Asymmetric Syntheses of Protected Derivatives of Carnosadine and Its Stereoisomers as Conformationally Constrained Surrogates for Arginine

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All four stereoisomers of carnosadine were shown to be accessible from the lactone 1 or the diester 10 (or their enantiomers). Members of the *cis* series (Z-cyclo-Arg') were obtained via a sequence involving opening lactone 1 with ammonia, Hofmann rearrangement, and incorporation of the guanidine group via an azide (3). The *trans* series (*i.e.* The E-cyclo-Arg' series) was prepared via a route which is similar, except that it begins with hydrolysis of the less hindered ester functionality of diester 10. Products from both series were manipulated into protected forms for peptide synthesis using the BOC or the FMOC approach.

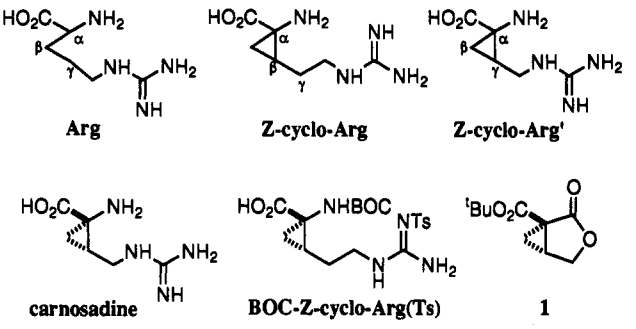
Introduction

Several studies have shown that stereoisomers of 2,3-methanoamino acids[1] can be substituted into peptides to give peptidomimetics which are relatively resilient to proteolytic degradation,[2-6] a property which could be exploited to prepare substances with increased bioavailability. Incorporation of 2,3-methanoamino acids into peptides also decreases their conformational flexibility.[7,8] Recent work from our laboratories[9] provides an illustration of this point: rigidity imposed by incorporating a 2,3-methanoamino acid greatly facilitated conformational analysis by NMR,[10,11] even in situations in which the parent (natural) peptide had no detectable secondary structure. Thus the nature of conformational restrictions by methanologs can be identified even in situations wherein the solution structure of the parent peptide cannot be analyzed by NMR due to equilibration between several well-populated conformations. This was an important observation because it implies that the shape of small peptide analogs could be systematically adjusted by adding different methanolog stereoisomers.

An intriguing possibility emerges from the discussion above. If a rigid peptidomimetic could be conformationally tailored to fit a particular receptor site, it should encounter minimum entropy factors opposing the docking process. Such entropy effects are critical to ligand receptor interactions.[12] Methanolog-containing peptidomimetics therefore have the potential to be highly potent, and bioavailable, pharmaceuticals.

Our group is investigating[6,13] cyclopropane-based analogs of FMRF-NH_2 and FLFQPQRF-NH_2 (one letter code for amino acids) to explore the properties of cyclopropane amino acids outlined above. Neuropeptides of this kind control opiate tolerance and withdrawal syndrome in the rat and other species,[14,15] two pharmacological properties that it would be advantageous to manipulate in humans. This activity is not a result of interactions with the μ-opioid receptors; they appear to interact with their own receptor site, for which the Arg-RF-NH_2 *C*-terminus is essential for good binding.[16] Systematic application of conformational constraints to the -RF-NH_2 "warhead" therefore would have revealing effects on the binding of peptidomimetics to the appropriate receptor(s).

Chart 1



Gram quantities of suitable arginine surrogates are required for the project outlined above, and for related efforts. Previously our group prepared a derivative of (*Z*)-2,3-methanoarginine, specifically 2R,3S-BOC-cyclo-Arg-(Ts)-OH.[17] This arginine surrogate is hard to prepare on a large scale by this route,[17] however, and the side chain still has appreciable flexibility. An analog of arginine which is both more accessible and constrained is therefore desirable. The design of such a compound is to be found in Nature; carnosadine from a red marine alga.[18] Car-

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nosadine and its stereoisomers are more closely related to arginine than is 2,3-methanoarginine (cyclo-Arg) insofar as the latter has an extra carbon, and they are *more* rigid at the side chain.

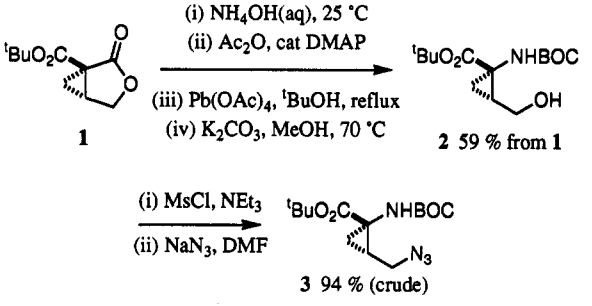
Synthesis of carnosadine and its stereoisomers (*i.e.* the cyclo-Arg′ series) (Chart 1) is a nontrivial exercise. Racemic carnosadine has been obtained via a 12-step route, then resolved,[19] but this is impractical for preparations of the gram quantities. Consequently, the work described in this paper was undertaken to develop convenient routes to carnosadine and its stereoisomers in protected forms suitable for peptide syntheses.

Results and Discussion

Most of the syntheses reported in this paper were performed twice. A small-scale run first to obtain/characterize the products and to develop conditions. A second series of experiments were then executed to test the feasibility of scale-up.

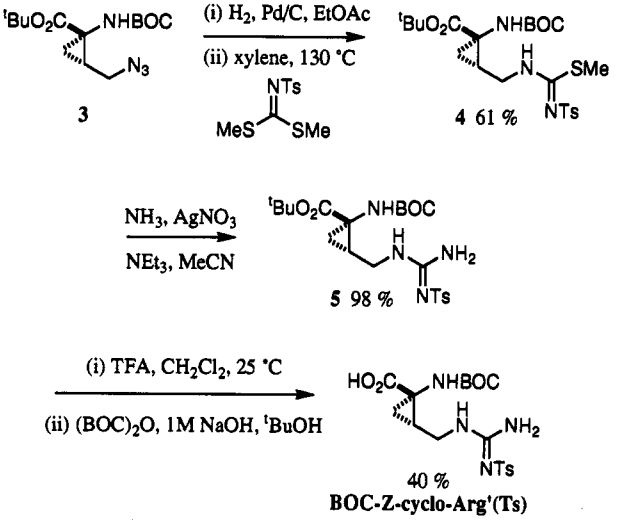
Derivatives of Z-cyclo-Arg′. Scheme 1 depicts the azide **3** which is a key intermediate for syntheses of derivatives of the Z-cyclo-Arg′ series. The mesylate of alcohol **2** was prepared in our previous synthesis of Z-cyclo-Met: *i.e.* opening the key lactone **1**[20] with ammonia, protection, Hofmann rearrangement, and deprotection. The yields shown in Scheme 1 are for the small-scale synthesis, with purifications. Comparable yields were obtained in the large-scale synthesis wherein alcohol **2** was obtained in 53% yield after recrystallization, and azide **3** (98%, crude) was used without purification. Multigram quantities of this azide were thereby prepared without column chromatography.

Scheme 1. Synthesis of Azide 3



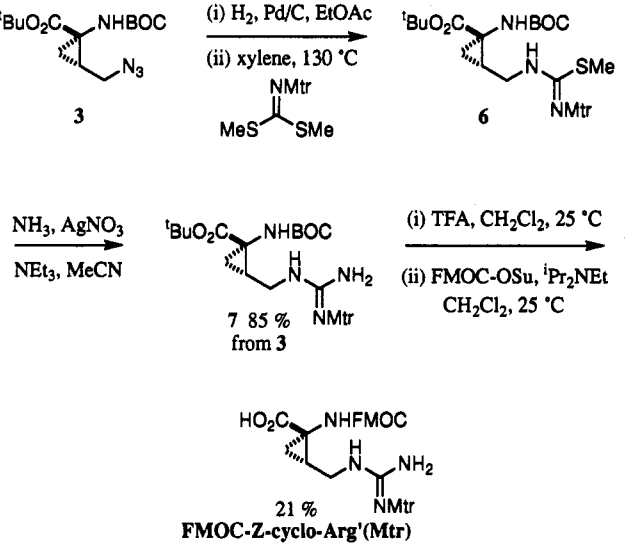
Arginine surrogates suitable for peptide syntheses using the FMOC or the BOC protection approach were required. Consequently, intermediate **3** was processed in two different ways to furnish suitably protected products. Scheme 2 shows the synthesis of BOC-Z-cyclo-Arg′(Ts). Azide **3** was reduced to an amine which was immediately reacted with S,S-dimethyl *N*-(4-methylbenzenesulfonyl)-carbonimidodithioate to give the masked guanidine **4**. Reaction with ammonia transformed this product into the tosylated guanidine **5**. This crude product was then deprotected with acid and N^{α}-protected to give the desired BOC-protected compound. This reprotection of the amine was one of the least efficient in the synthesis and reflects steric encumbrance of the α-amine group by the cyclopropane nucleus. Yields shown in Scheme 2 are for the small-scale procedure. In the large-scale reactions, introduction of the *N*-BOC group proceeded with better yield (62%), and only one single chromatographic separation, at the very end of this synthesis, was required to produce pure BOC-Z-cyclo-Arg′(Ts).

