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PAPER

Protecting-group-free synthesis of a dual CCK1/CCK2 receptor antagonist†

Jing Liu, Xiaohu Deng,* Anne E. Fitzgerald, Zachary S. Sales, Hariharan Venkatesan and Neelakandha S. Mani

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In our pursuit of an efficient, protecting-group-free synthesis of the dual CCK1/CCK2 receptor antagonist **1**, we have developed chemoselective conditions for sulfonamide formation reaction in pure water and a $PhNMe_2$ mediated carboxamide formation, both in the presence of a carboxylic acid. Practical synthesis of an unnatural, chiral β-aryl-α-amino acid is also described.

Introduction

Cholecystokinin (CCK), a 33 amino acid peptide, is a regulatory hormone predominantly found in the gastrointestinal tract as well as in the central nervous system. Two specific G protein coupled receptor subtypes (CCK1 and CCK2) regulate the major biological effects of CCK in the gastrointestinal system, which include motility, pancreatic enzyme secretion, gastric emptying, and gastric acid secretion.[1] In the past 20 years, selective inhibition of either of the two receptors has been vigorously pursued both in academia and in the pharmaceutical industry, which has resulted in a steady stream of CCK receptor antagonists into human clinical trials for the treatment of various gastrointestinal diseases.[2] It was recently proposed that dual CCK1/CCK2 receptor inhibition might provide a synergistic effect: CCK1 receptor inhibition improving lower esophageal sphincter smooth muscle function and increasing the rate of gastric emptying and the concurrent CCK2 receptor inhibition moderating gastric acid secretion, which might be beneficial for treatment of gastroesophageal reflux disease.[3] Our laboratories have been active contributors to the study of selective CCK1[4] and CCK2[5] receptor inhibition. Recent medicinal chemistry efforts identified compound **1** as a dual CCK1/CCK2 receptor antagonist with desirable pharmacologic properties (Fig. 1).[3] For the purpose of further profiling this compound, a large-scale synthesis of compound **1** was required.

Two routes were utilized in the drug discovery efforts to prepare compound **1**. The first-generation synthesis involved an eight step sequence starting from 4,5-dichlorophthalic anhydride (Scheme 1). It was noticed that one of the major contributors to the length of this sequence was the protection/deprotection manipulations of the carboxylic acid groups.

In the second-generation route (Scheme 2), the amide bond was formed prior to the sulfonamide. This obviated the manipulation

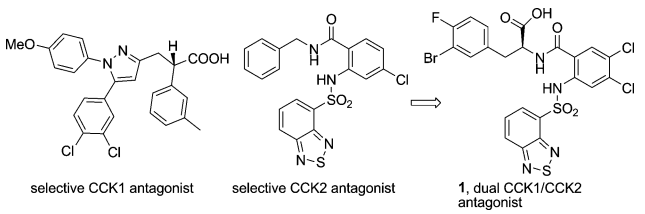
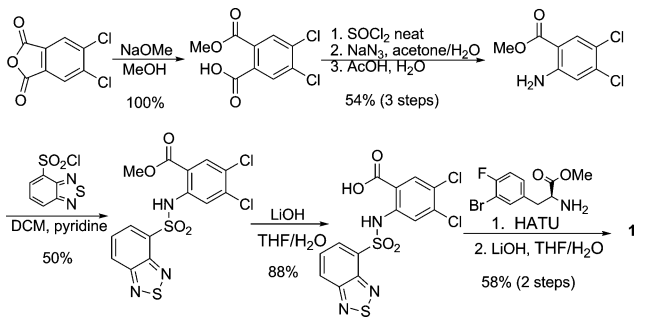


Fig. 1 Representative selective CCK1, CCK2 and dual CCK1/CCK2 antagonists identified in our laboratories.



Scheme 1 First-generation synthesis of compound **1**.

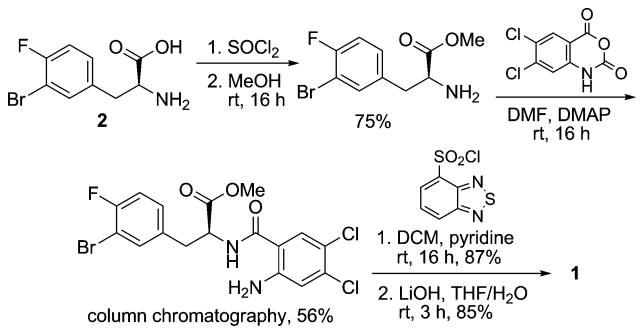
of the benzoic acid functional group, which significantly shortened the overall sequence to four steps. However, like the first generation synthesis, two chemical steps were committed to the introduction and removal of the acid protection group for the α-amino acid. To streamline the synthesis and avoid the use of protecting groups, we developed a two step route featuring a sulfonamide formation in *water* followed by a $PhNMe_2$ mediated amide formation, both in the presence of the unmasked acid group. In addition, a practical synthesis of unnatural, chiral β-aryl-α-amino acid **2** was developed featuring a Rh catalyzed asymmetric hydrogenation. Significant efforts were also devoted to the development of "green" conditions to minimize environmental impacts.

