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Efficient Large Scale Preparation of Neutral Endopeptidase/ Angiotensin-Converting Enzyme Dual Inhibitor CGS30440

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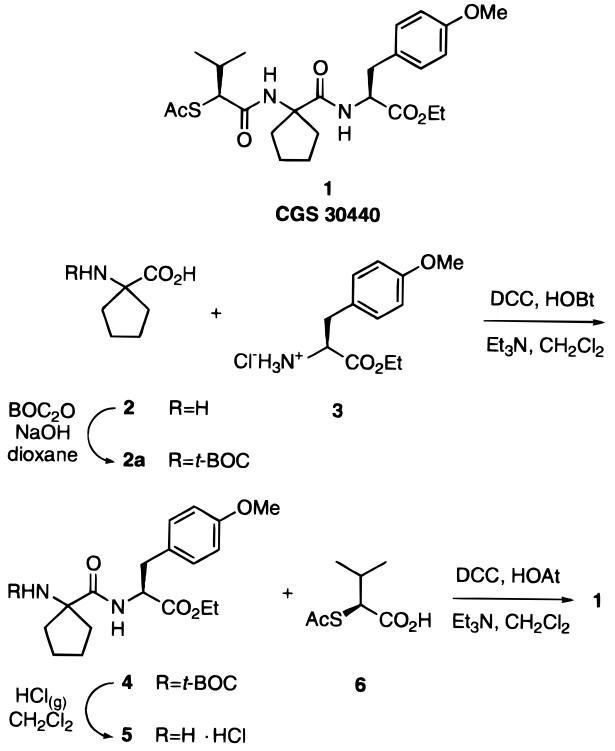
Abstract:

The development and piloting of a potential manufacturing process for ACE/NEP dual inhibitor CGS30440 is described. The synthesis proceeds sequentially from 1-aminocyclopentanecarboxylic acid via N-protection, peptide coupling with L-tyrosine ethyl ester, O-methylation of N-protected [(1-amino-1-cyclopentyl)carbonyl]-L-tyrosine ethyl ester, N-deprotection, peptide coupling of [(1-amino-1-cyclopentyl)carbonyl]-*O*-methyl-L-tyrosine ethyl ester with D-2-bromo-3-methylbutyric acid, and final displacement of bromide with thioacetate. This approach is superior to shorter Discovery routes based upon final peptide coupling of L-2-(acetylthio)-3-methylbutanoic acid to [(1-amino-1-cyclopentyl)carbonyl]-*O*-methyl-L-tyrosine ethyl ester.

Introduction

Intense interest in dual inhibitors of angiotensin-converting enzyme (EC 2.4.15.1, ACE) and neutral endopeptidase (EC 3.4.24.11, NEP) has arisen in the last five years.[1] Such therapeutic agents combine the proven effects of ACE and NEP inhibition (reduction of hypertension, mitigation of congestive heart failure[2] and diuresis, natriuresis, and vasodilation,[3] respectively). Furthermore, animal models indicate that dual inhibitors should show enhanced activity due to synergism[4] and complementarity.[1] Workers at Ciba (Novartis) recently prepared potent ACE/NEP inhibitor CGS30440 (**1**), showing exciting potential for clinical investigation.[1] Thus, the timelines for synthesizing the first 45 kg of **1** needed to be as short as possible. Additionally, we needed a head start toward fixing the final manufacturing process. Finally, the large number of alternative cardiovascular therapies available required that new treatments compete partly on cost; the preparation of **1** therefore needed to be short and use the most inexpensive raw materials possible.

Scheme 1



Discovery Synthesis

The synthesis used to prepare **1** (Scheme 1)[1] was deemed suboptimal, despite being quite short. Several deficiencies for scale-up were apparent. These included the use of DCC for both peptide couplings, mediocre yields for preparing **2a**, instability of **6**, cost of **3**, and especially at that time, no bulk availability of either **6** or *N*-hydroxy-7-azabenzotriazole (HOAt) coupling adjunct. Also, drug substance **1** was isolated by Discovery via column chromatography, so that little was known about either the extent of racemization during the final coupling, or purification through recrystallization.

The above-mentioned problems were addressed as follows. We wanted to exploit the advantages of using **6**;[5] however, we felt compelled to simultaneously explore its replacement with **6b** as a backup (to be followed by thioacetate displacement, Scheme 2). Substitution of **3** with **3a** (followed by methylation, Scheme 2) was clearly more cost-effective. Rework of both peptide couplings was also

