JOC The Journal of Organic Chemistry

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Synthesis of Fmoc-Protected Arylphenylalanines (Bip Derivatives) via Nonaqueous Suzuki-Miyaura Cross-Coupling Reactions

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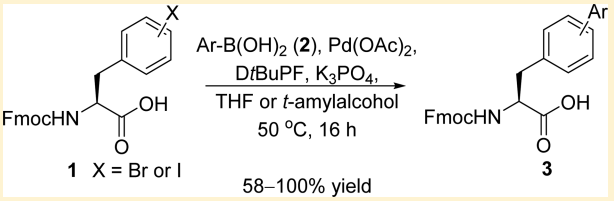
Supporting Information

ABSTRACT: A one-step synthesis of Fmoc-protected aryl/heteroaryl-substituted phenylalanines (Bip derivatives) using the nonaqueous palladium-catalyzed Suzuki−Miyaura cross-coupling (SMC) reaction of Fmoc-protected bromo- or iodophenylalanines is reported. This protocol allows for the direct formation of a variety of unnatural biaryl-containing amino acids in good to excellent yield, which can be readily used in subsequent Fmoc solid-phase peptide synthesis. The synthetic utility of this method is also demonstrated by the SMC reaction of bromophenylalanine-containing tripeptides.



Peptide-based drugs, in particular, macrocyclic peptides, are regaining interest in drug discovery because of their promising attributes to modulate challenging targets such as protein−protein interactions (PPIs), the binding surfaces of which are usually extensive and shallow. Unnatural amino acids incorporated into peptide-based drugs often provide improved binding affinity to the targeted protein and enhanced proteolytic stability. New methods and platforms have been developed to rapidly screen large peptide libraries that consist of both natural and unnatural amino acids for initial drug hits. Hit confirmation by distinct chemical synthesis followed by SAR optimization on side chains and backbones is the main objective for medicinal chemists. During these processes, Fmoc solid-phase peptide synthesis (SPPS) is commonly used for peptide elongation with specific monomeric (un)natural amino acids (AAs), assuring the fidelity of the molecule and, hence, an unambiguous SAR outcome. New methods to efficiently access Fmoc-protected unnatural amino acids are increasingly needed to allow modifications at the monomeric amino acid level or the peptide level to modulate PPIs.

The biaryl motif is viewed as a "privileged substructure" in drug design because of its defined and relatively rigid conformation and its ability to interact with not only aromatic (such as $\pi-\pi$ interactions) and hydrophobic residues but also polar groups and positively charged groups (such as π−cation interactions). In addition to naturally occurring cyclic bridged peptides,[1−5] synthetic (heterocyclic) biaryl-containing amino acids, such as (hetero)aryl-substituted phenylalanines (Phe), which are also called Bip derivatives, have been widely used in the modification of peptides and proteins to possess biological activities.[6−11] Additionally, biaryl-containing unnatural amino acids have been used as fluorescent species/dyes[12,13] and as nonreductive cross-linkers to mimic disulfide linkages.[14]

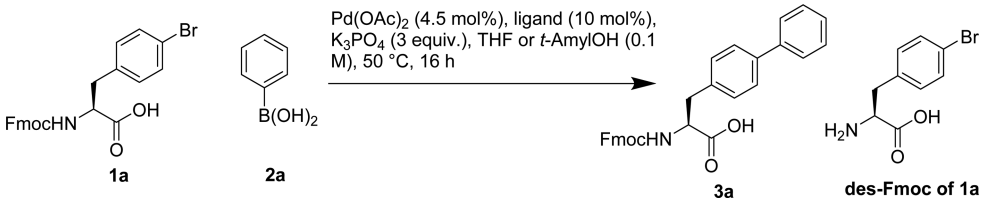
Palladium-catalyzed cross-couplings using the Suzuki−Miyaura coupling (SMC) reaction with aryl halides or aryl triflates have been developed to synthesize unnatural amino acids. For example, Bip derivatives were synthesized in SMC starting from *N*-acetyl- or *N*-Boc-protected iodo- or bromo-Phe,[2−4,6,9,10,15−19] the triflate of tyrosine (Tyr),[20−22] the boronic ester of Phe,[2−5,8,9,14,16] as well as unprotected organoborono- or bromo-Phe.[23,24] Recently, palladium-catalyzed C−H activation has also been actively studied for the synthesis of new amino acids.[25,26] Notably, Bip derivatives are commonly prepared via the SMC reaction from *N*-Boc-protected Phe precursors to provide *N*-Boc Bips. The protecting group is then switched to Fmoc prior to incorporating the new monomeric building blocks into Fmoc SPPS for peptide elongation.[22]