Results and discussion

Protecting groups have long been utilized by synthetic chemists to mask competitive reactivity from interfering with the desired

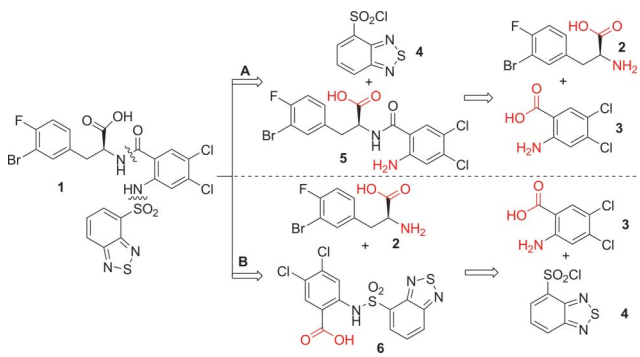
Johnson & Johnson Pharmaceutical Research and Development, L.L.C. 3210 Merryfield Row, San Diego, CA 92121, USA. E-mail: xdeng@its.jnj.com; Fax: +1-858-320-3354; Tel: +1-858-320-3338

† Electronic supplementary information (ESI) available: General experimental methods, ^1H and ^{13}C NMR spectra for compounds **1**, **2**, **5-8**, **10**, **11** and chiral HPLC chromatogram of compounds **1** and **8**. See DOI: 10.1039/c0ob01004a



Scheme 2 Second-generation synthesis of compound **1**.

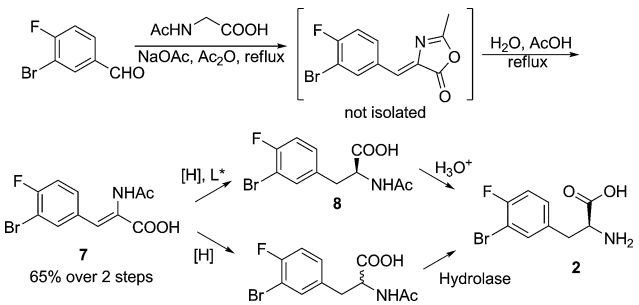
transformation. This strategy, offering security and predictability, has become a textbook approach for the precise control over the individual reactivity within a complex molecule. However, the use of protecting groups, which adds two steps to the overall synthesis, is against the "*ideal synthesis*" principle, which states that chemical syntheses should only consist of skeleton-building steps.[6] Recently, Hoffmann and Baran have been advocating "protecting-group-free" synthesis,[7] not only for achieving higher overall synthetic efficiency, but also as a unique opportunity for inventing novel reactions/conditions by taking advantage of the intrinsic reactivity of the unprotected functionalities.[8] We reasoned that a "protecting-group-free" synthesis of **1** might be feasible, if functionality compatibility was judiciously taken into consideration at the route designing stage. Guided by this principle, we started the retro-synthetic analysis exercise (Scheme 3). The natural disconnections of compound **1** are on the amide C–N bond and sulfonamide N–S bond. Depending on the order of constructing these two bonds, two routes were proposed, using three common building blocks. In either case, our challenge was to find suitable chemoselective conditions/strategies that would effect the desired transformation in the presence of the unmasked, competitive functionalities (COOH, NH_2). Before these two routes could be investigated, easy access to the three building blocks was needed. Compound **4** was commercially available and compound **3** was easily prepared in two steps in *water*.[9] However, practical multi-gram synthesis of unnatural, chiral β-aryl-α-amino acid **2** was not trivial.



Scheme 3 Retro-synthetic analysis of protecting-group-free synthesis of **1**.

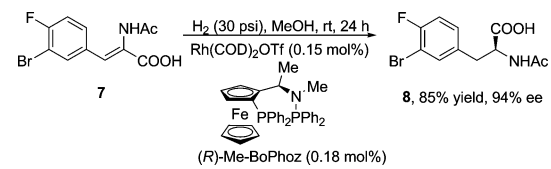
The initial synthesis of compound **2** and analogs used by our medicinal chemistry colleagues in their SAR work involved the enantioselective alkylation of glycine derivatives with benzyl halides.[10] The chirality was established in the alkylation step with the aid of either a pseudoephedrine chiral auxiliary or phase-transfer catalyst derived from a Cinchona alkaloid.[11] These methods were useful for providing milligram quantities of material, but scaling-up proved to be difficult due to expensive reagents, low yields, and variable enantioselectivity. It was clear that a practical, scalable route was required.

In line with our pursuit of the "ideal synthesis", we set a few criteria for the synthesis of **2**: 1) the stereogenic center should be established by a catalytic method rather than a chiral auxiliary mediated approach; 2) functional and protecting group manipulations should be minimized; 3) the reactions should be robust, easily scalable, and environmentally friendly. With these considerations in mind, we chose inexpensive 3-bromo-4-fluorobenzaldehyde as the starting point. Two readily scalable steps (Erlenmeyer–Plöchl azlactone synthesis and hydrolysis) provided intermediate **7** in high yield without the need for chromatographic purification (Scheme 4).[12] To establish the chirality, we envisioned two potential opportunities: the transition-metal catalyzed asymmetric hydrogenation of compound **7** or an enzymatic enantioselective amide hydrolysis of compound **8**.