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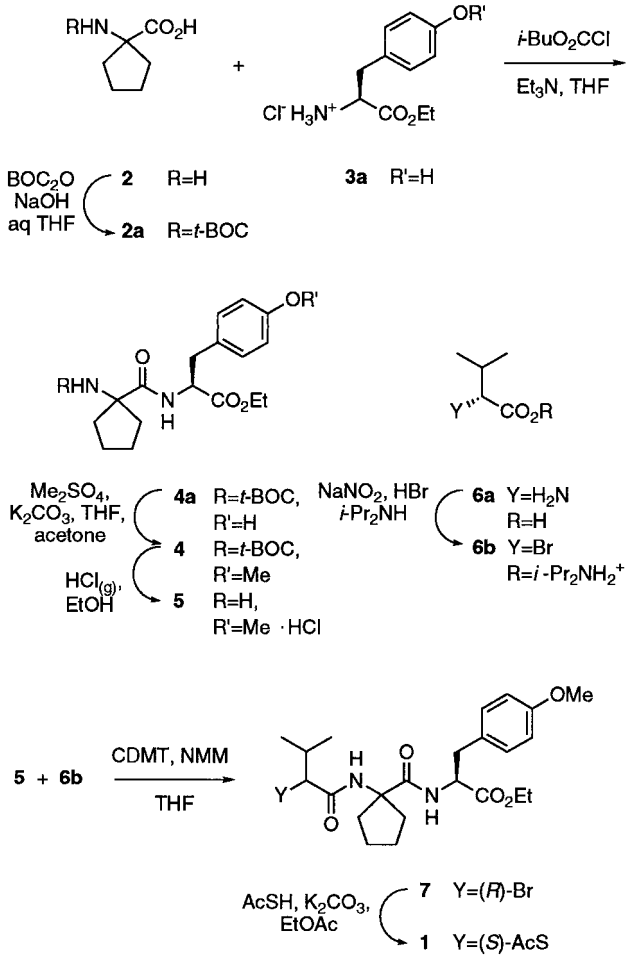
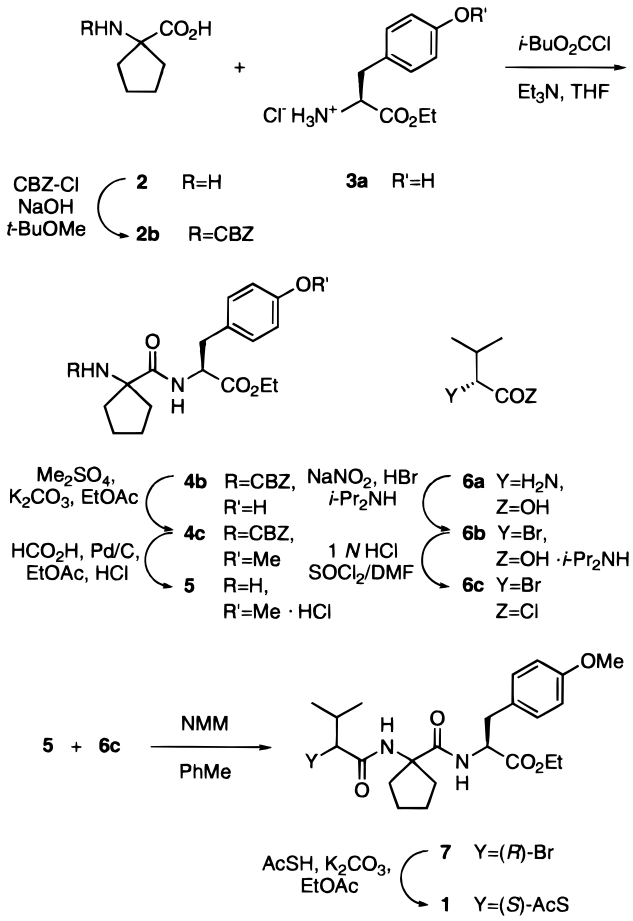
Scheme 2



Scheme 3



desirable. All these modifications were eventually implemented in the initial pilot run. Immediately upon completion of this first large-scale campaign, throughput and expense issues were addressed by a second synthesis (Scheme 3). This later effort replaced *t*-BOC protection with less expensive CBZ protection, utilized acid chloride **6c**, and demonstrated solvent replacement with increases in batch concentration for essentially all steps.

Toward a Multikilogram Synthesis of 1

Preparation of **2a**[6] was hampered by two factors: low solubility of **2** (Scheme 2) in essentially all organic solvents and competitive hydrolysis of BOC_2O in all aqueous biphasic reaction media. A variety of conditions using organic solvents (*t*-BuOH, THF, MeCN, DMF, MeOH, PhMe) was examined to minimize hydrolysis. None proved successful, all giving no reaction. Attempts were made to use phase transfer conditions (Et_3N in PhMe, THF and MeCN, or $Bu_4N^+HSO_4^-$ with the Li^+, Na^+, or K^+ salt of **2**), but this approach came to naught.[7] Among aqueous base−organic solvent biphasic mixtures, the best solvent appeared to be THF, as hydroxylic and very polar solvents led to rapid solvolysis of BOC_2O and nonwater miscible solvents gave no **2a** because **2** is not extracted from the aqueous phase. We optimized yields of **2a** by controlling the addition temperature (50−60 °C) and maintaining vigorous agitation. An added benefit associated with this addition temperature was avoidance of a sudden exotherm, leading to uncontrollable gas evolution that was observed when starting at lower addition temperatures. Isolated **2a** contained 3−5% *N*-*t*-BOC−**2** dimer, presumably arising via *N*-carboxyanhydride formation.[8] Fortunately, neither this impurity, nor its educt with **3a** carried through in the next step.

The first set of conditions tested for replacing DCC/HOBt coupling of **2a** and **3a** was mixed anhydride formation with isobutyl chloroformate (IBCF). The yield for this protocol was good; however, some 10% of **3a** was always lost regardless of solvent (at −25 to −10 °C) due to apparent attack upon the carbonate end of the mixed anhydride. Bases other than Et_3N were not screened for this reaction.[9] Isolation of **4a** from the IBCF reaction was nonetheless efficient, as all the unwanted urethane derivative of **3a** was removed during precipitation. Other protocols were examined in the attempt to limit urethane formation, but all gave

(6) Bardi, R.; Piazzesi, A. M.; Toniolo, C.; Sukumar, M.; Balaram, P. *Biopolymers* **1986**, *25*, 1635−1644.