Scheme 2. Synthesis of BOC-Z-cyclo-Arg′(Ts)



Scheme 3 shows the analogous preparation of FMOC-Z-cyclo-Arg′(Mtr). Yields shown in Scheme 3 are for a large-scale synthesis using minimal purification steps. At the end of the sequence, pure FMOC-Z-cyclo-Arg′(Mtr) was obtained via chromatography after the last step. The least efficient step in the whole synthesis was for incorporation of the FMOC protecting group on the hindered α-amino group.

Scheme 3. Synthesis of FMOC-Z-cyclo-Arg′(Mtr)



Derivatives of E-Cyclo-Arg′. Functionalized (*E*)-2,3-methanologs generally were more difficult to make than their *Z* isomers because in the latter case undesirable lactonization reactions predominated when an electrophilic side chain was produced *cis* to an amide substituent. An aborted synthesis illustrates this problem.[21] Amide **8** was mesylated to introduce side-chain functionality via nucleophilic displacement; instead lactone **9** was formed, the compound originally used to prepare amide **8**! Undesirable

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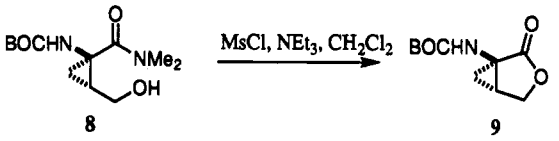
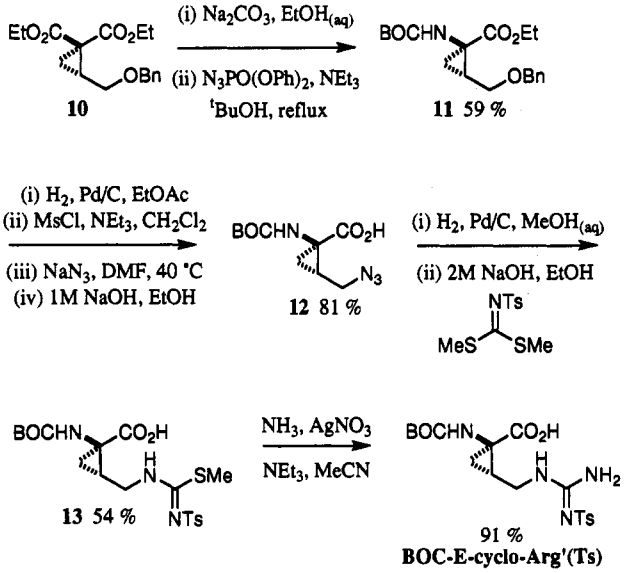


8 9

lactonization reactions of this kind can be prevented by temporarily dehydrating the amide functionality to a nitrile, but this sequence requires several steps that do not directly advance the construction of the product framework.[22]

Scheme 4 illustrates an alternative approach developed in the current study. Undesirable lactonization processes were avoided by performing side-chain manipulations on molecules with (less nucleophilic) *ester* groups *cis* to the electrophilic side chain. Previous work from our groups has shown that multigram amounts of diester **10** are available in opitcally pure form from mannitol.[20] Hydrolysis of the least encumbered ester functionality of **10**[23] and Curtius rearrangement gave the BOC-protected amino ester **11**. Hydrogenolysis of the benzyl group, mesylation, nucleophilic displacement, and hydrolysis generated the corresponding azide **12**. The alcohol and mesylate intermediates in this synthesis were used immediately; otherwise byproducts from lactonization processes were observed. Yields shown in Scheme 4 refer to the large-scale synthesis. The azide **12** was purified by an extraction procedure involving temporary formation of the sodium salt. After the next step, the functionalized acid **13** was purified by recrystallization. No chromatography was required; hence compound **12** was easily produced on a *ca.* 10-g scale. A sample of this was transformed into BOC-E-cyclo-Arg'(Ts) by reduction and introduction of a guanidine group as outlined in Scheme 4. The final product was purified via recrystallization; consequently no chromatography was performed in any of the steps leading to this material.

Scheme 4. Synthesis of BOC-E-cyclo-Arg'(Ts)

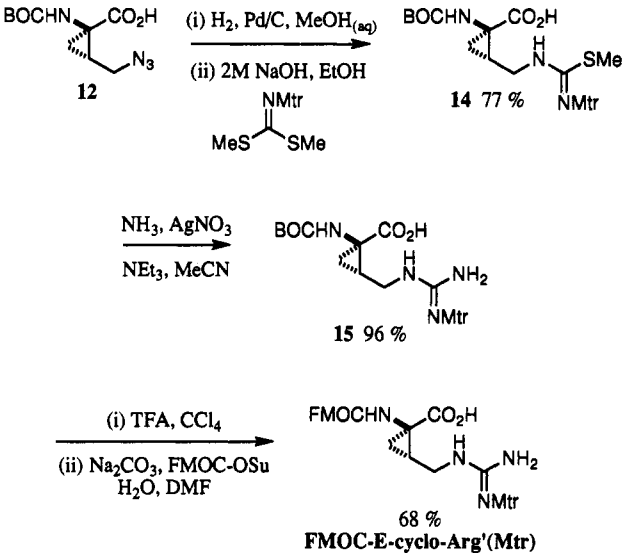


10 11 59 % 12 81 % 13 54 % 91 %

BOC-E-cyclo-Arg'(Ts)

The azide **12** was also used to prepare an E-cyclo-Arg' derivative protected for FMOC-based peptide syntheses. This sequence involved reduction of the azide, introduction of the guanidine group, removal of the BOC protecting group, and masking of the amine functionality with an FMOC (Scheme 5).

Scheme 5. Synthesis of FMOC-E-cyclo-Arg'(Mtr)



12 14 77 % 15 96 % 68 %

FMOC-E-cyclo-Arg'(Mtr)

Conclusions

Another synthesis of carnosadine was reported after this work was completed[24] and provides a useful comparison. It begins with a preparation of a nucleophile substituted with a chiral auxiliary. This is deprotonated and reacted with glycidol triflate (or with epibromohydrin) to form four diastereomeric products.[25] After this point the synthesis involved extensive chromatographic separations of diastereomeric mixtures. Finally, an intermediate analogous to alcohol **2** in Scheme 1 was produced.

Our group has also used glycidol triflate as a starting material for syntheses of 2,3-methanoamino acids,[17,22] but later developed an alternative route[20] since this triflate is expensive to prepare in optically active form and commercially available glycidol is typically of less than 95% enantiomeric purity. The route described here uses lactone **1** and diester **10** which can be prepared in 100-g amounts in optically pure form. Both enantiomers of these starting materials are accessible, and the subsequent steps in the syntheses do not perturb the stereocenters. Consequently, enantiomerically pure samples of all four cyclo-Arg' stereoisomers can be obtained via the route described in this paper. Syntheses of protected carnosadine, and stereoisomers of this, via the procedures described in this paper do not involve chromatographic separations of diastereomeric intermediates. In fact, little or no chromatography is necessary, which is an enormous advantage with respect to scale-up. The overall yields from D-mannitol to the final products are in the range 2.6–6.0% (BOC-Z-cyclo-Arg'(Ts), 3.5%; FMOC-Z-cyclo-Arg'(Mtr), 2.6%; BOC-E-cyclo-Arg'(Ts), 5.8%; FMOC-E-cyclo-Arg'-(Mtr), 6.0%). Finally, the products that were obtained are protected for peptide syntheses using either the BOC or the FMOC approach.