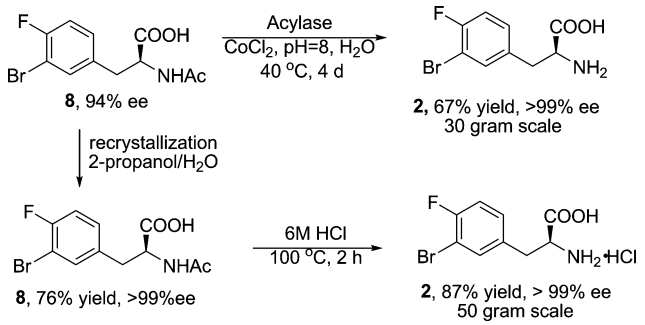
Scheme 4 Proposed enantioselective syntheses of **2**.

Transition-metal catalyzed asymmetric hydrogenation of α-amido cinnamic acids (or esters) for the synthesis of chiral phenylalanine derivatives is an extensively-investigated reaction. Ever since Knowles's landmark industrial scale synthesis of amino acid L-DOPA almost 40 years ago,[13] which garnered him the 2001 Nobel prize along with Noyori and Sharpless, numerous chiral ligands have been developed for this transformation.[14] However, the efficiency of chiral induction appeared to be highly substrate-dependent. For instance, the versatile ligand Me-DuPhos[15] failed to afford the desired product **8** in our case. In contrast, a much less utilized ferrocene-based ligand Me-BoPhoz[16] led to the complete conversion with 94% ee under low catalyst loading on a 40 gram scale (eqn (1)). Me-BoPhoz is commercially available, air stable, and does not require special handling, which satisfied our aforementioned criteria for a robust, scalable synthesis.



(1)

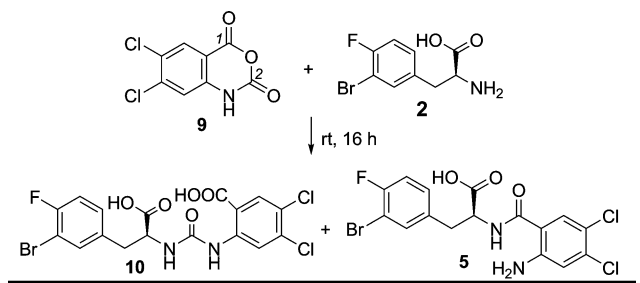
To increase the enantiomeric purity to >99% ee, two efficient strategies were successfully developed (Scheme 5). In the first route, an enzymatic amide hydrolysis was attempted to selectively hydrolyze the desired enantiomer **8** to acid **2**. Acylases operate



Scheme 5 Enantiomeric enrichment and amide hydrolysis.

in environmentally friendly conditions and have been widely adapted in the industry for asymmetric hydrolysis because of their unparalleled precision and efficiency.[17] Screening a number of commercially available acylases identified that the acylase "Amano"[18] provided superb selectivity. Under mild conditions (pH = 8.0, 40 °C, 4 days, with $CoCl_2$ as co-factor), the desired enantiomer **8** was hydrolyzed exclusively whereas the undesired minor enantiomer remained intact. Isolation of the desired product **2** as a single enantiomer (>99% ee) was easily achieved through pH adjustment followed by filtration. Alternatively, a classic recrystallization method was also developed to achieve the requisite enantiomeric enrichment. Systematic solvent screening revealed that a single recrystallization from 2-propanol/water provided a robust process for enantiomeric enrichment (>99% ee). Subsequent amide hydrolysis with 6 M HCl afforded the final product as the HCl salt in excellent yield. Both routes have been carried out on multi-gram scale to provide enantiomerically pure **2**.

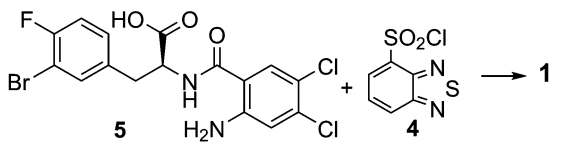
With all three building blocks in hand, route **A** from Scheme 3 was first investigated. To construct **5** directly from **2** and **3**, the requisite chemoselective amide bond formation would be difficult to achieve because of the existence of reactive amino and carboxylic acid groups in both **2** and **3**. To circumvent this problem, isatoic anhydride **9** was chosen as a surrogate with an intrinsically activated acid group (Scheme 6).[19] This strategy had been successfully utilized in the second-generation synthesis (Scheme 2), where the chemoselective ring-opening at the benzoic carbonyl (position *1*) was achieved with the methyl ester of **2**. However, when the same conditions were employed on **2** with an unprotected acid functional group, compound **10** was the only



entry	conditions	10	5
1	4 eq. Et_3N, DCM	90%	–
2	4 eq. Na_2CO_3, THF/H_2O	79%	–
3	Na_2CO_3, **H_2O**, pH=8	–	83%

Scheme 6 The chemo-selective ring-opening of isatoic anhydride **5**.