(7) A successful phase transfer reaction has since been reported: Khalil, E. M.; Subasinghe, N. L.; Johnson, R. L. *Tetrahedron Lett.* **1996**, *37*, 3441−3444.

(8) (a) Abou-Gharbia, M.; Moyer, J.; Patel, U. Webb, M.; Schieser, G.; Andree, T.; Haskins, J. T. *J. Med. Chem.* **1989**, *32*, 1024−1033. (b) Chen, F. M. F.; Benoiton, N. L. *Can. J. Chem.* **1987**, *65*, 619−625.

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distinctly worse results ($POCl_3$, $SOCl_2$/DMF, or *t*-BuCOCl).[10] In all instances, either low yields were obtained or isolation problems occurred.

Methylation of **4a** with K_2CO_3 and Me_2SO_4, the latter used in preference to MeI for volatility reasons, occurred without N-alkylation. Heterogeneous reaction conditions greatly simplified product isolation. Absence of side reactions, coupled with the relatively lower solubility of **4** as compared with **4a**, resulted in high yields and purities for this step. Even so, this solubility difference was a mixed blessing, as the reaction must be conducted with acetone cosolvent to avoid coating the K_2CO_3 with precipitating **4**. No racemization of **4** or **4a** was found to occur under even more forcing reaction conditions.

Deprotection of **4** proved tricky for technical reasons. Every solvent examined for this transformation had limitations. Surprisingly, **5** is very soluble in absolute EtOH and thus could not be crystallized. In dry EtOAc, because of the presence of a considerable excess of anhydrous HCl, sufficient AcOH was formed by acid-catalyzed cleavage to completely inhibit crystallization of **5**. Furthermore, this AcOH could not be completely removed from crude **5**, leading to formation of difficult-to-remove N-acetylated **5** during subsequent peptide coupling. Though these problems did not occur in PhMe solvent, its use afforded **5** as a very hygroscopic material that was difficult to handle. Ultimately, a workable method was developed that involved conducting the reaction in EtOH and then replacing that solvent with EtOAc for isolation.

We rapidly confirmed earlier results[1] that common alternative coupling methods for **5** and **6** were largely ineffective (IBCF, *t*-BuCOCl, and DCC/HOBt). In the case of IBCF as activator, poor reactivity per se was not a problem, as it was for the last two reagents. Rather, formation of significant quantities of impurities resulted (especially the urethane and the symmetrical urea derived from **5**).[9] After a concerted literature survey to look for potential solutions, one of the few reagents we found to be touted for hindered couplings,[11] and also available in bulk, was 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT).[11a] Application of this reagent to peptide coupling of **5** and **6** afforded 40−50% yields, which was not as high as we had hoped. Our difficulties with the reaction were compounded by lack of a means of both its purification and chiral assay. We were finally forced to develop an alternative for **6** when **7** prepared from **6** was determined to exhibit just 84% de: we had serious reservations that this de could be improved sufficiently without unacceptable loss of material.

(10) For an older but still helpful discussion of peptide coupling methods, see: *Chemistry of the Amino Acids*; Greenstein, J. P., Winitz, M., Eds.; Wiley: New York, 1961; Vol. 2, pp 943−1107.

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Realization of the Endgame

A contingency plan that called for replacing **6** with **6b** was developed (vide supra). Preparation of **6b** free acid was known,[12] as was its peptide coupling.[13] However, **6b** free acid is a low-melting solid that is difficult to crystallize. These characteristics made handling a challenge on large scale. Despite finding no reports of amine salts (**6a**) in the literature, we felt such a material would have distinct handling advantages. Thus, a variety of amines was screened for this purpose. Eventually, the *i*-Pr_2NH salt **6b** was identified as a stable compound that exhibited the highest optical purity (>98% ee). This met our requirements.[14] An added bonus of salt formation was minimization of α-hydroxy acid contamination to very low levels.

Success also depended upon finding a peptide coupling agent that gave minimal racemization of **7**. We unfortunately found that (less expensive) IBCF suffered the same drawbacks with **6b** as it did with **6** (urethane and symmetrical urea formation). However, CDMT routinely afforded a peptide coupling of **5** (>99% ee) and **6b** (98−98.5% ee) in good yield with only slight racemization (>97.5% de in crude **7**). Recrystallization of **7** removed most of the unwanted diastereomer (affording **7** of >99% de) and essentially all other impurities (99.5% chemical purity). Thus, the de of **7** obtained with the CDMT method was such that our initial pilot campaign could proceed.