The syntheses described here lay foundations for pharmacological/biophysical studies of many different peptidomimetics containing constrained arginine surrogates. One application was outlined in the Introduction

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of this paper, but there are many others. For instance, this work could be used to develop conformational probes of the Arg-Gly-Asp sequence central to glycoprotein IIbIIIa–fibrinogen interactions.[26-29]

Experimental Section

General Procedures. Melting points were uncorrected. High-field NMR spectra were recorded on a Varian XLAA 200 or a Gemini GEMA 200 (^{1}H at 200 MHz, ^{13}C at 50 MHz), ^{1}H chemical shifts are reported in δ relative to $CHCl_3$ (7.25 ppm) as internal standard, and ^{13}C chemical shifts are reported in ppm relative to $CHCl_3$ (77.0 ppm) unless specified otherwise. Multiplicities in ^{1}H NMR are reported as (br) broad, (s) singlet, (d) doublet, (t) triplet, (q) quartet, and (m) multiplet. Where necessary, the carbon multiplicities were determined via APT experiments. Thin layer chromatography was performed on silica gel 60 F_{254} plates from Whatman. Flash chromatography was performed on SP silica gel 60 (230–600-mesh ASTM). DMF was stored over 4-Å molecular sieves for a week before use; CH_2Cl_2 and *t*-BuOH were distilled from CaH_2. Other chemicals were purchased from commercial suppliers and used as received.

(1*S*,2*S*)-*tert*-Butyl 2-(Azidomethyl)-1-[*N*-(*tert*-butoxycarbonyl)amino]cyclopropane-1-carboxylate (3). The mesylate from the alcohol **2**[22] (130 mg, 0.355 mmol) was dissolved in 1 mL of DMF, and sodium azide (27.7 mg, 0.426 mmol, 1.2 equiv) was added with stirring at 25 °C. After 2 h at 25 °C, the DMF was evaporated under vacuum, diethyl ether (2 mL) was added to the crude product, and the solution was filtered through a short silica gel column with diethyl ether as eluent. After evaporation of the solvent, 102 mg of product was obtained (crude yield, 94%): mp 72–73 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.06 (br s, 1H), 3.44–3.25 (bd, 2H), 2.04–1.83 (bm, 1H), 1.71–1.55 (br, 1H), 1.41 (s, 9H), 1.39 (s, 9H), 1.10–0.92 (br, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.0, 156.3, 81.7, 80.1, 50.5, 38.5, 28.2, 27.9, 25.5, 21.1; IR ($CHBr_3$) 2986, 2097, 1720, 1484, 1367, 1289, 1246 cm^{-1}; $[\alpha]^{25}_D$ −4.79° (*c* = 1.23,CH_2Cl_2). Anal. Calcd for $C_{14}H_{24}N_4O_4$: C, 53.83; H, 7.74. Found: C, 53.78; H, 7.67. In the large-scale synthesis, a mixture of the mesylate of alcohol **2** (31.83 g), sodium azide (6.3 g), and DMF (200 mL) was stirred for 15 h at 60 °C under N_2. After concentration of the solution, the residue was extracted with Et_2O (400 mL × 2), and the organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated under vacuum to give 26.33 g of crude product **3** (crude yield, 98% for two steps). *Caution is recommended when manipulating this azide since it could conceivably decompose violently when activated by heat, shock, or friction.* No such problems were encountered in this work, however.

(1*S*,2*S*)-*tert*-Butyl 1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[[[(methylthio)(toluenesulfonylimido)methyl]amino]methyl]-cyclopropane-1-carboxylate (4). The azidomethyl derivative **3** (102 mg, 0.327 mmol) was dissolved in 2 mL of methanol. To this solution was added 10% Pd/C (51 mg, 0.048 mmol, 0.15 equiv), and the mixture was stirred at 25 °C for 2 h under an atmosphere of H_2. The resulting solution was filtered through Celite and silica gel in a short column and then washed with 10 mL of methanol. After evaporation of the solvent, the crude amine formed was used for the next step without purification. In the large-scale synthesis, a mixture of crude **3** (5.30 g) and 10% Pd/C (530 mg) in MeOH (150 mL) was stirred under an H_2 atmosphere for 12 h. The reaction solution was filtered and dried to yield 5.23 g of the crude amine: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.48 (br s, 1H), 2.98–2.82 (br m, 1H), 2.55–2.37 (br m, 1H), 1.82 (br s, 2H), 1.90-1.64 (overlapping, 1H), 1.50–1.30 (overlapping, 1H), 1.37 (s, 9H), 1.35 (s, 9H), 0.78–0.66 (br, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.8, 156.7, 81.0, 79.6, 41.4, 38.8, 30.3, 28.2, 27.9, 20.4.

A mixture of the crude amine (0.327 mmol) and *S*,*S*-dimethyl *N*-(4-methylbenzenesulfonyl)carbonimidodithioate (108 mg, 0.392 mmol, 1.2 equiv) in xylene (5 mL) was heated to 130 °C for 20 h under N_2. The reaction mixture was cooled to 25 °C, the solvent was removed, and the product was purified via flash chromatography using Et_2O/*n*-C_6H_{14} (1:1–2:1) as eluent. After evaporation of the solvent, 105 mg of the product was obtained (61% yield from **3**): mp 78–80 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.37–8.21, 7.49–7.28 (br, 1H, 2 rotamers), 7.80 (d, *J* = 7.8 Hz, 2H), 7.21 (d, *J* = 7.8 Hz, 2H), 5.29–5.20, 5.10–4.98 (br, 1H, 2 rotamers), 4.62–4.42 (br, 1H), 3.48-3.27 (br, 1H), 2.40–2.34 (s, 3H, 2 rotamers), 2.36 (s, 3H), 2.07–1.85 (br, 1H), 1.56–1.28 (br, 1H), 1.41 (s, 18H), 0.96–0.65 (br, 1H, 2 rotatmers); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.7, 169.1, 165.2, 157.9, 156.3, 142.6, 142.1, 139.9, 139.6, 129.2, 129.1, 129.0, 126.9, 126.8, 126.5, 126.4, 81.9, 80.9, 80.5, 43.1, 42.3, 39.0, 28.2, 27.9, 26.7, 26.0, 21.5, 20.1, 19.1, 14.3; IR ($CHBr_3$) 1712, 1579, 1494, 1291, 1249 cm^{-1}; $[\alpha]^{25}_D$ + 45.9° (*c* = 1.20, CH_2Cl_2). Anal. Calcd for $C_{23}H_{35}N_3O_6S_2$: C, 53.78; H, 6.87; N, 8.18. Found: C, 54.07; H, 7.21; N, 8.00. In a larger-scale synthesis, a mixture of the crude amine (5.23 g) and the tosyl derivative (4.67 g, 1 equiv) in xylene (150 mL) was refluxed for 5 h. After evaporation of the solvent, the crude product **4** was used for next step without purification.

(1*S*,2*S*)-*tert*-Butyl 1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[[2-(4-methylbenzenesulfonyl guanidino]methyl]cyclopropane-1-carboxylate (5). The methylthio derivative **4** (105 mg, 0.204 mmol) was dissolved in 4 mL of CH_3CN, and the solution was saturated with NH_3 at 0 °C. An CH_3CN (1 mL) solution of $AgNO_3$ (38.2 mg, 0.225 mmol, 1.1 equiv) was added over 30 min at 0 °C, and then the mixture was stirred for 3 h at 25 °C. The resulting yellow solid (AgSMe) was filtered and washed with CH_3CN (10 mL) and MeOH (5 mL). The filtrate was concentrated and the crude product was purified via column chromatography using Et_2O/*n*-C_6H_{14} (30%–70%) to give 96 mg of a white solid (98% yield). In a larger-scale reaction, the crude product **4** and 4.04 g of $AgNO_3$ were used to yield 7.12 g of crude **5** (crude yield; 87% for three steps): mp 175–177 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.2 Hz, 2H), 7.18 (d, *J* = 8.2 Hz, 2H), 6.72–6.20 (br, 3H), 5.97–5.65 (br, 1H), 3.83–3.45 (br, 1H), 2.98–2.72 (br, 1H), 2.34 (s, 3H), 1.91–1.64 (br, 1H), 1.58–1.25 (overlapping, 1H), 1.38 (s, 9H), 1.36 (s, 9H), 0.92–0.68 (br, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.8, 158.2, 156.7, 142.7, 129.8, 126.7, 82.1, 80.9, 40.7, 39.2, 28.5, 28.2, 27.5, 21.6, 20.2; IR ($CHBr_3$) 1706, 1550, 1253, 1181 cm^{-1}; $[\alpha]^{25}_D$ +36.8° (*c* = 0.66, CH_2Cl_2). Anal. Calcd for $C_{22}H_{34}N_4O_6S$: C, 54.75; H, 7.10; N, 11.61. Found: C, 54.37; H, 7.31; N, 11.43.