To the best of our knowledge, the direct cross-coupling of Fmoc-protected halogenated Phe with boronic acids has not been reported, except for one example in which a boronic ester was reacted with Fmoc-Phe(4-Br)-OH.[13] We envisioned that structurally diverse Fmoc-protected Bip derivatives could be prepared via the direct coupling of the corresponding arylboronic acids with commercially available bromo- or iodo-Fmoc-Phe-OHs in a one-step SMC reaction. At relatively low temperature (<80 °C) and using mild conditions such as a nonaqueous organic solvent, formation of the des-Fmoc byproduct would be minimized. By eliminating the conventional Boc deprotection and Fmoc reprotection steps, both atom economy and step economy would be greatly increased. Furthermore, the resulting Fmoc-protected Bip derivatives could be directly incorporated into peptide sequences using the Fmoc SPPS strategy. As part of this approach, it was anticipated

Received: August 10, 2016
Published: September 12, 2016

DOI: 10.1021/acs.joc.6b01965
J. Org. Chem. 2016, 81, 9499−9506

Table 1. Examples of Reaction Conversion and des-Fmoc Side Product (H_2N-Phe(4-Br)−OH) in the Phosphine Ligand Screen of the SMC Reaction between 1a and 2a[a]



entry	ligand	solvent	product HPLC area %	3a/1a	3a/des-Fmoc of 1a
1	D*t*BuPF	THF	85.9[b]	97:3	100:0
2	D*t*BuPF	*t*-AmylOH	86.5[b]	97:3	100:0
1	DCyPF	THF	86.5[b]	98:2	98:2
4	DCyPF	*t*-AmylOH	81.5	91:9	100:0
5	CX-POMetB	THF	76.5	96:4	97:3
6	A-taPhos	THF	78.0	92:8	100:0
7	DCEPhos	THF	79.5	92:8	99:1
8	XPhos	*t*-AmylOH	76.0	90:10	100:0
9	DPPF	THF	0	0:100	0:100
10	DPPF	*t*-AmylOH	23.3	36:64	98:2
11	XantPhos	THF	0	0:100	0:100
12	XantPhos	*t*-AmylOH	50.0	73:27	99:1

[a]Conditions: **1a** (10 μmol), **2a** (15 μmol), $Pd(OAc)_2$ (0.45 μmol, 4.5 mol %), ligand (0.5 μmol, 10 mol %), di-*t*Bu-biphenyl (internal standard, 0.10 equiv), K_3PO_4 or CsF (30 μmol), THF or *t*-AmylOH (100 μL), 50 °C for 16 h. For details, see the Supporting Information. [b]Note that these reactions were repeated on a 1 mmol scale (and carried to isolation) in near quantitative yield.

that, under basic conditions, the free carboxylic acids might serve to protect the chiral center from racemization, which is often a concern with reactions of protected amino acids at elevated temperature. Herein, we report the direct solution-phase synthesis of Fmoc-Bip-OH derivatives by nonaqueous SMC of iodo- or bromo-Fmoc-Phe-OHs with various boronic acids.