The final step depended upon displacement of Br^- with AcS^-, ordinarily an easy transformation. However, the potential for inversion of the chiral center bearing Br by solvated Br^- prior to AcS^- attack was real and to be avoided at all cost. With this in mind, we chose to explore biphasic (solid−liquid) reaction conditions in an attempt to limit Br^- concentration, thereby circumventing racemization. Under this protocol, formation of **1** was found to proceed cleanly in excellent yield essentially without Br^--induced inversion of **7**. The use of biphasic reaction conditions also facilitated product isolation. By far the most critical parameter influencing this transformation turned out to be the purity of AcSH. It was imperative that this material be assayed for dithioacetic acid content ($MeCS_2^-$ is more reactive than is AcS^-). The dithioacetate analogue of **1** was very difficult to remove from the bulk drug substance. When the dithioacetic acid content was kept below 1% in AcSH, recrystallization of **1** from aqueous EtOH provided product with an HPLC purity of 99.7%. Nearly all undesired diastereomer was also eliminated by recrystallization, affording >99.8% de in **1**. However, when recrystallization exposure time was not minimized, some solvolysis of the *S*-acetyl group was seen in the laboratory. The first piloting run using Scheme 2 prepared 48 kg of **1** of >99.7% chemical and >99.8% optical purity with an overall yield of 28%.

A More Cost Effective Synthesis

Analysis of the expenses associated with executing Scheme 2 emphasized the advantages of replacing the *t*-BOC

(12) Sierra, T.; Serrano, J. L.; Ros, M. B.; Ezcurra, A.; Zubia, J. *J. Am. Chem. Soc.* **1992**, *114*, 7645−7651.

(13) Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11580−11581.

(14) Fink, C. A. Eur. Pat. Appl. EP655, 461, 1993.

protecting group with CBZ, and the CDMT coupling agent with another activation method, i.e., **6c** (Scheme 3). We anticipated relatively straightforward process development for scale-up of the known preparation of **2b**.[15] Peptide coupling of **6c** was attractive as well, provided racemization was low, and had the added benefit of generating less waste. Knowing that the synthesis skeleton would not change, we also identified possibilities for fine-tuning the ecology and throughput of the subsequent steps.

Preparation of **2b** proved more efficient than that of **2a**. Elevated reaction temperature or a water-miscible cosolvent were not required to compensate for poor partitioning of **2** between the two liquid phases. This was apparently due to favorable relative acylation and hydrolysis rates of CBZ-Cl vs BOC_2O when the reaction pH was strictly controlled (10.5−11.5). This reaction was reminiscent of the *t*-BOC series in both the (less pronounced) beneficial effect of a high agitation rate and the formation of 1−2% *N*-CBZ−**2** dimer (which similarly was removed in the next step). We also found that TBME solvent was a waste and cost improvement over THF, simplifying both product isolation and solvent recovery.

Throughput, waste streams, and costs were all improved by combining peptide coupling of **2b** and **3a** with methylation of **4c**. This new process switched from THF, acetone, and water solvents to EtOAc and methylcyclohexane (due to favorable solubility of **4b** vs **4**). Coupling of **2b** and **3a** proceeded with the same 9:1 selectivity as did **2a** and **3a**.[9] The urethane impurity formed during this reaction was effectively removed later during crystallization of **4c**. The first pilot run of this reaction demonstrated an additional need to control the *i*-BuO_2CCl addition rate: symmetrical anhydride formation occurred when it was too slow, leading to unconsumed **2b**. The critical parameter for methylation of **4b** turned out to be the water content of the crude **4b** solution. Significant amounts of **4c** methyl ester impurity were obtained under "wet" conditions (derived from hydrolysis and methylation). Surprisingly, the methyl ester group was carried along through all subsequent transformations, appearing as **1** methyl ester at the end of the synthesis. Azeotropic drying of the crude **4b** solution (prior to methylation) was most convenient and greatly reduced this side reaction.

Deprotection of **4b** via transfer hydrogenation[16] was judged preferable to gaseous hydrogenation because of its operational simplicity and its less specialized equipment requirements; throughput should thus be increased. We found 10% Pd/C and a 10% excess of formic acid in EtOAc to be effective. However, one difficulty initially encountered under these conditions was significant formation of **5** diketopiperazine. Reasoning that this impurity arose from **5** free base, we added the proton source $NH_4^+HCO_2^-$ prior to starting the reaction. Through this technique, free amine concentration was limited, keeping diketopiperazine formation below 3%. Addition of a slight excess of concentrated aqueous HCl followed by azeotropic distillation with EtOAc to remove all H_2O provided crystalline **5** as a hygroscopic but handleable material. Note that formation of AcOH was not a problem under these conditions, allowing us to develop a much faster, simpler process for this step as compared with *t*-BOC removal. The cheaper, easier Scheme 3 synthesis gave **5** with an HPLC purity 2−3% lower, due essentially to diketopiperazine formation.

Cost analysis of the first pilot campaign identified a potential benefit in replacing CDMT for the second peptide coupling. It was also an opportunity to reduce process waste. Colleagues in Process Development (Basle) were able to demonstrate that the most direct way of accomplishing activation of **6b** was through **6c** and that yields were good and racemization was low on small scale. Scale-up fell to us and proceeded without incident. The free acid of **6b** was prepared and the solution was dried via azeotropic distillation. Use of PhMe (rather than several other solvents) for this sequence was especially advantageous due to its inertness and azeotropic behavior, the solubility characteristics of **7**, and the waste minimization attained. Coupling of **6c** with **5** afforded **7** in good yield and with high de (97−98%) after recrystallization. Although this was a lower de than the result with CDMT, it proved sufficient. We consciously chose to utilize the first pilot campaign purification method in the attempt to produce an impurity profile as similar to that of the first campaign as possible. Thus, gross chemical purity was very close to that obtained from the CDMT protocol (99.5% by HPLC), although with slight deviations in the impurity profile.