(1*S*,2*S*)-1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[[2-(4-methylbenzenesulfonyl)guanidino]methyl]cyclopropane-1-carboxylic Acid. The bis(*tert*-butoxycarbonyl)-protected compound **5** (96 mg, 0.199 mmol) was dissolved in CH_2Cl_2 (0.7 mL) and cooled to 0 °C. TFA (0.2 mL) was added and the reaction mixture was stirred for 1.5 h at 25 °C. The solution was concentrated and dried under vacuum, and the crude product was used for the following step after lyophilization. In a larger-scale synthesis, 7.12 g of crude **5** was added to a 50% TFA solution in CH_2Cl_2 at 0 °C, and the mixture was stirred for 3 h at 25 °C. After concentration, H_2O (150 mL) was added and then lyophilized to give 8.85 g of the crude solid: ^{1}H NMR (200 MHz, D_2O–DMSO-d_6 (7:3)) δ 7.70 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 3.54–3.36 (m, 1H), 3.27–3.10 (m, 1H), 2.31 (s, 3H), 2.06-1.76 (m, 1H), 1.72–1.59 (m, 1H), 1.32–1.16 (m, 1H).

The above sample from the small-scale reaction was dissolved in *t*-BuOH (2 mL) and H_2O (0.3 mL); then 2 N NaOH (0.3 mL, 3.0 equiv) was added and the mixture was stirred for 10 min at 25 °C. Di-*tert*-butyl dicarbonate (130.2 mg, 0.597 mmol, 3 equiv) was added, and the reaction mixture was stirred for 26 h at 25 °C. After lyophilization, the crude protected amine was purified via silica gel column chromatography (EtOAc/*n*-C_6H_{14}/AcOH, 95:4.0:1.0–95:0:5.0 eluant) to give 34 mg (40%) of the final product. In a larger-scale synthesis, the crude amino acid salt (8.85 g), *t*-BuOH (65 mL), 2 N NaOH (26.2 mL), and 3.69 g of di-*tert*-butyl dicarbonate were used. The reaction mixture was stirred for 12 h at 25 °C and then concentrated to remove the *t*-BuOH. After addition of water (50 mL), the aquous layer was washed with Et_2O (100 mL × 5), acidified with 1 M citric acid (30 mL), and then extracted with EtOAc (150 mL). The organic layer was

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washed with water and brine and then dried ($MgSO_4$), and the crude product was separated via silica gel column chromatography (CH_2Cl_2/MeOH/AcOH, 92:6.0:2.0 eluant). After concentration, EtOAc (100 mL) was added, and the organic solution was washed with water (100 mL × 6) to remove AcOH, dried ($MgSO_4$), and concentrated to about 10 mL. n-C_6H_{14} (120 mL) was added to precipitate 3.90 g of pure Boc-Z-Cyclo-Arg'(Ts) (62% for two steps): mp 150 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.63 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 6.97–6.70 (br, 2H), 3.28–3.10 (m, 1H), 3.03–2.84 (m, 1H), 2.32 (s, 3H), 1.78–1.59 (m, 1H), 1.43–1.23 (overlapping, 1H), 1.36 (s, 9H), 0.87–0.75 (m, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 173.8, 156.4, 141.1, 129.0, 125.5, 78.2, 38.3, 37.1, 28.0, 26.2, 20.8, 19.8; IR ($CHBr_3$) 1638, 1263 cm^{-1}; $[\alpha]^{25}_D$ +19.1° (c = 1.48, CH_2Cl_2). Anal. Calcd for $C_{18}H_{26}N_4O_6S$: C, 50.69; H, 6.15; N, 13.14. Found: C, 50.53; H, 6.54; N, 12.68.

(1*S*,2*S*)-*tert*-Butyl 1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[[[(methylthio)[(4-methoxy-2,3,5-trimethylbenzenesulfonyl)-imino]methyl]amino]methyl]cyclopropane-1-carboxylate (6). The azidomethyl derivative 3 (200 mg, 0.654 mmol) was dissolved in 5 mL of methanol, and 10% Pd/C (51 mg, 0.048 mmol, 0.073 equiv) was added. The mixture was stirred at 25 °C for 3 h under an atmosphere of H_2. The resulting solution was filtered through Celite and silica gel in a short column and then washed with 10 mL of methanol. After evaporation of the solvent, the crude product was used for the next step without purification. In the larger-scale synthesis, 7.0 g of the crude azide 3 and 700 mg of 10% Pd/C were used to give the crude amine by a procedure that was otherwise the same as that used in the small-scale synthesis: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.48 (br s, 1H), 2.98–2.82 (br m, 1H), 2.55–2.37 (br m, 1H), 1.82 (br s, 2H), 1.90–1.64 (overlapping, 1H), 1.50–1.30 (overlapping, 1H), 1.37 (s, 9H), 1.35 (s, 9H), 0.78–0.66 (br, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.8, 156.7, 81.0, 79.6, 41.4, 38.8, 30.3, 28.2, 27.9, 20.4.

A mixture of the crude amine (0.654 mmol) and *S*,*S*-dimethyl *N*-(4-methoxy-2,3,5-trimethylbenzenesulfonyl)carbonimidodithioate (262 mg, 0.785 mmol, 1.2 equiv) in xylene (5 mL) was heated to 130 °C for 12 h under N_2. The reaction mixture was cooled to 25 °C, the solvent was removed, and the product was purified via flash chromatography (Et_2O/n-C_6H_{14}/CH_2Cl_2, 1.0:1.0:0.1 eluant). After evaporation of the solvent, 204 mg of the product was obtained (54.6% yield from 3). In a larger-scale synthesis, the crude amine and the Mtr derivative were used to yield the crude coupling product 6 by the procedure used previously in the synthesis of 4: mp 75 °C dec; ^{1}H NMR (200 MHz, acetone-d_6) δ 8.32–8.20 (br, 0.43H), 7.32–7.13 (br, 0.57H), 6.92–6.57 (br, 1H), 6.70 (s, 1H), 6.36–6.07 (br, 1H), 3.84 (s, 3H), 3.60–3.32 (br, 1H), 2.68 (s, 3H), 2.60 (s, 3H), 2.56–2.27 (br, 3H), 2.10 (s, 3H), 2.10–1.87 (m, 1H), 1.43 (s, 9H), 1.41 (s, 9H), 1.57–1.33 (overlapping, 1H), 1.08–0.91 (m, 1H); ^{13}C NMR (50 MHz, acetone-d_6) δ 181.4, 171.6, 159.1, 139.1, 137.8, 134.0, 124.7, 112.3, 81.3, 80.0, 55.7, 42.9, 39.3, 32.0, 28.3, 27.9, 24.0, 19.6, 18.5, 14.1, 11.8; IR ($CHBr_3$) 1712, 1579, 1295 cm^{-1}; $[\alpha]^{25}_D$ +28.7° (c = 1.2, CH_2Cl_2). Anal. Calcd for $C_{26}H_{41}N_3O_7S_2$: C, 54.62; H, 7.23; N, 7.35. Found: C, 54.35; H, 7.55; N, 7.14.

