-MEM with 10% fetal bovine serum and 250ug/ml G418 (Gibco, Grand Island, NY, USA). For competitive binding assay, hV1a cells were plated into 12-well culture plate at 1:16 dilution from a confluency flask, and maintained in culture for at least two days. Culture medium was then removed, cells were washed with 2ml binding buffer (25mM HEPES, 0.25% BSA, 1x DMEM, PH = 7.0). To each well, 990ul binding buffer containing 1nM ³H-arginine-vasopressin was added, and followed by 10ul series diluted test compounds dissolved in DMSO. All incubations were in triplicate, and dose-inhibition curves consisted of total binding (DMSO) and 5 concentrations (usually 0.1, 1.0, 10, 100, and 1000nm) of test agent encompassing the IC₅₀. 100nM unlabeled arginine-vasopressin (Sigma) was used to assess non-specific binding. Cells were incubated for 30 minutes at 37°C, assay mixture was removed and each well was washed three times with PBS (PH = 7.4). 1ml 2% SDS was added per well and plates were let sit for 30 minutes. The whole content in a well was transferred to a scintillation vial. Each well was rinsed with 0.5ml PBS, which was then added to the corresponding vial. Scintillation fluid (Ecoscint, National Diagnostics, Atlanta, Georgia) was then added at 3ml per vial. Samples were counted in a liquid scintillation counter (Beckman LS6500). IC₅₀ values were calculated by Prism Curve fitting software. The conversion factor for computing K_i from IC₅₀ was 0.61.

V1a Receptor Binding Assay (Eli Lilly & Co):

The V1a binding assay employed at Eli Lilly used the same cell line expressing the human V1a receptor in CHO cells, but the assay differed inasmuch as binding was carried out on membranes using a filtration method²². IC₅₀ values measured by the two methods usually differed by 2-fold or less.

Measurement of plasma and brain levels in dogs after oral dosing:

Samples were collected from a single dog dosed orally with compound at 10 mg/kg in 10% DMSO/90% PEG300. Blood and brain samples (cortex, brain stem, cerebellum, hypothalamus and hippocampus regions) were harvested and frozen in liquid nitrogen and stored at -70 °C prior to analysis by LC/MS mass spectrometry. Samples were thawed on ice, homogenized in pH 9.4 buffer on ice extracted with ethyl acetate after addition of a reference compound to measure extraction efficiency. Extracts were removed and taken to dryness, reconstituted in acetonitrile and injected on a Finnigan ESI LC/MS/MS system. Spiked control brain samples were prepared in similar fashion for use as quantitation standards.

Measurement of plasma and brain levels in rats after oral dosing:

Initial experiments showed that in most cases our compounds could be dissolved at 20 mg/ml in 23% hydroxypropyl beta-cyclodextrin with 0.68% lactic acid. Sprague-Dawley rats (Charles River Laboratory) were dosed by gavage with a combination of three compounds (20 mg/kg each): **22** + **41** + **48** or **42** + **46** + **47**. Blood (approximately 3 mL) was collected from 3 animals per group per time point at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours postdose. Blood was collected via exsanguination (cardiac puncture) under carbon dioxide anesthesia into tubes containing sodium heparin anticoagulant and plasma was separated by centrifugation within 1 h. Brains were collected at the same time points. Plasma and brain levels were determined by LC/MS/MS.

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26. Thibonnier M, Auzan C, Madhun Z, Wilkins P, Berti-Mattera L, Clauser E. J Biol Chem 1994;269:3304. [PubMed: 8106369]

Figure 1.
Receptor crosstalk as a screening tool

Figure 2.

Figure 3.
X-ray structure/absolute configuration of **7**

Figure 4.
SAR overview of Zone A, 20 compounds prepared

Figure 5.
SAR overview of Zone B, 3 compounds prepared

Figure 6.
SAR overview of Zone C, 35 compounds prepared

Figure 7.
Acceptability of benzylamide in Zone D

Figure 8.
SAR overview of Zone D

Figure 9.
Non-additivity of the Zone C pivaloyl and Zone D piperidyl-ethyl-piperidine substitutions.