Performing the final reaction and recrystallization as per the first pilot campaign protocol (again for impurity profile reasons) yielded 165 kg of drug substance **1** of identical de (>99.8%) and very similar chemical purity (99.6%). The difference in the two campaigns was due essentially to the presence of 0.1−0.2% of **1** methyl ester in the latter, derived apparently from 0.1−0.2% of **3a** methyl ester present in the material from the vendor! The impurity profiles of the two batches were otherwise judged to be inconsequentially different. The 32% overall yield was significantly higher for the second campaign, helping to lower the cost per kilo of **1** by 33%. Lastly, throughput was raised between 5% and 50% (averaging 25%) for all but one step, which, in tandem with solvent and reagent changes, led to impressive waste (and cost) minimization.

Conclusions

Strategically, postponing introduction of the acetylthio group until the end, rather than opting for greater convergence with ∼8% racemization in the second peptide coupling, proved critical to the overall success of large scale preparation of **1**. Replacement of the *t*-BOC protecting group with CBZ turned out to have cost and throughput advantages beyond the simple cost differential of BOC_2O and CBZ-Cl. In particular, greater reaction concentrations were possible throughout the entire CBZ series. Finally, the greater optical fidelity obtained for the second peptide coupling with CDMT was not sufficient to warrant its use over the cheaper, less

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(16) El Amin, B.; Anantharamaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442−3444.

wasteful acid chloride method, due to facile purification of **1**.

Experimental Section

General Information. All reactions were carried out under a nitrogen atmosphere. Solvents and reagents were obtained from commercial sources and used as obtained. Critical starting materials **2**, **3**, and **6a** were assayed for chemical and chiral purity prior to use. IR spectra were obtained from KBr mulls. ^{1}H NMR spectra were recorded at 270 or 300 MHz in $CDCl_3$ unless otherwise noted. Coupling constants (J) were recorded in hertz. Melting points were determined by differential scale calorimetry and are uncorrected. Optical rotations were obtained in EtOH at $c = 1$ unless otherwise noted. Reaction progress was monitored by HPLC. Combustion analyses were performed by Robertson Microlit Inc., Chatham, NJ.

1-[[(1,1-Dimethylethoxy)carbonyl]amino]-1-cyclopentanecarboxylic Acid (2a).[6,7] A solution of di-*tert*-butyl dicarbonate (504 g, 2.31 mol) in THF (400 mL) was added at 55 °C to an emulsion of **2** (200 g, 1.55 mol) and NaOH (74.4 g, 1.86 mol) in H_2O (560 mL) and THF (850 mL) over 30 min. After addition was complete, the reaction was stirred at 55 °C for 18 h. The reaction mixture was concentrated to 1000 mL (40% of original volume) and diluted with EtOAc (500 mL) and then by addition of 2 N $KHSO_4$ over 30 min (caution: gas evolution) to pH 2.5−3. The bottom aqueous layer was extracted with EtOAc (500 mL), and the combined organic extracts were washed with H_2O (250 mL). After azeotropic drying and concentration to 550 mL (40% of the original volume), **2a** was precipitated with heptane (1200 mL). The suspension was stirred at 0 °C for 2 h and filtered, and the filter cake was rinsed with heptane/EtOAc (85/15, 150 mL) to give **2a** as a white solid (303 g, 85%): mp 131 °C; ^{1}H NMR (CD_3OD) δ 10.63 (br s, 1 H) 4.92 (br s, 1 H), 2.15 (m, 2 H), 1.91 (m, 2 H), 1.75 (m, 4 H), 1.43 (s, 9 H); IR 3314, 3252, 1706, 1646 cm^{-1}.

1-[[(Phenylmethoxy)carbonyl]amino]-1-cyclopentanecarboxylic Acid (2b).[15] A solution of benzyl chloroformate (1013 g, 5.94 mol) in *t*-BuOMe (TBME, 990 mL) was added at 23 °C to a solution of **2** (700 g, 5.42 mol) in 4 N NaOH (1350 mL) over 2 h. As needed, 4 N NaOH (1917 mL) was added simultaneously to maintain the pH at 10.5−11.5. After addition was complete, the reaction was stirred at 23 °C for 3 h. The top organic layer was discarded. The aqueous layer was washed with TBME (1060 mL), cooled to 0 °C, and acidified with 12 N HCl (ca. 685 g) to pH 2−2.5. The resulting mixture was extracted with EtOAc (2 × 1750 mL), and the combined organic extracts were washed with 1% NaCl (2000 mL). After the extracts were concentrated to 2000 mL (30% of original volume), methylcyclohexane (3500 mL) was added to precipitate **2b**. The crude product was filtered, and the filter cake was rinsed with methylcyclohexane (500 mL) to give **2b** as a white solid (1234 g, 80%): mp 96 °C; ^{1}H NMR (DMSO-d_6) δ 7.62 (s, 1 H), 7.34 (m, 5 H), 5.00 (s, 2 H), 2.05−1.80 (m, 4 H), 1.70−1.65 (m, 4 H); IR 3376, 2390, 1740, 1637 cm^{-1}.