Table 1 Formation of **1** through final stage sulfonamide formation



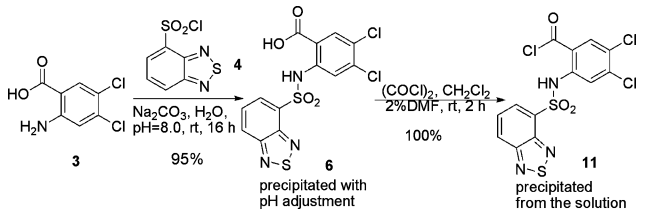
entry	base	solvent	conditions	yield[a]
1	pyridine	DCM	rt, 16 h	0%
2	Et_3N	DCM	rt, 16 h	0%
3	pyridine	-	70 °C, 16 h	0%
4	2,6-lutidine	THF	reflux, 16 h	0%
5	Na_2CO_3	acetone/H_2O	rt, 16 h	12%
6	Na_2CO_3	THF/H_2O	rt, 16 h	4%
7	Na_2CO_3	H_2O	rt, 16 h, pH=8	20%
8	Na_2CO_3	H_2O	10% $Bu_4N^+Br^-$ rt, 16 h, pH=8	5%

a. Quantitative HPLC yield.

product in 90% isolated yield. It is noteworthy that the ring-opening reaction of isatoic anhydride at the *2* position is only rarely found in the literature, and usually in low yields.[20] Apparently, the intrinsic reactivity of the free acid functionality of **2** facilitated the usually more difficult ring-opening reaction. Nevertheless, to access the desired compound **5**, new conditions specific for the unprotected amino acid **2** were required. Adding water as co-solvent has been shown to promote the ring opening reaction of isatoic anhydrides at the *1* position;[21] unfortunately in our case this did not provide the desired chemoselectivity (Scheme 6, entry 2). In contrast, with water as the sole solvent and pH control at 8.0, the chemoselectivity of this ring-opening reaction completely reversed to afford the desired compound **5** exclusively.[22] Simple acidification of the reaction solution with aqueous HCl solution precipitated pure **5** in 83% yield.

Unfortunately, the subsequent sulfonamide formation between compound **5** with **4** was surprisingly difficult. In contrast to the 87% yield obtained in the second-generation synthesis with the methyl ester of **5** (Scheme 2), the same sulfonamide formation conditions (pyridine/DCM) failed to produce any desired product **1** (Table 1, entry 1). Employment of stronger base (Et_3N) or an increase of the reaction temperature did not promote this reaction (entries 2–4). Schotten–Baumann conditions[23] in mixed organic/water solvent systems provided small amounts of **1** (entries 5 and 6). The best yield of **1** (20%) was obtained in pure water with pH control at 8.0 (entry 7).[24] Addition of a phase transfer catalyst ($Bu_4N^+Br^-$) was detrimental to this reaction (entry 8). The difficulty of forming the sulfonamide bond is probably due to the intramolecular hydrogen bonding between the carboxylic acid proton and the free amino group of compound **5**. This route was not pursued further.

Route **B** (Scheme 3) was next investigated. Based on methodology we have developed previously,[24] direct sulfonamide formation with unprotected 2-amino-4,5-dichloro-benzoic acid and compound **4** was achieved in pure water (Scheme 7). Compound **6** precipitated in 95% yield after simple acidification with concentrated

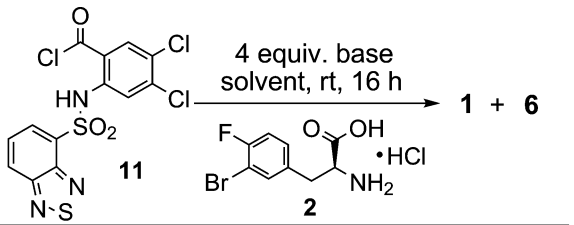


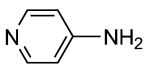
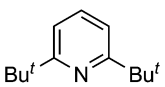
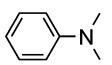
Scheme 7 Direct sulfonamide formation in water.

HCl solution. The reaction was exceptionally green, proceeding in high yield and generating no organic waste.[25] To avoid the use of expensive coupling reagents and the associated waste disposal problems, we chose to use acid chloride **11** for the subsequent amide bond forming reaction. The acid chloride **11** was readily prepared in quantitative yield under standard conditions.

With **11** in hand, direct amide formation with unprotected **2** was investigated. Under classic and modified Schotten–Baumann conditions,[26] hydrolysis of **11** was simply too fast for the amide formation process to compete (Table 2, entries 1–4). The typical non-aqueous conditions (DCM/Et_3N) also afforded the hydrolyzed acid **6** as the major product (entry 5). Switching to THF as the solvent significantly suppressed the hydrolysis reaction (entry 6).[27] It appeared that stronger bases (entries 7, 8, 10) tended to promote the hydrolysis process, whereas weaker bases such as pyridine favoured the formation of **1** (entry 9). A weak and sterically hindered base, 2,6-lutidine, provided a synthetically useful 10 : 1 ratio of compound **1** and **6** (entry 11). Attempts to enhance this ratio with a bulkier base (2,6-di-*t*-butyl pyridine, entry 12) was counterproductive. Finally, we found that *N*,*N*-dimethylaniline provided the best ratio of **1**:**6** (20 : 1, entry 13). The reaction profile was rather clean and pure compound **1** was afforded in 70% isolated yield after recrystallization from ^{i}PrOAc. Since racemization of amino acids is often observed in amide coupling reactions, it is important to note that no loss of ee was observed during the above process. The same conditions were successfully applied to the synthesis of related analogs as well.