Figure 10.
Rationale for aminomalonate platform

Figure 11.
D,L-aminomalonyl platform and attendant VIa affinity

Figure 12.
L-aspartyl platform and attendant VIa affinity

Figure 13.
D-aspartyl platform and attendant V1a affinity

Figure 14.
L-glutamyl platform and attendant V1a affinity

Figure 15.
D-glutamyl platform and attendant V1a affinity

50, $K_i = 0.3$ nM

51, $K_i = 0.66$ nM

53, $K_i = 1.27$ nM

Figure 16.
Structures and human V1a binding affinities of **50**, **51** and **53**

Figure 17.
Oral pharmacokinetics of **SRX246** and **SRX251** in rats.

Figure 18.
Aminomalonic acid derivatives.

Figure 19.
L-aspartic acid derivatives.

Figure 20.
D-aspartic acid derivatives.

Figure 21.
Additional D-aspartic acid derivatives

Figure 22.
L-glutamic acid derivatives.

Figure 23.
Additional L-glutamic acid derivatives.

Figure 24.
D-glutamic acid derivatives

Scheme 1.

Scheme 2.
Synthesis of **33** and analogs

Scheme 3.

Syntheses of L-aspartic and L-glutamic acid derivatives. R = -OSu, i: HNR₁R₂/THF; R = -OH, ii: HNR₁R₂/HOBT/EDC, HCl/CH₂Cl₂.

Scheme 4.
 Syntheses of D-aspartic and D-glutamic acid derivatives

Scheme 5.
Synthesis of additional L-glutamic acid derivatives

Table 1

Structures, HRMS (FAB), V1a Ki values.

Cpd #	HNR ₁ R ₂	Calcd for: and equal to	Found: (m+H)	Ki (nM)
33	4-[2-(1-Piperidinyl)ethyl]piperidine	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3711	1.17
80	4-(1-Piperidinyl)piperidine	C ₄₁ H ₄₅ F ₃ N ₅ O ₅ 744.3373	744.3399	3.39
81	4-Butylpiperazine	C ₃₉ H ₄₃ F ₃ N ₅ O ₅ 718.3216	718.3194	6.5
82	1-Benzyl-4-aminopiperidine	C ₄₃ H ₄₃ F ₃ N ₅ O ₅ 766.3216	766.3191	13.3
83	4-Isopropylpiperazine	C ₃₈ H ₄₁ F ₃ N ₅ O ₅ 704.3060	704.3038	18.2
84	4-Cyclohexylpiperazine	C ₄₁ H ₄₅ F ₃ N ₅ O ₅ 744.3377	744.3367	26.4
85	2-(1-Piperidinyl)ethylamine	C ₃₈ H ₄₁ F ₃ N ₅ O ₅ 704.3060	704.3064	61

Table 2

Structures, HRMS (FAB), V1a Ki values.

Cpd #	HNR ₁ R ₂	Calcd for: and equal to	Found: (m+H)	Ki (nM)
37	1-Benzyl-4-aminopiperidine	C ₄₄ H ₄₅ F ₃ N ₅ O ₅ 780.3373	780.3356	1.13
42	4-(1-Piperidiny)l)piperidine	C ₄₂ H ₄₇ F ₃ N ₅ O ₅ 758.3529	758.3554	1.89
86	(+)-3(S)-1-Benzyl-3-aminopyrrolidine	C ₄₃ H ₄₃ F ₃ N ₅ O ₅ 766.3216	766.3201	3.60
87	4-Benzylhomopiperazine	C ₄₅ H ₄₇ F ₃ N ₅ O ₅ 794.3524	794.3511	5.12
88	4-[2-(1-Piperidiny)ethyl]piperidine	C ₄₄ H ₅₁ F ₃ N ₅ O ₅ 786.3842	786.3860	6.00
89	1-Benzyl-4-aminopiperidine ^(*) (Zone B = 2-phenylethyl)	C ₄₄ H ₄₇ F ₃ N ₅ O ₅ 782.3534	782.3535	23
90	4-(1-Pyrrolidiny)l)piperazine	C ₄₁ H ₄₅ F ₃ N ₅ O ₅ 744.3373	744.3355	~ 30
91	4-Cyclohexylpiperazine	C ₄₂ H ₄₇ F ₃ N ₅ O ₅ 758.3529	758.3555	~ 30
92	4-Cyclopentylpiperazine	C ₄₁ H ₄₅ F ₃ N ₅ O ₅ 744.3373	744.3397	< 60
93	4-[2-(1-Pyrrolidiny)ethyl]piperazine	C ₄₂ H ₄₈ F ₃ N ₆ O ₅ 773.3638	773.3638	< 60