***N*-[[1-[[(1,1-Dimethylethoxy)carbonyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine Ethyl Ester (4a).** IBCF (59.6 g, 0.436 mol) was added over 20 min to a suspension of **2a** (100.0 g, 0.436 mol) and NEt_3 (44.0 g, 0.436 mol) in THF (1500 mL) at −10 °C. After stirring for 30 min, **3a** (107.2 g, 0.436 mol) was added in one portion followed by NEt_3 (44.0 g, 0.436 mol) over 10 min. This mixture was stirred for 2 h at −5 °C. After warming to 23 °C, it was filtered and the filter cake was rinsed with THF (100 mL). The filtrate was concentrated to 590 mL (30% of original volume), and heptane (800 mL) was added over 10 min. The precipitate was filtered, and the filter cake was rinsed with heptane (100 mL) to give **4a** as a white solid (150 g, 82%): mp 166 °C; ^{1}H NMR δ 8.21 (br s, 1 H), 7.02 (br s, 1 H), 6.87 (d, 2 H, $J = 8.5$), 6.69 (d, 2 H, $J = 8.5$), 5.18 (br s, 1 H), 4.68 (dd, 1 H, $J = 6.9, 7.1$), 4.03 (q, 2 H, $J = 7.2$), 2.94 (m, 2 H), 2.12 (m, 2 H), 1.78 (m, 2 H), 1.61 (m, 4 H), 1.32 (s, 9 H), 1.13 (t, $J = 7.2$); IR 3450, 3200, 1742, 1698 cm^{-1}; $[\alpha]^{25}_D = +6.2°$ (MeOH). Anal. Calcd for $C_{22}H_{32}N_2O_6$: C, 62.84; H, 7.67; N, 6.67. Found: C, 62.51; H, 7.62; N, 6.49.

***N*-[[1-[[(1,1-Dimethylethoxy)carbonyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine Ethyl Ester (4).** Neat Me_2SO_4 (291 g, 2.28 mol, caution: toxic) was added over 1 min to a suspension of **4a** (460 g, 1.094 mol) and K_2CO_3 (302.4 g, 2.19 mol, 325 mesh) in acetone (3680 mL) and THF (1380 mL). The mixture was heated to reflux for 4.5 h, cooled to 23 °C, and filtered, and the filter cake was rinsed with acetone/THF (8/3, 150 mL). The filtrate was reduced to 1500 mL (30% of original volume), and H_2O (550 mL) was added with rapid stirring over 10 min. The suspension was cooled to 0 °C, stirred for 4 h, and filtered, and the filter cake was rinsed with acetone/H_2O (2/1, 150 mL) to give **4** as a white solid (420 g, 88%): mp 107 °C; ^{1}H NMR δ 7.07 (br s, 1 H), 7.03 (dd, 2 H, $J = 8.6, 8.6$), 6.77 (dd, 2 H, $J = 8.6, 8.6$), 4.89 (br s, 1 H), 4.75 (dd, 1 H, $J = 7.0, 7.1$), 4.10 (q, 2 H, $J = 7.3$), 3.74 (s, 3 H), 3.03 (m, 2 H), 2.20 (m, 2 H), 1.82 (m, 2 H), 1.71 (m, 4 H), 1.48 (s, 9 H), 1.19 (t, $J = 7.3$); IR 3450, 3325, 1732, 1706 cm^{-1}; $[\alpha]^{25}_D = +13.5°$; Anal. Calcd for $C_{23}H_{34}N_2O_6$: C, 63.57; H, 7.89; N, 6.45. Found: C, 63.51; H, 7.78; N, 6.36.

***N*-[[1-[[(Phenylmethoxy)carbonyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine Ethyl Ester (4c).** Neat NEt_3 (162 g, 1.60 mol) was added to a solution of **2b** (400 g, 1.52 mol) in EtOAc (3000 mL), and the mixture was cooled to −8 °C. IBCF (222 g, 1.60 mol) was added over 25 min, with the temperature kept below −2 °C. The resulting mixed anhydride was stirred for 30 min, cooled to −10 °C, and added to **3** (411 g, 1.67 mol) in EtOAc (1000 mL) at −15 °C. More NEt_3 (188 g, 1.85 mol) was added over 45 min, with the temperature below −6 °C. After this addition was complete, the mixture was warmed to 15 °C and stirred for 1.5 h. The reaction was quenched with H_2O (1000 mL). The top organic layer was washed sequentially with 3% HCl (900 mL), 6% K_2CO_3 (2 × 900 mL), and brine (750 mL) and then azeotropically dried to give a solution of *N*-[[1-[[(phenylmethoxy)carbonyl]amino]-1-cyclopentyl]carbonyl]-L-tyrosine ethyl ester (**4b**) in EtOAc. The solution of **4b** was combined with K_2CO_3 (473 g, 3.42 mol) and Me_2SO_4 (291 g, 2.28 mol). The resulting suspension was heated to reflux for 4 h. The mixture was cooled to 23 °C and

filtered, and the filter cake was rinsed with EtOAc (700 mL). The volume was reduced to 2200 mL (30% of original volume) and methylcyclohexane (5000 mL) was added to the residue over 45 min at 60 °C. The solution was cooled to 0 °C to induce crystallization and was stirred for 4 h. The product was filtered, and the filter cake was rinsed with methylcyclohexane/EtOAc (6/1, 800 mL) to give **4c** as a white solid (536 g, 71% from **2b**): mp 129 °C; ^{1}H NMR δ 7.33 (br s, 5 H), 7.05−6.99 (m, 3 H), 6.88−6.65 (m, 2 H), 5.30 (s, 1 H), 5.07 (s, 2 H), 4.77 (br s, 1 H), 4.24−4.02 (m, 2 H), 3.72 (s, 3 H), 3.03 (br s, 3 H), 2.23−1.70 (m, 8 H), 1.25−1.20 (m, 3 H); IR 3450, 1745, 1686, 1521 cm^{-1}; $[\alpha]^{25}_D$ = +24.0° ($CHCl_3$). Anal. Calcd for $C_{26}H_{32}N_2O_6$: C, 66.65; H, 6.88; N, 5.98. Found: C, 66.29; H, 6.61; N, 5.90.