(1*S*,2*S*)-*tert*-Butyl 1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[[2-(4-methoxy-2,3,5-trimethylbenzenesulfonyl)guanidino]-methyl]cyclopropane-1-carboxylate (7). The methylthio derivative 6 (145 mg, 0.254 mmol) was dissolved in 5 mL of CH_3CN, and the solution was saturated with NH_3 at 0 °C. An CH_3CN (1 mL) solution of $AgNO_3$ (47.5 mg, 0.279 mmol, 1.1 equiv) was added over 20 min at 0 °C, and the mixture was stirred for 3.5 h at 25 °C. The resulting yellow solid (AgSMe) was filtered and washed with CH_3CN (10 mL) and then MeOH (5 mL). The filtrate was concentrated under vacuum to give 182 mg of a white solid. This solid was resolidified from Et_2O/n-C_6H_{14} solution. In a larger-scale synthesis, crude 6 and 4.85 g of silver nitrate were used to give 10.34 g of crude product by a procedure that was analogous to that used in the synthesis of 5 (crude yield, 85% for three steps): mp 148 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 6.62 (s, 1H), 3.75 (s, 3H), 3.24–2.85 (m, 2H), 2.56 (s, 3H), 2.48 (s, 3H), 2.01 (s, 3H), 1.72–1.52 (m, 1H), 1.35 (s, 9H), 1.32 (s, 9H), 1.22 (dd, J = 9.4, 4.3 Hz, 1H), 1.80–1.68 (m, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 191.5, 171.2, 157.4, 156.2, 156.0, 137.5, 123.5, 111.6, 79.5, 78.2, 55.3, 40.1, 38.0, 28.0, 27.5, 25.8, 23.6, 18.0, 11.6; IR ($CHBr_3$) 3444, 3313, 2983, 1695, 1546, 1384, 1264, 1182, 1123 cm^{-1}; $[\alpha]^{25}_D$ +9.22° (c = 1.48, CH_2Cl_2). Anal. Calcd for $C_{25}H_{40}N_4O_7S$: C, 55.53; H, 7.46; N, 10.36. Found: C, 55.26; H, 7.55; N, 10.26.

(1*S*,2*S*)-1-[*N*-[(9-Fluorenylmethoxy)carbonyl]amino]-2-[[2-(4-methoxy-2,3,5-trimethylbenzenesulfonyl]guanidino]-methyl]cyclopropane-1-carboxylic Acid. The BOC-protected amine 7 (41 mg, 0.076 mmol) was dissolved in $CDCl_3$ (0.6 mL) in an NMR tube. TFA (0.3 mL) was added and the reaction was allowed to stand at 25 °C until the *t*-BOC peak had disappeared (*ca.* 16 h). The solution was evaporated, and the crude product was purified on Dowex 50 × 8–100, H^+ form. After lyophilization, 17 mg of the Z-cyclo-Arg'(Mtr) was obtained (58%). In a large-scale synthesis, crude 7 (5.17 g) was dissolved in a 50% TFA solution in CH_2Cl_2 (100 mL) at 0 °C and the solution was stirred for 3 h at 25 °C. After concentration of the reaction, H_2O (400 mL) was added and the solution was lyophilized. The crude product was separated on an ion exchange column and lyophilized to yield 2.06 g of the deprotected amino acid: ^{1}H NMR (200 MHz, D_2O) δ 6.69 (s, 1H), 3.72 (s, 3H), 3.51–3.33 (m, 1H), 3.21–2.99 (m, 1H), 2.51 (s, 3H), 2.43 (s, 3H), 1.99 (s, 3H), 1.75–1.56 (m, 1H), 1.38–1.22 (m, 1H), 0.90–0.65 (br, 1H).

The above compound from the small-scale reaction (0.045 mmol) and diisopropylethylamine (5.77 mg, 0.045 mmol, 1 equiv) were mixed in 1 mL of $CDCl_3$ and stirred for 10 min at 25 °C. FMOC-OSu (15.05 mg, 0.045 mmol, 1 equiv) was added under N_2, and the solution was stirred for 12 h. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/n-C_6H_{14}; 90:10:2.0) and then recrystallized from CH_2Cl_2/Et_2O/n-C_6H_{14} to give 24 mg (89%) of FMOC-Z-cyclo-Arg'(Mtr): mp 141–142 °C; ^{1}H NMR (200 MHz, acetone-d_6) δ 7.84 (d, J = 7.5 Hz, 2H), 7.75–7.64 (m, 2H), 7.45–7.22 (m, 4H), 6.65 (s, 1H), 6.40–6.21 (br, 1H), 4.43–4.17 (m, 3H), 3.80 (s, 3H), 3.77–3.61 (br, 1H), 2.98–2.81 (br, 1H), 2.68 (s, 3H), 2.64 (s, 3H), 2.07 (s, 3H), 2.02–1.86 (br, 1H), 1.50 (dd, J = 9.7, 5.0 Hz, 1H), 1.06–0.94 (m, 1H); ^{13}C NMR (50 MHz, acetone-d_6) δ 188.6, 173.6, 158.6, 141.8, 138.9, 136.8, 135.5, 128.3, 127.7, 125.9, 124.6, 120.5, 112.2, 67.0, 55.6, 47.7, 40.1, 30.3, 28.1, 24.0, 20.3, 18.4, 11.8; IR ($CHBr_3$) 1725, 1657, 1640, 1535, 1306 cm^{-1}; $[\alpha]^{25}_D$ 5.8° (c = 0.95, CH_3OH). Anal. Calcd for $C_{31}H_{34}N_4O_7S{\cdot}H_2O$: C, 59.60; H, 5.81; N, 8.97. Found: C, 60.21; H, 5.89; N, 8.99. In a large-scale synthesis, 2.0 g of the deprotected amino acid, diisopropylethylamine (0.917 mL), FMOC-OSu (1.771 g), and CH_2Cl_2 (50 mL) were used to yield 1.22 g of the final product via a similar procedure (21% for two steps).

(*R*)-(−)-Diethyl 2-(Benzyloxymethyl)cyclopropane-1,1-dicarboxylate (10). Diethyl malonate (11.23 g, 70.11 mmol) was added to a well-stirred solution of sodium hydride (3.39 g, 147.23 mmol, 2.1 equiv) in dimethoxyethane (250 mL) at 25 °C under N_2. After 20 min of stirring at 25 °C, (*S*)-4-(benzyloxymethyl)-2,2-dioxo-1,3,2-dioxathiolane[30] (17.12 g, 70.11 mmol, 1.0 equiv) was added slowly over 50 min, and the solution was heated to reflux for 20 h. The reaction mixture was cooled to 25 °C, and the solvent evaporated under reduced pressure. The residue was extracted with EtOAc (200 mL × 2), and the extract was washed with saturated $NaHCO_3$ solution, H_2O, and brine and dried over Na_2SO_4. After evaporation of the solvent, 19.05 g of the crude product was obtained (yield, 89%). In a larger-scale synthesis, 58.45 g of cyclic sulfate and 38.34 g of diethyl malonate were used to yield 69.28 g of crude product by an analogous procedure (crude yield, 98%). The crude product was used for next step without purification. An analytically pure sample was obtained via flash chromatography (10–20% Et_2O/n-C_6H_{14}): ^{1}H NMR (200 MHz, acetone-d_6) δ 7.31 (br s, 5H), 4.45 (br s, 2H), 4.25–3.97 (m, 4H), 3.60 (dd, J = 10.5, 5.8 Hz, 1H), 3.42 (dd, J = 10.5, 7.5 Hz, 1H), 2.27–2.00 (br m, 1H), 1.45 (dd, J = 7.4, 4.4 Hz, 1H), 1.32 (dd, J = 9.4, 4.4 Hz, 1H), 1.27–1.11 (two overlapping triplets, J = 7.1, 7.1 Hz, 6H); ^{13}C NMR (50 MHz, acetone-d_6) δ 170.0, 167.8, 139.2, 128.8, 128.0, 127.9, 72.9, 68.4, 61.7, 61.5, 33.6, 26.9, 18.3, 14.1; IR (neat) 2983, 1724, 1712, 1370, 1318, 1285, 1206, 1129, 1113 cm^{-1}; $[\alpha]^{25}_D$ −35.5° (c = 1.18, CH_2Cl_2). Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.62; H, 7.36.