* A suspension of 0.05 g (0.064 mmol) of **37** and palladium (5% wt. on activated carbon, 0.024 g) in 5 mL of methanol was held under an atmosphere of hydrogen until complete disappearance of **37** as determined by thin layer chromatography (95:5 dichloromethane/methanol eluent). The reaction was filtered to remove the palladium over carbon and the filtrate was evaporated to give 0.04 g of crude **89**. It was purified by column chromatography (95:5 dichloromethane/methanol eluent) to afford 0.011 g (23%) of **89**. HRMS (FAB) calcd for C₄₄H₄₇F₃N₅O₅ 782.3534, found 782.3535 (M+H)⁺.

Table 3

Structures, HRMS (FAB), V1a Ki values.

Cpd #	HNR ₁ R ₂	Calcd for: and equal to	Found: (m+H)	Ki (nM)
94	3-(Trifluoromethyl)benzylamine	C ₄₄ H ₄₅ F ₃ N ₅ O ₅ 780.3373	780.3344	6100
95	2-(Dimethylamino)ethylamine	C ₄₀ H ₄₉ F ₃ N ₆ O ₅ 693.3764	693.3757	> 600
96	3-(Dimethylamino)propylamine	C ₄₁ H ₅₁ N ₆ O ₅ 707.3921	707.3912	> 600

Table 4

Structures and HRMS (FAB), V1a Ki values.

Cpd #	HNR ₁ R ₂	Calcd for: and equal to	Found: (m+H)	Ki (nM)
41	4-(1-Piperidinyl)piperidine	C ₄₂ H ₄₇ F ₃ N ₅ O ₅ 758.3529	758.3510	1.49
97	4-[(1-Piperidinyl)methyl]piperidine	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3666	3.7
98	1-Benzyl-4-amino-piperidine	C ₄₄ H ₄₅ F ₃ N ₅ O ₅ 780.3373	780.3353	5.58
99	4-[2-(1-Piperidinyl)ethyl]piperidine	C ₄₄ H ₅₁ F ₃ N ₅ O ₅ 786.3842	786.3840	7.9
100	4-(1-Pyrrolidinyl)piperazine	C ₄₁ H ₄₅ F ₃ N ₅ O ₅ 744.3373	744.3398	27.5
101	3-(S)-(1-Benzyl)-3-amino-pyrrolidine	C ₄₃ H ₄₃ F ₃ N ₅ O ₅ 766.3216	766.3223	> 30

Table 5

HRMS (FAB), V1a Ki values.

Cpd #	Calcd for: and equal to	Found: (m+H)	Ki (nM)
50	C ₄₂ H ₅₀ N ₅ O ₅ 704.3812	704.3801	0.30
51	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3674	0.66
102	C ₄₂ H ₄₆ F ₄ N ₅ O ₅ 776.3435	776.3463	1.13
53	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3703	1.27
104	C ₄₄ H ₅₄ N ₅ O ₅ 732.4125	732.4127	8.7
105	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3666	> 6
52	C ₄₂ H ₅₀ N ₅ O ₅ 704.3812	704.3806	> 30
103	C ₄₄ H ₅₄ N ₅ O ₅ 732.4125	732.4108-	179