***N*-[(1-Amino-1-cyclopentyl)carbonyl]-*O*-methyl-L-tyrosine Ethyl Ester Monohydrochloride (5). From 4.** Gaseous HCl (80.1 g, 2.19 mol) was passed into a cooled solution of **4** (60.0 g, 0.14 mol) in absolute EtOH (360 mL) at 23 °C over 20 min (caution: exothermic). After stirring for 1 h, the mixture was reduced to 104 mL (23% of the original volume). EtOAc (800 mL) was added to the concentrate over 5 min, and seeds of authentic **5** were added. The suspension was cooled to 0 °C and stirred for 1 h. The product was collected on a filter, and the filter cake was rinsed with EtOAc (40 mL) to give **5** as a hygroscopic white solid (42.8 g, 84%).

From 4c. A solution of HCO_2H (7.14 g, 0.155 mol) in EtOAc (54 mL) was added to a suspension of **4c** (66.12 g, 0.141 mol), 10% Pd/C (3.0 g, 0.003 mol), and $NH_4^+HCO_2^-$ (4.45 g, 0.071 mol) in EtOAc (535 mL) at 23 °C over 1.5 h. After addition was complete, the mixture was stirred for 1.5 h. Catalyst was filtered off, and the filter cake was rinsed with EtOAc (55 mL). Concentrated HCl (13.90 g, 0.141 mol) was added to the filtrate, and the solvent was removed under vacuum. EtOAc (300 mL) was added to the residue, and seeds of authentic **5** were added. The suspension was cooled to 0 °C, stirred for 1 h, and filtered, and the filter cake was rinsed with EtOAc (55 mL) to give **5** as a hygroscopic white solid (47.3 g, 90%): mp 129 °C; ^{1}H NMR (DMSO-d_6) δ 8.68 (d, 1 H, J = 5.7), 8.26 (s, 3 H), 7.20 (d, 2 H, J = 8.5), 6.83 (d, 2 H, J = 8.5), 4.54−4.39 (m, 1 H), 4.09 (q, 2 H, J = 7.1), 3.71 (s, 3 H), 3.12−2.90 (m, 2 H), 2.20−1.70 (m, 8 H), 1.16 (t, 3 H, J = 7.1); IR 3298, 3225, 1721, 1675 cm^{-1}; $[\alpha]^{25}_D$ = −13.7°. Anal. Calcd for $C_{18}H_{27}ClN_2O_4$: C, 58.29; H, 7.34; N, 7.55; Cl, 9.56. Found: C, 57.97; H, 7.14; N, 7.38; Cl, 9.52.

(*R*)-2-Bromo-3-methylbutanoic Acid (1:1) *N*-(1-Methylethyl)-2-propanamine (6b). A solution of $NaNO_2$ (16.0 g, 0.225 mol) in H_2O (29 mL) was added over 2.5 h to a solution of **6a** (20.0 g, 0.171 mol), 48% HBr (60 mL, 0.53 mol), and H_2O (39 mL) at −5 °C. The mixture was stirred at 0 °C for 1 h, then warmed to 23 °C, and extracted with TBME (3 × 50 mL). The combined organic extracts were washed sequentially with 10% $Na_2S_2O_3$ (40 mL), H_2O (40 mL), and brine (40 mL). After the organic portion was filtered and cooled to −4 °C, diisopropylamine (23.8 mL, 0.17 mol) was added over 12 min, with the temperature maintained below 5 °C. The resulting suspension was stirred at 0 °C for 1 h and filtered, and the filter cake was washed with cold TBME (30 mL) to give **6b** as a white solid (32.7 g, 68%): mp 118 °C (dec); ^{1}H NMR δ 9.49 (br s, 2 H), 4.05 (d, 1 H, J = 6.8), 3.28 (septet, 2 H, J = 6.5), 2.17 (m, 1 H), 1.28 (d, 12 H, J = 6.5), 0.99 (d, 3 H, J = 6.6), 0.98 (d, 3 H, J = 6.5); IR 3036, 2850, 1625, 1583 cm^{-1}; $[\alpha]^{25}_D$ = +4.51° (H_2O). Anal. Calcd for $C_{11}H_{24}BrNO_2$: C, 46.81; H, 8.57; N, 4.96; Br, 28.31. Found: C, 46.89; H, 8.66; N, 5.01; Br, 28.22.

***N*-[[1-[((2-*R*)-Bromo-3-methyl-1-oxobutyl)amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine Ethyl Ester (7). Method A.** *N*-Methylmorpholine (31.1 g, 0.34 mol) was added over 3 min to a solution of **6b** (79.52 g, 0.28 mol) and CDMT (47.22 g, 0.27 mol) in THF (600 mL) at −10 °C. The mixture was stirred for 2 h and then a solution of **5** (95.0 g, 0.26 mol) in THF (350 mL) was added over 15 min. This mixture was stirred for 2.5 h at −10 °C, then warmed to 23 °C, and stirred for 16 h. Water (1110 mL) was added over 1 h and then the mixture was cooled to 0 °C and held for 1 h. The suspension was filtered, and the filter cake was rinsed with H_2O/THF (3/1, 800 mL) to give crude **7** (95.6 g). Purification by recrystallization (930 mL of EtOH/400 mL H_2O) gave pure **7** as a white solid (94.4 g, 74% based on **5**).