(1*R*,2*R*)-Ethyl 2-(Benzyloxymethyl)-1-[*N*-(*tert*-butoxycarbonyl)amino]cyclopropane-1-carboxylate (11). The crude

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diethyl dicarboxylate derivative **10** *6.0 g, 19.6 mmol) and Na_2CO_3 (4.56 g, 43.1 mmol, 2.2 equiv) were mixed in EtOH–H_2O (20 mL/50 mL) and maintained at 60 °C for 48 h. The reaction mixture was concentrated and extracted with ether (50 mL). The aqueous layer was acidified with concd. HCl, extracted with EtOAc (80 mL X 2), and then dried over Na_2SO_4. After evaporation of the solvent, 5.2 g of the crude product was obtained (98%). In a larger-scale synthesis, 69.28 g of **10** was used to give 43.31 g of the product by an analogous procedure (crude yield, 69%; 9% of starting material was recovered by extraction). This product was used for the next step without purification. A small amount of the crude **(1*S*,2*R*)-(+)-2-(benzyloxymethyl)-1-(ethoxycarbonyl)cyclopropane-1-carboxylic acid** was purified by flash chromatography (Et_2O/*n*-C_6H_{14}/AcOH; 50:50:1.0) and characterized spectroscopically: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40–7.20 (m, 5H), 4.50 (d, *J* = 12.2 Hz, 1H), 4.41 (d, *J* = 12.0 Hz, 1H), 4.21–4.05 (two q, *J* = 7.2 Hz, 2H), 3.78 (dd, *J* = 10.7, 5.6 Hz, 1H), 3.47 (dd, *J* = 10.7, 8.9 Hz, 1H), 2.51–2.33 (m, 1H), 1.89 (dd, *J* = 9.3, 4.4 Hz, 1H), 1.76 (dd, *J* = 8.1, 4.4 Hz, 1H), 1.18 (t, *J* = 7.2, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.6, 172.0, 137.6, 128.4, 127.9, 127.7, 72.8, 67.6, 62.7, 34.1, 30.3, 22.2, 13.9; IR ($CHBr_3$) 3097, 2983, 1706, 1624, 1553, 1265, 1166 cm^{-1}; $[\alpha]^{25}_D$ +11.0° (*c* = 1.35, CH_2Cl_2).

The crude carboxylic acid derivative prepared above (6.08 g, 21.87 mmol) and NEt_3 (3.66 mL, 26.24 mmol, 1.2 equiv) were mixed with dry *t*-BuOH (60 mL) at 25 °C under N_2. Diphenyl phosphorazidate (6.62 g, 24.06 mmol, 1.1 equiv) was added and the reaction mixture was stirred under reflux for 12 h. The solution was concentrated and the crude product was extracted with Et_2O (100 mL × 2), washed with H_2O and brine, and dried over Na_2SO_4. After evaporation of the solvent, the residue was separated via flash chromatography (20–50% Et_2O/*n*-C_6H_{14}) to give 4.134 g of product as a colorless oil (yield, 54%). In a large-scale synthesis, 44.61 g of starting material was used to yield 48.21 g of the crude product which was used for next step without purification (yield, 86%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.27 (br s, 5H), 5.55–5.30 (br, 1H), 4.42 (s, 1H), 4.21–4.00 (m, 2H), 3.82–3.67 (br m, 1H), 3.56–3.40 (br m, 1H), 1.86–1.67 (br m, 1H), 1.67–1.54 (br, 1H), 1.41 (s, 9H), 1.40–1.20 (br, 1H), 1.20 (t, *J* = 11.9 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.2, 155.9, 138.3, 128.3, 127.8, 127.6, 79.8, 72.9, 67.7, 61.3, 38.2, 29.9, 28.3, 21.4, 14.2, 14.2; IR ($CHBr_3$) 3037, 2869, 1717, 1490, 1368, 1324, 1250, 1179, 1098 cm^{-1}; $[\alpha]^{25}_D$ +6.2° (*c* = 1.31, CH_2Cl_2). Anal. Calcd for $C_{19}H_{27}NO_5$: C, 645.29; H, 7.79; N, 4.01. Found: C, 65.22; H, 7.82; N, 4.36.

(1*R*,2*S*)-2-(Azidomethyl)-1-[*N*-(*tert*-butoxycarbonyl)amino]cyclopropane-1-carboxylic Acid (12). A mixture of the BOC-protected amide **11** (1.914 g, 5.48 mmol) and 10% Pd/C (70 mg) in EtOAc (40 mL) was stirred for 48 h at 25 °C under a hydrogen balloon. The solution was concentrated under vacuum, and the residue was filtered through a pad of silica gel and Celite and washed with EtOAc (20 mL). The filtrate was evaporated to give 1.48 g of crude **(1*R*,2*R*)-ethyl 1-[*N*-(*tert*-butoxycarbonyl)amino]-2-(hydroxymethyl)cyclopropane-1-carboxylate** (100%) which was used for next step without purification. In a larger-scale synthesis, 25.0 g of starting material and 2.5 g of 10% Pd/C were used to yield 20 g of crude product by an analogous method: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.41 (br s, 1H), 4.14 (q, *J* = 7.1 Hz, 2 H), 3.94 (dd, *J* = 11.6, 4.9 Hz, 1H), 3.66 (dd, *J* = 11.6, 9.1 Hz, 1H), 2.90 (br s, 1H), 1.97–1.73 (br m, 1H), 1.58 (dd, *J* = 8.3, 5.1 Hz, 1H), 1.40 (s, 9H), 1.31 (dd, *J* = 9.6, 5.1 Hz, 1H), 1.22 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.8, 156.4, 80.1, 61.6, 59.5, 37.8, 33.8, 28.2, 21.3, 14.1; IR ($CHBr_3$) 3029, 2983, 1722, 1489, 1370, 1323, 1265, 1185, 1024 cm^{-1}; $[\alpha]^{25}_D$ +9.6° (*c* = 1.60, CH_2Cl_2).

To a solution of the hydroxymethyl derivative (1.48 g, 5.48 mmol) in CH_2Cl_2 (20 mL) was added NEt_3 (0.666 g, 6.58 mmol, 1.2 equiv), followed by methanesulfonyl chloride (0.753 g, 6.58 mmol, 1.2 equiv). After 10 h of stirring at 25 °C under N_2, the reaction mixture was concentrated under vacuum and the residue was filtered through a pad of silica gel eluting with Et_2O (50 mL). The filtrate was evaporated to give the crude product (1.778 g, 96%) which was used for the next step without purification. To a solution of the mesylate (1.40 g, 4.154 mmol) in DMF (5 mL) was added NaN_3 (449 mg, 6.907 mmol, 1.66 equiv), and the reaction mixture was stirred for 24 h at 25 °C under N_2. The solvent was evaporated under vacuum, and the residue was filtered through a pad of silica gel with the elution of MeOH/Et_2O (20 mL). The filtrate was concentrated and then purified by flash chromatography (Et_2O/*n*-C_6H_{14}, 1:1). Removal of the solvent gave 1.05 g (89%) of the product as a colorless oil. In a larger synthesis, 20 g of starting material was used to give 25 g of the crude mesylate, which was mixed with NaN_3 (5.59 g) in DMF (70 mL) and then stirred for 18 h at 40 °C. The reaction solution was concentrated and extracted with Et_2O (300 mL × 2). The organic layer was washed with water and brine and then dried ($MgSO_4$), and the organic solution was concentrated to give 19.94 g of the crude azide **(1*R*,2*S*)-ethyl 2-(azidomethyl)-1-[*N*-(*tert*-butoxycarbonyl)amino]cyclopropane-1-carboxylate** (98% yield from **11**): ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.26 (br s, 1H), 4.27–4.06 (m, 2H), 3.64–3.38 (m, 2H), 1.85–1.68 (m, 1H), 1.60 (dd, *J* = 7.6, 5.0 Hz, 1H), 1.42 (s, 9H), 1.33–1.17 (overlapping m, 1H), 1.25 (t, *J* = 7.2 Hz, 3H): ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.2, 155.8, 80.3, 61.8, 49.0, 38.0, 29.6, 28.2, 22.2, 14.1; IR ($CHBr_3$) 2981, 2095, 1719, 1499, 1392, 1369, 1320, 1268 cm^{-1}; $[\alpha]^{25}_D$ +11.7° (*c* = 1.20, CH_2Cl_2). Anal. Calcd for $C_{12}H_{20}N_4O_4$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.77; H, 7.24; N, 19.51.