Table 2 Formation of **1** through final stage amide formation



entry	base	solvent	ratio **1** : **6**[a]
1	Na_2CO_3	acetone/H_2O	– 100%
2	Na_2CO_3	H_2O, pH=8	– 100%
3	NaOH	H_2O, pH=8 10% $Bu_4N^+Br^-$	– 100%
4	pyridine	THF/H_2O	– 100%
5	Et_3N	DCM	1 3
6	Et_3N	THF	2.5 1
7	$^{i}Pr_2NEt$	THF	1 2.5
8		THF	1.3 1
9	pyridine	THF	3 1
10		THF	1 10
11	2,6-Lutidine	THF	10 1
12		THF	1 3
13		THF	20 1[b]

a. Ratio determined base on HPLC peak area integration.
b. The yield was 70% after recrystallization from ^{i}PrOAc

Conclusions

Two "protecting-group-free" routes were explored for the preparation of compound **1**, a dual CCK1/CCK2 receptor antagonist. In the presence of unprotected, competitive functional groups, development of new chemoselective conditions are often required even for the "easy" reactions such as amide and sulfonamide formation. Notable new reactions/conditions include a selective ring-opening reaction of isatoic anhydride in *water*, a sulfonamide formation in *water* and a $PhNMe_2$ mediated amide formation reaction. In the end, a highly efficient, two-step route for the synthesis of **1** was developed.

Experimental

General techniques

Proton and carbon NMR spectra were recorded on a 500 MHz or a 600 MHz NMR spectrometer. Flash column chromatography was performed using Merck silica gel 60. HRMS (ESI) was performed on a μTof apparatus. Melting points were determined using an electrothermal apparatus and are uncorrected. Unless specified, all the reagents and solvents were purchased from commercial sources and used without further purification. Optical rotation was measured on a Perkin–Elmer 341 polarimeter. Chiral HPLC analysis was performed on a Hewlett Packard 1100.

Synthetic procedures

2-Acetylamino-3-(3-bromo-4-fluoro-phenyl)-acrylic acid (7). A 500 mL, round-bottom flask equipped with a magnetic stir bar, an internal thermometer and a reflux condenser was charged with 3-bromo-4-fluorobenzaldehyde (50.0 g, 0.25 mol, 1.1 equiv.), *N*-acetylglycine (26.2 g, 0.22 mol, 1.0 equiv.), sodium acetate (13.8 g, 0.17 mol, 0.77 equiv.), and acetic anhydride (52 mL). The mixture was heated to 130 °C and stirred for 10 h. After cooling to room temperature, the precipitate formed was collected by filtration and the filter cake rinsed with water. The resulting wet solid was suspended in acetic acid (250 mL) and heated at 100 °C for 1 h. After cooling to 0 °C, the precipitated solid was collected by filtration, washed with water, and dried to afford the title

compound as a yellow solid (53.5 g, 0.17 mol, 76%). Mp: 215–216 °C. ^{1}H NMR (600 MHz, d_6-DMSO) δ 12.80 (br s, 1H), 9.52 (br s, 1H), 7.93 (dd, J = 6.9, 1.9 Hz, 1H), 7.66 (ddd, J = 8.4, 4.8, 1.9 Hz, 1H), 7.42 (t, J = 8.7 Hz, 1H), 7.19 (s, 1H), 1.98 (s, 3H). ^{13}C NMR (151 MHz, d_6-DMSO) δ 169.1, 166.1, 158.0 (d, J_{C-F} = 248.3 Hz, 1C), 134.3, 132.2 (d, J_{C-F} = 3.7 Hz, 1C), 131.0 (d, J_{C-F} = 7.6 Hz, 1C), 128.2, 128.1 (d, J_{C-F} = 1.8 Hz, 1C), 116.8 (d, J_{C-F} = 22.4 Hz, 1C), 108.1 (d, J_{C-F} = 21.2 Hz, 1C), 22.5. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{11}H_{10}BrFNO_3$ 301.9828; found, 301.9824.

(*S*)-2-Acetylamino-3-(3-bromo-4-fluoro-phenyl)-propionic acid (8). Bis(1,5-cyclooctadiene)rhodium(I) trifluoromethanesulfonate (0.11 g, 0.23 mmol, 0.0015 equiv.) and (*R*)-*N*-diphenylphosphino-*N*-methyl-1-[(*S*)-2-diphenylphosphino) ferrocenyl]ethylamine [(*R*)-methyl *BoPhoz*] (0.17 g, 0.27 mmol, 0.0018 equiv.) were added to anhydrous methanol (10 mL) under nitrogen atmosphere and stirred for 30 min. A 2.25 L Parr hydrogenation flask was charged with 2-acetylamino-3-(3-bromo-4-fluoro-phenyl)-acrylic acid (**7**, 47.0 g, 0.16 mol, 1.0 equiv.) and anhydrous methanol (650 mL) under nitrogen atmosphere. The preformed catalyst solution was transferred into the Parr hydrogenation flask. The mixture was shaken under hydrogen atmosphere (30 psi) at room temperature overnight. The solvent was removed under reduced pressure and the residue was suspended in Et_2O (100 mL) and cooled to 0 °C. The precipitated solid was collected by filtration to afford the title compound as a light tan solid (40.0 g, 0.14 mol, 85%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.50 (dd, J = 6.7, 2.1 Hz, 1H), 7.24 (ddd, J = 8.4, 4.7, 2.2 Hz, 1H), 7.13 (t, J = 8.6 Hz, 1H), 4.65 (dd, J = 9.0, 5.2 Hz, 1H), 3.19 (dd, J = 14.0, 5.2 Hz, 1H), 2.93 (dd, J = 14.0, 9.0 Hz, 1H), 1.93 (s, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 174.2, 173.2, 159.4 (d, J_{C-F} = 244.9 Hz, 1C), 136.5 (d, J_{C-F} = 3.8 Hz, 1C), 135.3, 131.3 (d, J_{C-F} = 7.3 Hz, 1C), 117.3 (d, J_{C-F} = 22.4 Hz, 1C), 109.3 (d, J_{C-F} = 21.0 Hz, 1C), 54.9, 37.3, 22.3. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{11}H_{12}BrFNO_3$ 303.9985; found, 303.9970. Chiral HPLC: ChiralPak AD-H 4.6 × 250 mm column, 15% ethanol in hexanes (1% trifluoroacetic acid in ethanol), 0.8 mL min^{-1}, retention time: 8.650 min [(*R*)-isomer: 8.092 min], The enantiomeric purity was 94% ee.