At the initiation of this effort, we first screened the $Pd(OAc)_2$/ligand-catalyzed nonaqueous SMC reaction of Fmoc-Phe(4-Br)-OH (**1a**) and phenylboronic acid (**2a**) using K_3PO_4 or CsF as the base, and *t*-amylalcohol (*t*-AmylOH) or THF as the solvent (Table 1). Microscale high-throughput experimentation (HTE) techniques[27] were used to rapidly evaluate a series of electronically and sterically diverse phosphine ligands (10 μmol scale; see Chart 1 in the Supporting Information for details). The HTE revealed that K_3PO_4 was the preferred base, whereas CsF produced little or no desired product and the des-Fmoc starting bromide was observed for almost all the catalyst conditions that were examined. Of the 24 ligands evaluated, D*t*BuPF provided 97% conversion in both THF and *t*-AmylOH with <2% of the des-Fmoc side product (Table 1, entries 1 and 2). DCyPF gave excellent performance in THF (98% conversion), with slightly lower conversion in *t*-AmylOH (91%) (entries 3 and 4). There were several other ligand/solvent combinations, such as CX-POMetB/THF, A-taPhos/THF, DCEPhos/THF, and X-Phos/*t*-AmylOH which also gave good conversion (>90%) with little or no des-Fmoc side product detected (entries 5−8). Note that some low-performing ligands such as DPPF and XantPhos in *t*-AmylOH gave much better conversion as well as retention of the Fmoc group, whereas THF gave no desired product with total loss of the Fmoc group (entries 9−12).

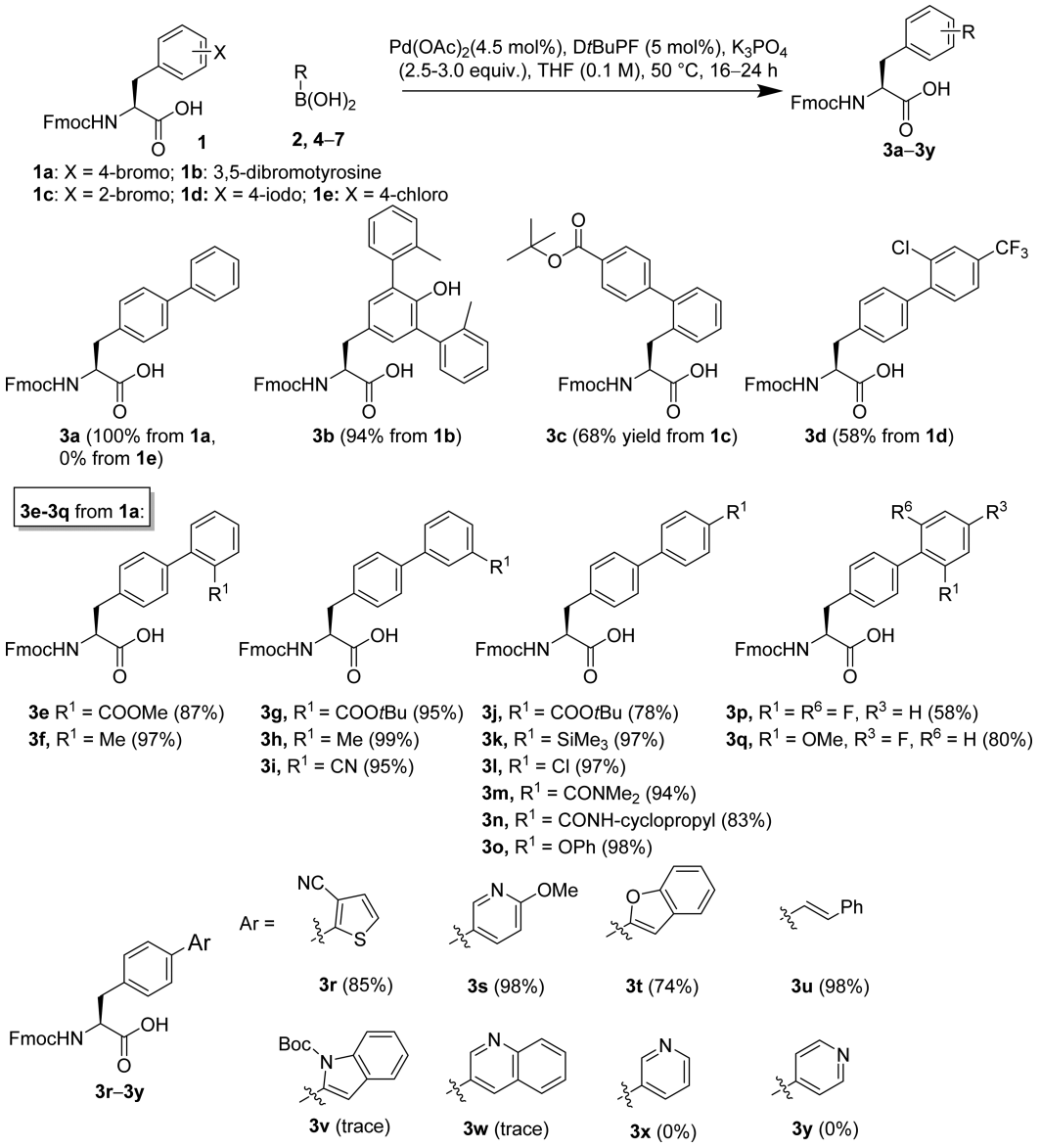
Importantly, the reactions for the four best ligand/solvent combinations (D*t*BuPF or DCyPF, K_3PO_4, and THF or *t*-AmylOH) were examined by chiral HPLC and showed no ee erosion. The three best results (entries 1−3 in Table 1) were repeated on a 1 mmol scale and isolated accordingly. Fmoc-Bip-OH (**3a**) was obtained in nearly quantitative yields using either DCyPF or D*t*BuPF while being heated in THF or *t*-AmylOH at 50 °C for 16 h.