Method B. A suspension of **6b** (101.4 g, 0.359 mol) in toluene (600 mL) was washed with 1 N HCl (2 × 250 mL). The resulting **6b** free acid was dried by azeotropic distillation. More toluene (200 mL) was added, and the solution was treated with DMF (1.4 mL, 0.018 mol) and $SOCl_2$ (25 mL, 0.343 mol). This mixture was heated to 55 °C and stirred for 3 h. The volume was reduced to 194 mL (85% of original volume) under vacuum to remove HCl and SO_2. The solution of (*R*)-2-bromo-3-methylbutanoyl chloride (**6c**) in toluene was added to a suspension of **5** (74.96 g, 0.202 mol) in toluene (675 mL) at −5 °C. Neat *N*-methylmorpholine (60 mL, 0.546 mol) was added to the mixture over 9 min while the temperature was maintained below 7 °C. After addition was complete, the mixture was stirred for 2 h at 7 °C. Water (187 mL) was added, and the mixture was heated to 80 °C to dissolve the solids. The separated top organic layer was washed with 1 N HCl (2 × 180 mL) and H_2O (180 mL) at 75 °C. The still hot solution was filtered, and the filtrate was cooled to 0 °C over 3 h. The product was filtered, and the filter cake was rinsed with TBME (300 mL) to give crude **7** (81.6 g). Purification by recrystallization (720 mL of EtOH/325 mL H_2O) gave pure **7** as a white solid (75.0 g, 75% based on **5**): mp 156 °C; ^{1}H NMR δ 7.06 (m, 2 H), 6.99 (br d, 1 H, J = 8.7), 6.81 (m, 2 H), 6.68 (br s, 1 H), 4.74 (m, 1 H), 4.18 (d, 1 H, J = 6.4), 4.16 (q, 2 H, J = 7.2), 3.80 (s, 3 H), 3.06 (m, 2 H), 2.29 (m, 3 H), 1.97 (m, 2 H), 1.75 (m, 4 H), 1.25 (t, 3 H, J = 7.2), 1.04 (d, 3 H, J = 7.0), 0.96 (d, 3 H, J = 7.0); IR 3382, 3259, 1730, 1681 cm^{-1}; $[\alpha]^{25}_D$ = +30.7°. Anal. Calcd for $C_{23}H_{33}BrN_2O_5$: C, 55.54; H, 6.69; N, 5.63; Br, 16.06. Found: C, 55.44; H, 6.71; N, 5.85; Br, 16.01.

***N*-[[1-[[(*S*)-2-(Acetylthio)-3-methyl-1-oxobutyl]amino]-1-cyclopentyl]carbonyl]-*O*-methyl-L-tyrosine Ethyl Ester (1).** Thioacetic acid (80.7 g, 1.06 mol) was added over 15

min to a suspension of **6** (478 g, 0.96 mol) and K_2CO_3 (159 g, 1.15 mol, 325 mesh) in EtOAc (4800 mL) at 23 °C. After stirring for 4 h, the mixture was filtered, and the filter cake was washed with EtOAc (480 mL). The filtrate was washed with H_2O (3 × 1000 mL) and concentrated to 2000 mL (35% of original volume). The solution was heated to 50 °C, and heptane (4780 mL) was added over 45 min. The suspension was cooled to 0 °C, stirred for 4 h, and filtered, and the filter cake was rinsed with cold heptane/EtOAc (9/1, 950 mL) to give crude **1** (438.2 g). Purification by recrystallization (5115 mL of EtOH/5115 mL H_2O) gave pure **1** as a white solid (75.0 g, 88%): mp 108 °C; ^{1}H NMR δ 7.04 (m, 1 H), 7.02 (m, 2 H), 6.76 (m, 2 H), 6.40 (br s, 1 H), 4.48 (m, 1 H), 4.07 (q, 2 H, J = 7.1), 3.72 (s, 3 H), 3.63 (d, 1 H, J = 8.4), 2.98 (m, 2 H), 2.31 (s, 3 H), 2.18 (m, 3 H), 1.90 (m, 2 H), 1.67 (m, 4 H), 1.16 (t, 3 H, J = 7.2), 0.94 (d, 3 H, J = 7.5), 0.93 (d, 3 H, J = 7.5); ^{13}C NMR (75.5 MHz) δ 197.0, 173.0, 171.5, 170.9, 158.5, 130.4, 128.2, 113.8, 67.6, 61.1, 55.2, 54.3, 54.0, 37.1, 36.8, 36.5, 30.5, 28.6, 24.0, 20.9, 19.9, 14.1; IR 3419, 3338, 1740, 1676 cm^{-1}; $[\alpha]^{25}_D$ = +50.8°. Anal. Calcd for $C_{25}H_{36}N_2O_6S$: C, 60.95; H, 7.37; N, 5.63; S, 6.51. Found: C, 60.91; H, 7.42; N, 5.74; S, 6.46.

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