To a solution of the azide ester as prepared above (600 mg, 2.11 mmol) in EtOH (12 mL) was added a 1 N NaOH solution (2.54 mL, 2.54 mmol, 1.2 equiv), and the reaction mixture was stirred for 48 h at 25 °C. The solution was concentrated, and the residue was purified by column chromatography (EtOAc/*n*-C_6H_{14}/AcOH, 25:50:1, then 50:50:1). Removal of the solvent gave 495 mg (92%) of the product **12** as a colorless solid. In a larger synthesis, 19.94 g of the crude ester was mixed with EtOH (84 mL) and 1 M NaOH (91.25 mL). After 30 h of stirring at 25 °C, the reaction solution was washed with 33% Et_2O/*n*-C_6H_{14} (100 mL × 3), saturated (NaCl), acidified (concd. HCl), and extracted with EtOAc (300 mL × 2). The organic layer was washed with water and brine and then dried ($MgSO_4$), and the organic solution was concentrated to give the crude acid **12**. This was recrystallized (50% Et_2O in *n*-C_6H_{14}) to give 14.89 g (83%) of pure **12**: mp 135–136 °C dec; ^{1}H NMR (200 MHz, acetone-d_6) δ 6.78 (br s, 1H), 3.63 (dd, *J* = 13.1, 6.5 Hz, 1H), 3.45 (dd, *J* = 13.1, 8.5 Hz, 1 H), 1.96–1.77 (m, 1H0, 1.50 (dd, *J* = 7.8, 5.1 Hz, 1H), 1.40 (s, 9H), 1.34 (dd, *J* = 9.5, 5.1 Hz, 1H); ^{13}C NMR (50 MHz, acetone-d_6) δ 173.2, 156.2, 79.0, 49.7, 38.0, 29.7, 28.3, 22.2; IR ($CHBr_3$), 2087, 1697, 1645, 1296, 1255, 1207, 1161 cm^{-1}. Anal. Calcd for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29. Found: C, 46.43; H, 6.14. *Caution is recommended when manipulating this azide since it could conceivably decompose violently when activated by heat, shock, or friction.* No such problems were encountered in this work, however.

(1*R*,2*S*)-1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[[[(methylthio)(toluenesulfonylimino)methyl]amino]cyclopropane-1-carboxylic Acid (13). To a solution of **12** (310 mg, 1.21 mmol) in MeOH/H_2O (10 mL:0.1 mL) was added 10% Pd/C (30 mg), and the reaction mixture was stirred for 4 h under an H_2 atmosphere at 25 °C. The reaction mixture was filtered through a pad of Celite and washed with 90% MeOH/H_2O (20 mL). After concentration of the filtrate, H_2O (10 mL) was added and the solution was lyophilized to give 290 mg of the product (crude yield, 100%). In a larger synthesis, 5 g of **12** and 10% Pd/C (1.0 g) were used to give 4.26 g of the crude amine by a similar procedure (yield, 95%): mp 138–140 °C; ^{1}H NMR (200 MHz, D_2O) δ 3.30–3.05 (br, 2 H), 1.63–1.41 (m, 1H), 1.49–1.27 (overlapping m, 1H), 1.29 (s, 9H), 1.17 (dd, *J* = 9.7, 5.3 Hz, 1H); ^{13}C NMR (50 MHz, D_2O) δ 176.9, 158.1, 81.2, 39.3, 38.2, 27.7, 25.2, 20.0; $[\alpha]^{25}_D$ −11.4° (*c* = 1.26, CH_3OH).

To a solution of the compound from the small-scale synthesis (55.7 mg, 0.242 mmol) and *S*,*S*-dimethyl *N*-(toluenesulfonyl)-carbonimidothioate (106.3 mg, 0.386 mmol, 1.59 equiv) in EtOH (3 mL) was added a 2 N NaOH solution (0.133 mL, 0.266 mL, 1.1 equiv), and the reaction mixture was heated to reflux for 13 h. After concentration, the residue was purified by column chromatography (EtOAc/*n*-C_6H_{14}/AcOH, 30:70:1, then 50:50:1). Evaporation of the solvent gas 83 mg of the product (75%). In a larger synthesis, a mixture of the crude amine (4.26 g), the tosyl derivative (5.61 g), and 2 N NaOh (10.18 mL) in EtOH (100 mL) was stirred for 12 h at 60 °C. After concentration, H_2O (50 mL), was added and the aqueous solution was washed with Et_2O (40 mL × 5), acidified with 1 N HCl (22 mL), and extracted with

50% EtOAc in Et_2O (200 mL × 2). The organic layer was washed with water and brine and then dried ($MgSO_2$), and the organic solution was concentrated to give crude **13**. The crude product was recrystallized in pure EtOH to give 4.84 g of pure **13** (57%): mp 156–158 °C; ^1H NMR (100 MHz, acetone-d_6) δ 7.75 (d, J = 7.9 Hz, 2H), 7.30 (d, J = 7.9 Hz, 2H), 3.92–3.66 (br m, 1H), 3.51–3.32 (br m, 1H), 2.38 (s, 3H), 2.41 (s, 1.5H), 2.32 (s, 1.5H), 2.00–1.80 (br, 1H), 1.64–1.54 (m, 1H), 1.54–1.40 (overlapping, 1H), 1.42 (s, 9H); ^{13}C NMR (50 MHz, acetone-d_6) δ 172.7, 158.3, 142.4, 129.5, 127.3, 80.1, 43.7, 37.3, 28.3, 22.0, 21.2, 14.2, IR ($CDBr_3$) 1691, 1538, 1496, 1451, 1278 cm^{-1} $[\alpha]^{25}_D$ +6.84° (c = 0.92, CH_2Cl_2). Anal. Calcd for $C_{19}H_{27}N_3O_6S_2$: C, 49.87; H, 5.95; N, 9.18. Found: C, 49.80; H, 6.00; N, 9.16.

(1*R*,2*S*)-1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[(2-toluenesulfonyl)guanidino)methyl]cyclopropane-1-carboxylic Acid. The methylthio derivative **13** (52 mg, 0.114 mmol) was dissolved in 3 mL of CH_3CN, and the solution was saturated with NH_3 at 0 °C. A CH_3CN (1 mL) solution of $AgNO_3$ (21.23 mg, 0.125 mmol, 1.1 equiv) was added over 20 min at 0 °C, and the mixture was stirred for 3.5 h at 25 °C. The resulting yellow solid (AgSMe) was filtered and washed with CH_3CN (10 mL) and MeOH (5 mL). The filtrate was concentrated under vacuum to give 30 mg of **BOC-E-cyclo-rg′(Ts)** as a colorless solid (62%). This solid was purified by precipitation from Et_2O/n-C_6H_{14}. In a larger synthesis, 4.51 g of the starting material **13** was used in a similar way. After concentration of the filtrate, the crude product was partitioned between ETOAc (250 mL) and 0.5 M citric acid (60 mL). The organic layer was washed with water and brine and then dried ($MgSO_4$), and the organic solution was concentrated to give crude material, which was recrystallized from H_2O to give 3.83 g (91%) of pure **BOC-E-cyclo-Arg′(Ts)**: mp 145–148 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.49 (d, J = 7.8 Hz, 2H), 6.97 (d, J = 7.8 Hz, 2 H), 6.45–6.18 (br, 2H), 6.00–5.76 (br, 1H), 3.48–3.20 (br, 1H), 3.15–2.94 (br, 1H), 2.13 (s, 3H), 1.55–1.34 (br, 1H), 1.33–0.94 (br, 2 H), 1.17 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.5, 156.1, 141.3, 128.8, 125.7, 125.6, 79.1, 31.2, 28.1, 21.8, 21.4, 21.1; IR ($CHBr_3$) 1739, 1635, 1542, 1280 cm^{-1}; $[\alpha]^{25}_D$ +56.3° (c = 0.75, CH_2Cl_2). Anal. Calcd for $C_{18}H_{26}N_4O_6S(H_2O)$: C, 48.63; H, 6.35; N, 12.61. Found: C, 48.52; H, 6.14; N, 12.29.