After the more active ligand systems were identified, we surveyed a range of boronic acids and several Fmoc-halo-Phe-OHs to evaluate the scope of the reaction (Scheme 1). For simplicity, only the $Pd(OAc)_2$/D*t*BuPF catalyst system in THF was used. *para*-Bromo-Phe **1a**, 3,5-dibromo-Tyr **1b**, and *ortho*-bromo-Phe **1c** gave good to excellent yields of the 4-Bip analogue **3a**, the tyrosine derivative **3b**, and the *ortho*-biaryl analogue **3c**, respectively. The 4-iodo-Phe **1d** generated **3d** in 58% yield, while the corresponding chloride **1e** did not provide any of the desired **3a**. Phenylboronic acids substituted at the *ortho*, *meta*, or *para* position with either electron-withdrawing groups (e.g., compounds **3e**, **3g**, **3i**, **3j**, and **3l**−**n**) or electron-donating groups (e.g., compounds **3f**, **3h**, **3k**, and **3o**) gave good to excellent yields. Challenging coupling partners such as 2,6-difluoroboronic acid and 2-benzofuran boronic acid, which are believed to quickly deboronate under basic conditions, still gave **3p** and **3t** in 58 and 74% yield, respectively. Several heteroaryl boronic acids gave satisfactory yields to provide compounds such as **3r** and **3s**; the styrene analogue **3u** was also efficiently prepared. These monomeric amino acids were then used directly as building blocks in subsequent Fmoc SPPS peptide elongation to form 12- to 15-mer AA linear sequences, which were then cyclized to construct a variety of macrocyclic peptides containing Bip derivatives as potent inhibitors that disrupt specific protein−protein interactions. The synthesis and SAR of these Bip-containing cyclic peptides will be disclosed in due course.

While most of the substrates afforded the desired substituted Bips in good yields, as shown in Scheme 1, substrates **4**−**7** gave little or none of the desire products **3v**−**3y** in the $Pd(OAc)_2$/D*t*BuPF/K_3PO_4/THF system. Further ligand screening indicated that divergent catalyst systems worked for Boc-2-indole