(*S*)-2-Acetylamino-3-(3-bromo-4-fluoro-phenyl)-propionic acid (8, >99% ee)—enantiomeric enrichment *via* recrystallization. (*S*)-2-Acetylamino-3-(3-bromo-4-fluoro-phenyl)-propionic acid (42 g, 88% ee)[28] was dissolved in 2-propanol (210 mL) at 70 °C. Hot water (preheated to 70 °C) was added drop wise with stirring until the solution became slightly cloudy (~580 mL). The solution was cooled to room temperature and left overnight without stirring. The precipitated crystals were collected, washed with H_2O and dried to afford the title compound as an off-white solid (32.0 g, 76% recovery). Mp: 153–154 °C. The enantiomeric purity was >99% ee. $[\alpha]_D^{20}$ = +35.1° (c = 1.37, MeOH).

3-Bromo-4-fluoro-L-phenylalanine (2). *N*-Acetyl-3-bromo-4-fluoro-L-phenylalanine (**8**, 50.0 g, 0.16 mol, 94% ee, 1.0 equiv.) was suspended in water (1.0 L). Aqueous KOH (2.0 M) was added to adjust pH to 8.0. Warmed to 40 °C, Acylase "Amano" (4.0 g, Amano Enzyme Inc., Japan) and $CoCl_2$ (26 mg, 0.2 mmol, 0.00125 equiv.) were then added. The pH of the solution was maintained at 8.0 by the addition of aqueous KOH (2.0 M) through a syringe pump. It took 4 days at 40 °C to achieve complete conversion. Aqueous HCl (2.0 M) was added to adjust pH to 1. The precipitated solid, mostly the unreacted minor *S*-enantiomer, was filtered off. The filtrate solution was concentrated to ~20 mL and adjusted to pH 6.0 with 2.0 M KOH. After stirring at 0 °C overnight, the desired product crystallized out. The solid was collected by filtration to afford the title compound as a light tan solid (29 g, 67% yield). Mp: 197–200 °C. ^{1}H NMR (600 MHz, AcOD) δ 7.58 (dd, J = 6.6, 2.0 Hz, 1H), 7.35 (ddd, J = 8.3, 4.6, 2.1 Hz, 1H), 7.16 (t, J = 8.6 Hz, 1H), 4.36 (dd, J = 7.8, 5.1 Hz, 1H), 3.38 (dd, J = 14.7, 4.9 Hz, 1H), 3.22 (dd, J = 14.7, 8.0 Hz, 1H). ^{13}C NMR (151 MHz, AcOD) δ 173.1, 158.5 (d, J_{C-F} = 245.8 Hz, 1C), 134.7, 132.7 (d, J_{C-F} = 3.7 Hz, 1C), 130.7 (d, J_{C-F} = 7.5 Hz, 1C), 116.6 (d, J_{C-F} = 22.4 Hz, 1C), 108.6 (d, J_{C-F} = 21.1 Hz, 1C), 55.7, 34.8. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_9H_{10}BrFNO_2$ 261.9879; found, 261.9838. The enantiomeric purity was >99.5% ee.[29]

3-Bromo-4-fluoro-L-phenylalanine hydrochloride (2, HCl salt). (*S*)-2-Acetylamino-3-(3-bromo-4-fluoro-phenyl)-propionic acid (**8**, 40.0 g, 0.13 mol, 99% ee) was added to hydrochloric acid (6.0 M in water, 88 mL). The suspension was heated to 100 °C and stirred for 3 h. Heating was removed and the reaction mixture cooled to 0 °C. The solid obtained was collected by filtration to give the title compound as a white solid (38.1 g, 97%). Mp: 229–231 °C. ^{1}H NMR (600 MHz, D_2O) δ 7.50 (dd, J = 6.6, 2.1 Hz, 1H), 7.21 (ddd, J = 8.4, 4.7, 2.2 Hz, 1H), 7.14 (t, J = 8.7 Hz, 1H), 3.90 (dd, J = 7.7, 5.5 Hz, 1H), 3.16 (dd, J = 14.7, 5.4 Hz, 1H), 3.04 (dd, J = 14.7, 7.7 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 171.3, 158.4 (d, J_{C-F} = 244.9 Hz, 1C), 134.2, 131.7 (d, J_{C-F} = 3.7 Hz, 1C), 130.4 (d, J_{C-F} = 7.7 Hz, 1C), 116.9 (d, J_{C-F} = 22.5 Hz, 1C), 108.7 (d, J_{C-F} = 21.2 Hz, 1C), 54.0, 34.5. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_9H_{10}BrFNO_2$ 261.9879; found, 261.9859. The enantiomeric purity was >99% ee. $[\alpha]_D^{20}$ = +6.2 (c = 1.54, MeOH).