Table 6[structures, HRMS (FAB), V1a IC₅₀ and Ki values]

Cpd #	HNR ₁ R ₂	Calcd for: and equal to	Found: (m+H)	Ki (nM)
48	4-Cyclohexylpiperazine	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3672	0.27
106	4-(Cyclohexylmethyl)piperazine	C ₄₄ H ₅₁ F ₃ N ₅ O ₅ 786.3842	786.3862	0.46
46	4-(2-Phenylethyl)piperazine	C ₄₅ H ₄₇ F ₃ N ₅ O ₅ 794.3529	794.3502	1.17
47	4-(1-Piperidiny)l)piperidine	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3685	1.45
107	4-Propylpiperazine	C ₄₀ H ₄₅ F ₃ N ₅ O ₅ 732.3373	732.3371	1.19
108	4-[(1-Piperidiny)methyl]piperidine	C ₄₄ H ₅₁ F ₃ N ₅ O ₅ 786.3842	786.3821	1.95
109	2-(1-Piperidiny)ethylamine	C ₄₀ H ₄₅ F ₃ N ₅ O ₅ 732.3373	732.3374	9
110	4-[2-(1-Piperidiny)ethyl]piperidine	C ₄₅ H ₅₃ F ₃ N ₅ O ₅ 800.3999	800.4019	9.9

Table 7

Structures, HRMS (FAB), V1a Ki values.

Cpd #	HNR ₁ R ₂	Calcd for: and equal to	Found: (m+H)	Ki (nM)
111	2-Fluoro-3-(trifluoromethyl)benzylamine	C ₄₃ H ₄₈ F ₄ N ₅ O ₅ 790.3592	790.3572	0.20
112	2,3-Dichlorobenzylamine	C ₄₂ H ₄₈ Cl ₂ N ₅ O ₅ 772.3033	772.3011	0.38
113	2-Fluoro-5-(trifluoromethyl)benzylamine	C ₄₃ H ₄₈ F ₄ N ₅ O ₅ 790.3592	790.3568	0.41
114	3-Fluoro-5-(trifluoromethyl)benzylamine	C ₄₃ H ₄₈ F ₄ N ₅ O ₅ 790.3592	790.3577	0.51
115	3-Chlorobenzylamine	C ₄₂ H ₄₉ ClN ₅ O ₅ 738.3422	738.3441	0.66
116	3,5-Difluorobenzylamine	C ₄₂ H ₄₈ F ₂ N ₅ O ₅ 740.3624	740.3624	0.82
117	2-(Trifluoromethyl)benzylamine	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3692	0.93
118	3,5-Bis-(trifluoromethyl)benzylamine	C ₄₄ H ₄₈ F ₆ N ₅ O ₅ 840.3560	840.3583	4.45
119	4-Fluoro-3-(trifluoromethyl)benzylamine	C ₄₃ H ₄₈ F ₄ N ₅ O ₅ 790.3592	790.3588	< 30

Table 8

Structures, HRMS (FAB), V1a Ki values.

Cpd #	HNR ₁ R ₂	Calcd for: and equal to	Found: (m+H)	Ki (nM)
49	4-Cyclohexylpiperazine	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3707	5.84
120	4-Butylpiperazine	C ₄₁ H ₄₇ F ₃ N ₅ O ₅ 746.3529	746.3503	10.5
121	4-Isopropylpiperazine	C ₄₀ H ₄₅ F ₃ N ₅ O ₅ 732.3373	732.3347	13
122	4-(2-Phenylethyl)piperazine	C ₄₅ H ₄₇ F ₃ N ₅ O ₅ 794.3529	794.3552	33
123	4-(Cyclohexylmethyl)- piperazine	C ₄₄ H ₅₁ F ₃ N ₅ O ₅ 786.3842	786.3823	> 12
124	4-(1-Piperidiny)piperidine	C ₄₃ H ₄₉ F ₃ N ₅ O ₅ 772.3686	772.3688	~ 30