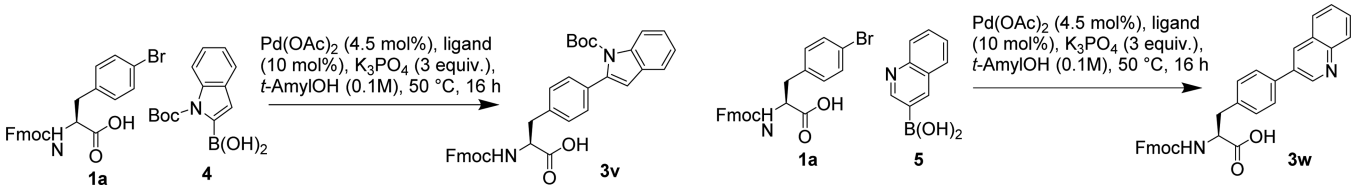
DOI: 10.1021/acs.joc.6b01965
J. Org. Chem. 2016, 81, 9499−9506

Scheme 1. Synthesis of Aryl/Heteroaryl-Substituted Phenylalanines[a,b]



[a]Reactions were run on 0.5 or 1.0 mmol scale. [b]Yields (%) are in parentheses and were not optimized.

Table 2. Examples of the Phosphine Ligand Screen for Substrates 4 and 5[a]



ligand	3v/1a	3v/des-Fmoc of 1a	ligand	3w/1a	3w/des-Fmoc of 1a
P(*o*-tol)$_3$	75:25	73:27	PPh_3	64:36	95:5
rac-BINAP	73:27	69:31	DPPF	56:44	93:7
DPEPhos	66:34	94:6	PXy_3	59:41	90:10
QPhos	63:37	60:40	DtBuPF	52:48	94:6

[a]Conditions: **1a** (10 μmol), **4** or **5** (15 μmol), $Pd(OAc)_2$ (0.45 μmol, 4.5 mol %), ligand (0.5 μmol, 10 mol %), biphenyl internal standard, 0.10 equiv), K_3PO_4 (30 μmol), *t*-AmylOH (100 μL), 50 °C for 16 h. For details, see the Supporting Information.

boronic acid **4** and 3-quinolineboronic acid **5** (see Supporting Information for details). Of the 24 ligands evaluated, *t*-AmylOH generally gave better yields than THF. Ligands such as DPEPhos, *rac*-BINAP, P(*o*-tol)$_3$, and QPhos provided **3v** in

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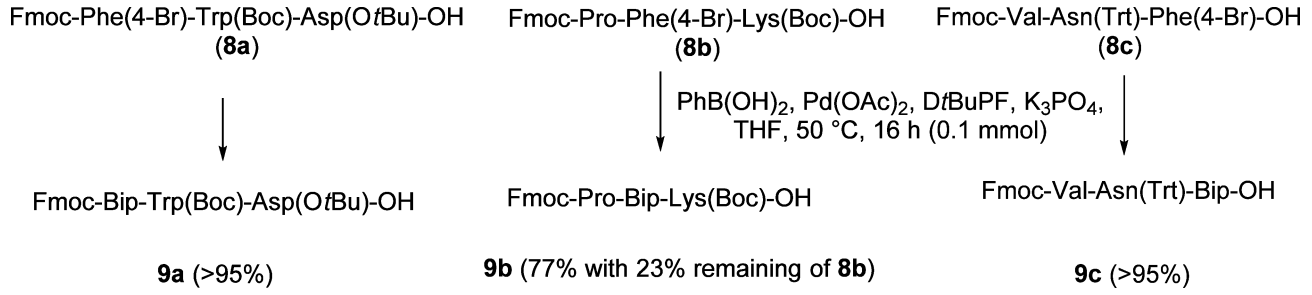
Scheme 2. Synthesis of Bip-Containing Tripeptides[a,b]

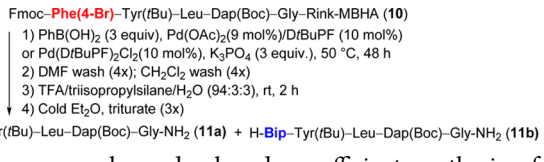


[a]Tripeptides **8a**–**8c** were prepared on chlorotrityl resin followed by cleavage from the resin using HFIP/CH_2Cl_2 (1:4) for 1 h (2×), concentration, and then hexanes/Et_2O trituration. [b]HPLC yields.