2-(2-Amino-4,5-dichloro-benzoylamino)-3-(3-bromo-4-fluoro-phenyl)-propionic acid (5). A mixture of 6,7-dichloro-1*H*-benzo[d][1,3]oxazine-2,4-dione (**9**, 1.78 g, 7.7 mmol, 1.0 equiv.) and 2-amino-3-(3-bromo-4-fluoro-phenyl)-propionic acid, HCl salt (**2**, **HCl salt**, 2.3 g, 7.7 mol, 1.0 equiv.) was suspended in water (100 mL). Under vigorous stirring, Na_2CO_3 aqueous solution (2.0 M) was added *via* a syringe pump to adjust and maintain the pH at 8.0 ± 0.2. After 16 h at room temperature, the reaction mixture became a clear solution. Concentrated HCl was added to adjust pH to 2.0. The precipitated solid was collected by filtration, washed with water, dried to afford the title compound as a white solid (2.9 g, 6.3 mmol, 83%). Mp: 167–169 °C. ^{1}H NMR (500 MHz, d_6-DMSO) δ 12.89 (s, 1H), 8.68 (d, J = 8.2 Hz, 1H), 7.71 (s, 1H), 7.66 (dd, J = 6.8, 2.1 Hz, 1H), 7.38–7.32 (m, 1H), 7.29 (t, J = 8.7 Hz, 1H), 6.94 (s, 1H), 6.64 (s, 2H), 4.58 (ddd, J = 10.7, 8.2, 4.7 Hz, 1H), 3.19 (dd, J = 13.8, 4.7 Hz, 1H), 3.02 (dd, J = 13.8, 10.7 Hz, 1H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 172.7, 167.0, 157.0 (d, J_{C-F} = 243.5 Hz, 1C), 149.5, 136.3 (d, J_{C-F} = 3.7 Hz, 1C), 134.2, 133.9, 130.3 (d, J_{C-F} = 7.3 Hz, 1C), 129.7, 117.0, 116.3 (d, J_{C-F} = 22.0 Hz, 1C), 115.3, 113.8, 107.4 (d, J_{C-F} = 20.8 Hz, 1C), 53.5, 35.0. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{16}H_{13}N_2O_3FCl_2Br$ 448.9465; found, 448.9449. $[\alpha]_D^{20}$ = –29.3° (c = 1.00, MeOH).

2-{3-[2-(3-Bromo-4-fluoro-phenyl)-1-carboxy-ethyl]-ureido}-4,5-dichloro-benzoic acid (10). To a suspension of 6,7-dichloro-1*H*-benzo[d][1,3]oxazine-2,4-dione (**9**, 200 mg, 0.86 mmol,

1.0 equiv.) and 2-amino-3-(3-bromo-4-fluoro-phenyl)-propionic acid, HCl salt (**2**, 256 mg, 0.86 mmol, 1.0 equiv.) in DCM (20 mL), Et_3N (0.48 mL, 3.4 mmol, 4.0 equiv.) was added to form a clear homogenous solution. The reaction solution was stirred at room temperature overnight. The DCM layer was extracted with water (40 mL) twice. The aqueous layers were combined and the pH adjusted to 2.0 with concentrated HCl. The precipitate was collected by filtration, washed with water and dried to afford the title compound as a white solid (0.19 g, 0.39 mmol, 45%).[30] Mp: 160–162 °C. ^{1}H NMR (500 MHz, d_6-DMSO) δ 10.85 (s, 1H), 8.60 (s, 1H), 8.11 (d, J = 6.4 Hz, 1H), 8.03 (s, 1H), 7.62 (dd, J = 6.8, 1.8 Hz, 1H), 7.34 (ddd, J = 6.9, 5.0, 1.9 Hz, 1H), 7.30 (t, J = 8.6 Hz, 1H), 4.37 (ddd, J = 9.9, 8.2, 4.9 Hz, 1H), 3.19–3.05 (m, 1H), 2.91 (dd, J = 13.9, 10.1 Hz, 1H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 173.2, 167.8, 157.0 (d, J_{C-F} = 243.5 Hz, 1C), 154.3, 141.9, 136.2 (d, J_{C-F} = 3.6 Hz, 1C), 134.9, 133.8, 132.0, 130.4 (d, J_{C-F} = 7.3 Hz, 1C), 121.5, 119.9, 117.5, 116.3 (d, J_{C-F} = 21.9 Hz, 1C), 107.4 (d, J_{C-F} = 20.8 Hz, 1C), 54.2, 35.4. HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{17}H_{13}N_2O_5FCl_2Br$ 492.9363; found, 492.9355. $[\alpha]_D^{20}$ = +11.3° (c = 0.02, MeOH).