(1*R*,2*S*)-1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[[[(methylthio)[(4-methoxy-2,3,5-trimethylbenzenesulfonyl)imino]methyl]amino]methyl]cyclopropane-1-carboxylic Acid (14). To a solution of **12** (6.0 g, 23.42 mmol) in 140 mL of MeOH/H_2O (10:1) was added 10% Pd/C (620 mg) under an N_2 atmosphere, and the reaction mixture was stirred for 12 h under an H_2 atmosphere at 25 °C. The reaction mixture was filtered through a pad of Celite and washed with 90% MeOH/H_2O (100 mL). The filtrate was concentrated under vacuum to give 4.61 g of the crude amine: mp 138–140 °C; ^1H NMR (200 MHz, D_2O) δ 3.30–3.05 (br, 2H), 1.63–1.41 (m, 1H), 1.49–1.27 (overlapping m, 1H), 1.29 (s, 9H), 1.17 (dd, J = 9.7, 5.3 Hz, 1H); ^{13}C NMR (50 MHz, D_2O) δ 176.9, 158.1, 81.2, 39.3, 38.2, 27.7, 25.2, 20.0; $[\alpha]^{25}_D$ −11.4 °C (c = 1.26, CH_3OH).

To a solution of the crude amine (4.61 g, 20.04 mmol) and *S*,*S*-dimethyl *N*-)4-methoxy-2,3,5-trimethylbenzenesulfonyl)carbonimidodithioate (6.01 g, 18.04 mmol, 0.9 equiv) in EtOH (100 mL) was added a 2 N NaOH solution (11.02 mL, 22.04 mL, 1.1 equiv), and the reaction mixture was heated to reflux for 4.5 h. After concentration of the reaction solution, the residue was dissolved in 100 mL of H_2O. The aquous solution was washed with Et_2O (100 mL × 5), acidified with solid citric acid (10 g), and extracted with EtOAc (150 mL). The organic layer was washed with water and brine and then dried ($MgSO_4$), and the organic solution was concentrated to give 9.31 g of the crude acid **14** (77%): mp 107–109 °C dec; ^1H NMR (200 MHz, $CDCl_3$) δ 8.25–8.12, 7.39–7.29 (br, 1H, 2 rotamers), 7.78 (s, 2H), 6.51 (s, 1H), 5.58 (s, 1H), 3.80 (s, 3H), 3.98–3.37 (br, 2H), 2.68, 2.67 (s, 3H, 2 rotamers), 2.57 (s, 3H), 2.41, 2.29 (s 3H, 2 rotamers), 2.09 (s, 3H), 1.97–1.76 (br, 1H), 1.73–1.57 (br, 1H), 1.53–1.33 (overlapping, 1H), 1.39 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.5, 168.0, 164.2, 158.8, 158.6, 157.3, 138.8, 137.6, 132.4, 124.7, 111.7, 80.8, 55.4, 42.8, 37.0, 30.5, 28.2, 24.0, 22.6, 18.5, 14.1, 11.9; $[\alpha]^{25}_D$ + 11.4° (c = 1.24, CH_2Cl_2). Anal. Calcd for $C_{22}H_{33}N_3O_7S_2$: C, 51.24; H, 6.45; N, 8.15. Found: C, 51.46; H, 6.87; N, 7.71.

(1*R*,2*S*)-1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[[2-(4-methoxy-2,3-,5-trimethylbenzenesulfonyl)guanidino]methyl]cyclopropane-1-carboxylic Acid (15). The methylthio derivative **14** (9.01 g, 17.48 mmol) was dissolved in 30 mL of CH_3CN, and the solution was saturated with NH_3 gas at −20 °C. A solution of $AgNO_3$ (4.09 g, 24.07 mmol, 1.38 equiv) in CH_3CN (100 mL) was added over 2.5 h at about −10 °C, and the mixture was stirred for 12 h at 25 °C. The resulting yellow solid (AgSMe) was filtered and washed with CH_3CN (100 mL) and MeOH (50 mL). The filtrate was concentrated under vacuum, and the residue was dissolved in 10% K_2CO_3 solution (100 mL). The aqueous solution was washed with Et_2O (120 mL × 3) and EtOAc (100 mL × 2), acidified with 2 N citric acid (30 mL), and extracted with EtOAc (100 mL). The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated under vacuum to give 8.35 g of crude **15** (96%). A small amount of this material was purified by silica gel column chromatography (CH_2Cl_2/MeOH/AcOH, 93:5.0:2.0) and characterized spectroscopically: mp 170–173 °C dec; ^1H NMR (200 MHz, $CDCl_3$) δ 7.03–6.67 (br, 2H), 6.51 (s, 1H), 6.55–6.25 (br, 2H), 6.05–5.93 (br, 1H), 3.80 (s, 3H), 3.75–3.53 (br, 1H), 3.36–3.12 (br, 1H), 2.65 (s, 3H), 2.58 (s, 3H), 2.10 (s, 3H), 1.90–1.69 (br, 1H), 1.67–1.53 (m, 1H), 1.39 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.2, 158.5, 156.9, 156.2, 156.1, 138.6, 136.7, 124.8, 111.7, 80.5, 55.4, 39.6, 37.4, 30.6, 28.3, 24.1, 22.6, 18.3, 14.1, 11.9; IR ($CHBr_3$) 1631, 1553, 1398, 1307, 1127, 1169, 1122 cm^{-1}; $[\alpha]^{25}_D$ +4.0.4° (c = 1.55, CH_2Cl_2). Anal. Calcd for $C_{21}H_{32}N_4O_7S$: C, 52.05; H, 6.66; N, 11.56. Found: C, 51.66, H, 7.05; N, 11.09.

(1*R*,2*S*)-1-[*N*-[(9-Fluorenylmethoxy)carbonyl]amino]2-[[2-(4-methoxy†2,3,5-trimethylbenzenesulfonyl)guanidino]methyl]cyclopropane-1-carboxylic Acid. Crude compound **15** (3.95 g, 8.16 mmol) was dissolved in CCl_4 (42 mL) at 0 °C, TFA (18 mL) was added dropwise, and the reaction was stirred for 20 min at 25 °C. The solution was concentrated, neutralized by adding 2 N NaOH solution, and lyophilized, and the residue was used for next step. Thus, this amine was dissolved in 1 M Na_2CO_3 (14.68 mL), DMF (20 mL) and H_2O (5 mL) were added, and the solution was cooled to 0 °C; FMOC-OSu (2.47 g, 7.32 mmol, 1 equiv) was added in one portion, and the mixture was stirred for 8 h at 25 °C. Water (250 mL) was added and the solution was washed with Et_2O (100 mL × 5), acidified with citric acid (18 g), and extracted with EtOAc (500 mL). The organic layer was washed with H_2O and brine, and dried ($MgSO_4$), and the product was purified on a silica gel column (CH_2Cl_2/MeOH/AcOH, 92:7.0:1.0). AFter concentration of the product batches, Et_2O (50 mL) was added, followed by n-C_6H_{14} (300 mL) to precipitate the product. The white solid was filtered and dried to give 3.37 g of pure **FMOC-E-Cyclo-Arg′(Mtr)** (68%): mp 141–143 °C dec; ^1H NMR (200 MHz, $CDCl_3$) δ 7.69 (d, J = 7.4 Hz, 2H), 7.53 (d, J = 7.4 Hz, 2H), 7.40–7.14 (m, 4H), 6.63–6.51 (br, 1H), 6.46 (s, 1H), 6.42 (br, 1H), 5.82–5.35 (br, 2H0, 4.47–4.19 (br, 2H), 4.18–4.05 (m, 1H), 3.75 (s, 3H), 3.71–3.45 (br, 1H), 3.40–3.13 (br, 1H), 2.63 (s, 3H), 2.56 (s, 3H), 2.06 (s, 3H), 1.92–1.72 (br, 1 H), 1.70–1.56 (br, 1H), 1.50–1.33 (br, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.1, 158.6, 157.5, 156.1, 143.7, 141.2, 138.6, 136.7, 127.7, 127.1, 125.2, 124.9, 119.9, 111.7, 67.2, 55.4, 47.0, 37.6, 24.1, 22.8, 18.3, 11.9; IR ($CHBr_3$) 2253, 1712, 1624, 1552, 1465, 1308, 1253, 1121 cm^{-1}; $[\alpha]^{25}_D$ +13.9° (c = 1.0, CH_2Cl_2). Anal. Calcd for $C_{11}H_{34}N_4O_7S{\cdot}H_2O$: C, 59.60; H, 5.81; N, 8.97. Found: C, 59.56; H, 5.91; N, 8.87.

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Supplementary Material Available: Copies of ^1H and ^{13}C NMR spectra of **7**, **14**, **15**, **FMOC-Z-cyclo-Arg′(Mtr)**, and **FMOC-E-cyclo-Arg′(Mtr)** (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.