moderate yields, but only DPEPhos showed <10% of the des-Fmoc side product. Ligands such as PPh_3, DPPF, PXy_3, and D*t*BuPF worked for **5**, and all showed less than 10% of the des-Fmoc side product despite lower conversion (Table 2). 3-Pyridinylboronic acid (**6**) and 4-pyridinylboronic acid (**7**) did not give the desired products (**3x** and **3y**) with any of the ligands tested, presumably resulting from their instability and slow rate of transmetalation as stated in the literature.[28]

Having established the SMC reaction with the monomeric bromo- or iodo-Fmoc-Phe-OH, we next evaluated its extended application to modify 4-bromo-Phe-containing short peptides bearing a free COOH C-terminus and an Fmoc-protected N-terminus (e.g., compounds **8a**, **8b**, and **8c**) (Scheme 2). The biaryl-containing peptides that are generated can then be incorporated into Fmoc SPPS for peptide elongation or other modifications. Preliminary data suggested that the aforementioned general reaction conditions gave the desired tripeptides **9a**–**9c** in good yields regardless of the position of the 4-Br-Phe, that is, at the C-terminus, at the N-terminus, or in the middle of the peptide.

The above general catalyst system was also applied to the solid-phase synthesis of an Fmoc-protected pentapeptide on Rink resin (**10**, average loading 0.22 mmol/g). Preliminary results suggested that removal of the Fmoc group is significant, and that the reactions are generally much slower. After being heated at 50 °C for 48 h using the $Pd(OAc)_2$/D*t*BuPF/K_3PO_4/THF system, followed by cleavage from the resin (TFA/triisopropylsilane = 95:5), the reaction gave **11a**/**11b** = 3:1 (**11a**, des-Fmoc starting bromide; **11b**, des-Fmoc product) based on UV peak areas from HPLC analysis. Changing $Pd(OAc)_2$/D*t*BuPF to the air-stable $Pd(DtBuPF)_2Cl_2$ as well as replacing THF with DMF/H_2O (10:1) gave similar results and provided **11a**/**11b** = 2.7:1. Catalyst systems suitable for the solid-phase synthesis of Bips are currently being studied.



In summary, we have developed an efficient synthesis of Fmoc-protected Bip derivatives via the $Pd(OAc)_2$/D*t*BuPF-catalyzed nonaqueous SMC of bromo- or iodo-Fmoc-Phe-OHs with various boronic acids in good to excellent yields. Applying the SMC reaction to Fmoc-protected bromophenylalanine containing linear tripeptides also gave the desired Bip-containing tripeptides in high yields.

EXPERIMENTAL SECTION

General Methods. Commercial reagents were used as received unless otherwise stated. All reactions were performed under a nitrogen atmosphere. All reactions were monitored by LCMS using the following conditions: BEH C18 column = 1.7 μm (2.1 × 50 mm); solvent A = 100% water with 0.05% TFA; solvent B = 100% ACN with 0.05% TFA; gradient = 2–98% B over 1 min, then hold at 98% B for 0.5 min; flow = 0.8 mL/min, wavelength = 220 nm. All ^1H NMR and ^{13}C NMR spectra were recorded using DMSO-d_6 or MeOD as the solvent operating at frequencies as follows: 500 MHz for and 125 MHz for ^{13}C NMR. Spectra data are reported in the following format: chemical shift (multiplicity, coupling constants, and number of hydrogens). Chemical shifts are specified in parts per million downfield of a tetramethylsilane internal standard (δ units, tetramethylsilane = 0 ppm) and/or referenced to solvent peaks, which in ^1H NMR spectra appear at 2.49 ppm for CD_3SOCD_3, 3.30 ppm for CD_3OD, and which in ^{13}C NMR spectra appear at 39.7 ppm for CD_3SOCD_3, 49.0 ppm for CD_3OD, 77.0 ppm for $CDCl_3$, and 164.20 and 116.60 ppm for CF_3COOD. All ^{13}C NMR spectra were proton decoupled, that is, $^{13}C\{1H\}$NMR.

The purity of the compounds was checked using the following two HPLC conditions.