2-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-4,5-dichloro-benzoic acid (6). In a 3-necked, round-bottom flask equipped for mechanical stirring and fitted with a pH meter was placed a mixture of 2-amino-4,5-dichloro-benzoic acid (**3**, 40 g, 0.19 mol, 1.0 equiv.) and benzo[1,2,5]thiadiazole-4-sulfonyl chloride (**4**, 45 g, 0.19 mol, 1.0 equiv.) suspended in 700 mL H_2O. Under vigorous stirring, aqueous Na_2CO_3 (2.0 M) was added with a syringe pump to adjust and maintain the pH at 8.0 ± 0.2. The reaction took about 16 h to complete (about 1.2 equivalents of Na_2CO_3 were needed). Concentrated HCl was carefully added upon stirring to adjust pH < 2.0. The precipitated solid was collected by filtration, washed with water and dried under reduced pressure. The crude product was stirred in hot EtOAc (*ca.* 1 g/2 mL EtOAc, 50 °C) for 20 min. and cooled to room temperature. The solid was collected by filtration to give the pure title compound (66 g, 0.16 mol, 85%). Mp: 237–239 °C. ^{1}H NMR (500 MHz, d_6-DMSO) δ 11.93 (br. s, 1H), 8.44 (ddd, J = 9.7, 8.0, 0.9 Hz, 2H), 7.92 (s, 1H), 7.89 (d, J = 8.8, 7.1 Hz, 1H), 7.72 (s, 1H). ^{13}C NMR (125.7 MHz, d_6-DMSO): δ 165.9, 153.3, 146.6, 137.6, 134.8, 130.9, 130.8, 127.9, 127.2, 125.9, 123.4, 117.5, 116.0. IR (dry film, cm^{-1}): 3108 (br. m), 1675 (s), 1375 (s), 1244 (s), 1157 (s). HRMS-ESI (m/z): [M – H]$^-$ calcd for $C_{13}H_7Cl_2N_3O_4S_2$ 401.9171; found, 401.9177.

2-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-4,5-dichloro-benzoyl chloride (11). 2-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-4,5-dichloro-benzoic acid (**6**, 5 g, 12.4 mmol, 1.0 equiv.) was suspended in 40 mL CH_2Cl_2 and DMF (48 μL, 5 mol%). Oxalyl chloride (1.3 mL, 14.9 mmol, 1.2 equiv.) was added drop wise at room temperature. After stirring at room temperature for 16 h, the precipitated solid was collected by filtration and washed with DCM to afford the pure title compound (5.5 g, 12.4 mmol, 100%). Mp: 210–212 °C. ^{1}H NMR (600 MHz, d_6-DMSO) δ 11.61 (s, 1H), 8.47 (ddd, J = 9.7, 8.0, 0.9 Hz, 2H), 7.92 (s, 1H), 7.91 (dd, J = 8.8, 7.1 Hz, 1H), 7.73 (s, 1H). ^{13}C NMR (151 MHz, d_6-DMSO) δ 167.5, 154.7, 148.0, 138.6, 136.4, 132.5, 132.5, 129.0, 128.7, 127.6, 125.2, 118.9, 117.2.

2-[2-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-4,5-dichloro-benzoylamino]-3-(3-bromo-4-fluoro-phenyl)-propionic acid (1). A mixture of 2-(benzo[1,2,5]thiadiazole-4-sulfonylamino)-4,5-dichloro-benzoyl chloride (**11**, 5.5 g, 12.4 mmol, 1.0 equiv.) and 2-amino-3-(3-bromo-4-fluoro-phenyl)-propionic acid, HCl salt (**2**, **HCl salt**, 3.7 g, 12.4 mmol, 1.0 equiv.) was suspended in dry THF (200 mL). $PhN(CH_3)_2$ (5 mL, 37.5 mmol, 3.0 equiv.) was added drop wise over 5 min to form a clear solution. After stirring at room temperature for 2 h, THF was evaporated under reduced pressure and the residue was re-dissolved in EtOAc. The organic layer was washed with 1 M HCl and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product thus obtained was recrystallized from hot ^{i}PrOAc to afford the title compound as a white solid (5.6 g, 8.6 mmol, 70%). Mp: 144–145 °C. ^{1}H NMR (600 MHz, d_6-DMSO) δ 13.10 (s, 1H), 11.69 (s, 1H), 9.11 (s, 1H), 8.42 (t, J = 8.5 Hz, 2H), 7.90–7.83 (m, 2H), 7.67 (s, 1H), 7.63 (dd, J = 6.8, 1.9 Hz, 1H), 7.36–7.24 (m, 2H), 4.60 (ddd, J = 10.2, 7.9, 5.1 Hz, 1H), 3.20 (dd, J = 13.9, 4.9 Hz, 1H), 2.99 (dd, J = 13.9, 10.3 Hz, 1H). ^{13}C NMR (151 MHz, d_6-DMSO) δ 171.9, 165.9, 157.1 (d, J_{C-F} = 243.8 Hz, 1C), 154.7, 147.9, 137.5, 135.7 (d, J_{C-F} = 3.5 Hz, 1C), 135.0, 133.9, 132.5, 130.3 (d, J_{C-F} = 7.3 Hz, 1C), 130.2, 129.0, 128.6, 127.5, 125.2, 119.5, 119.3, 116.4 (d, J_{C-F} = 22.0 Hz, 1C), 107.5 (d, J_{C-F} = 20.8 Hz, 1C), 54.0, 34.8. HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{22}H_{15}N_4O_5S_2FCl_2Br$ 646.9023; found, 646.9015. $[\alpha]_D^{20}$ = −79.9° (c = 0.38, MeOH).

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30 The solid contained $Et_3N{\cdot}HCl$. The amount varied from batch to batch.