Method A: A linear gradient using solvent A (5% acetonitrile, 95% water, 0.05% TFA) and solvent B (95% acetonitrile, 5% water, 0.05% TFA); 10–100% of solvent B over 10 min and then 100% of solvent B over 5 min. Column: Sunfire C18 3.5 μm (3.0 × 150 mm); flow rate was 0.5 mL/min, and UV detection was set to 220 and 254 nm. The LC column was maintained at room temperature.

Method B: A linear gradient using solvent A (5% acetonitrile, 95% water, 0.05% TFA) and solvent B (95% acetonitrile, 5% water, 0.05% TFA); 10–100% of solvent B over 10 min and then 100% of solvent B over 5 min. Column: Xbridge phenyl 3.5 μm (3.0 × 150 mm); flow rate was 0.5 mL/min, and UV detection was set to 220 and 254 nm. The LC column was maintained at room temperature.

All of the compounds were confirmed for molecular weight using high-resolution MS (HRMS). An LTQ Orbitrap mass spectrometer in line with UPLC allowed collection of molecular ion data with accuracy of <5 ppm.

General Procedures for SMC Reactions in Scheme 1. To a N_2-flushed 20 mL scintillation vial equipped with a magnetic stir bar were added Fmoc-halo-Phe-OH (0.5 mmol), boronic acid (1.5–2.5 equiv), and anhydrous THF (6 mL). The suspension was degassed by bubbling N_2 into the vial for several minutes. Palladium(II) acetate (4.5 mol %), D*t*BuPF (5 mol %), and then anhydrous K_3PO_4 (2.5 equiv) were added. The suspension was degassed for several minutes, and then the vial was capped with a septum. The reaction mixture was stirred at 50 °C for 16 h. After being cooled, 20% citric acid was added to acidify the reaction. The organic layer was separated, and the aqueous layer was extracted with EtOAc (2×). Silica gel was added to the combined organic layers, and the mixture was concentrated to dryness. The residue was dry-loaded on a silica gel column (ISCO system) and eluted with hexanes/EtOAc to give the desired product. Sometimes for compounds which are tailing in a hexanes/EtOAc

DOI: 10.1021/acs.joc.6b01965
J. Org. Chem. 2016, 81, 9499–9506

system, further eluting with MeOH/CH_2Cl_2 is also needed. Small portions of the sample can be further purified by preparative supercritical fluid chromatography (SFC) (MeOH/CO_2) or reversed-phase HPLC (CH_3CN/H_2O/TFA), using conditions that were optimized for characterization and purity considerations. SFC general conditions: the preparative SFC was achieved on a Chiralpak AS-H column, 30 × 250 mm, 5 μm. The mobile phase was composed of 45% methanol in CO_2. The flow rate was set at 85 mL/min, system temperature at 40 °C, and the column back pressure at 100 bar.

The SMC reactions of the tripeptides in Scheme 2 were performed similarly according to those in Scheme 1, but on a 0.1 mmol scale.

Compounds **3a**, **3f**,[22] **3h**,[22] and **3l** are known compounds.

*(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-3-[4-hydroxy-3,5-bis(2-methylphenyl)phenyl]propanoic Acid (**3b**):* 94% yield (274 mg); off-white solids; ^{1}H NMR (500 MHz, methanol-d_4) δ 7.77 and 7.68 (d, J = 7.1 Hz, total 1H), 7.75 and 7.68 (d, J = 7.0 Hz, total 1H), 7.60 and 78.53 (d, J = 7.3 Hz, total 1H), 7.59 and 7.50 (d, J = 7.4 Hz, total 1H), 7.36 (td, J = 7.6, 2.4 Hz, 2H), 7.30−7.04 (m, 10H), 6.96 (s, 2H), 4.45 (dd, J = 9.0, 4.8 Hz, 1H), 4.30 (t, J = 8.5 Hz, 1H), 4.24 (dd, J = 10.4, 7.2 Hz, 1H), 4.14 (t, J = 7.1 Hz, 1H), 3.18 (dd, J = 13.9, 4.8 Hz, 1H), 2.95 and 2.81 (dd, J = 14.0, 9.0 Hz, total 1H), 2.16 (s, 6H); ^{13}C NMR (126 MHz, methanol-d_4) δ 175.17, 158.36, 150.74, 145.24, 145.19, 142.54, 142.51, 139.69, 138.33, 131.87, 131.35, 130.90, 130.82, 129.55, 128.76, 128.74, 128.53, 128.19, 128.17, 126.69, 126.29, 126.21, 120.89, 120.87, 68.08, 56.97, 48.39, 37.92, 20.24; ESI-HRMS calcd for $C_{38}H_{34}NO_5$ $[M + H]^+$ 584.24315, found 584.24322, mass difference 0.12 ppm. Orthogonal HPLC purity: 100%, retention time = 12.18 min (Method A); 100%, retention time = 11.47 min (Method B).

*(2S)-3-(2-{4-[(tert-Butoxy)carbonyl]phenyl}phenyl)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)propanoic Acid (**3c**):* 68% yield (192 mg); off-white solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.52 (s, 1H), 7.92 (d, J = 8.3 Hz, 2H), 7.87 (d, J = 7.5 Hz, 2H), 7.63 (dd, J = 7.5, 3.1 Hz, 2H), 7.60 (d, J = 8.7 Hz, 1H), 7.46−7.36 (m, 5H), 7.35−7.24 (m, 4H), 7.17−7.09 (m, 1H), 4.20−4.09 and 4.10−4.03 (m, total 3H), 3.97 (ddd, J = 10.5, 8.6, 4.8 Hz, 1H), 3.14 (dd, J = 14.2, 4.7 Hz, 1H), 2.82 (dd, J = 14.3, 10.5 Hz, 1H), 1.54, and 1.47 (s, total 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.97, 164.76, 155.72, 145.47, 143.69, 140.95, 140.64, 140.61, 134.83, 130.15, 129.86, 129.54, 129.26, 128.90, 127.61, 127.56, 126.97, 126.52, 125.23, 125.18, 120.04, 80.68, 65.52, 54.09, 46.50, 33.77, 27.75; ESI-HRMS calcd for $C_{35}H_{34}NO_6$ $[M + H]^+$ 564.23806, found 564.23837, mass difference 0.542 ppm. Orthogonal HPLC purity: 100%, retention time = 13.18 min (Method A); 100%, retention time = 11.85 min (Method B).

*(2S)-3-{4-[2-Chloro-4-(trifluoromethyl)phenyl]phenyl}-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)propanoic Acid (**3d**):* 58% yield (163 mg); off-white solids; ^{1}H NMR (500 MHz, methanol-d_4) δ 7.80−7.71 (m, 3H), 7.64−7.51 (m, 3H), 7.49−7.28 (m, 8H), 7.30−7.16 (m, 3H), 4.50 (dd, J = 9.8, 4.6 Hz, 1H), 4.32 (dd, J = 10.5, 6.9 Hz, 1H), 4.19 (dd, J = 10.5, 7.2 Hz, 1H), 4.11 (t, J = 7.1 Hz, 1H), 3.43−3.21 (m, 1H), 3.00 (dd, J = 13.8, 9.9 Hz, 1H); ^{13}C NMR (126 MHz, methanol-d_4) δ 174.93, 158.39, 145.51, 145.31, 145.13, 142.56, 142.52, 139.21, 137.72, 134.23, 133.28, 131.77 (q, J = 33.1 Hz), 130.37, 130.32, 128.76, 128.13 (d, J = 1.7 Hz), 127.82 (q, J = 3.9 Hz), 126.37, 126.24, 124.88 (q, J = 271.6 Hz), 124.86 (d, J = 4.0 Hz), 120.89, 120.86, 68.01, 56.65, 48.36, 38.39; ESI-HRMS calcd for $C_{31}H_{24}ClF_3NO_4$ $[M + H]^+$ 566.13467, found 566.13488, mass difference 1.471 ppm. Orthogonal HPLC purity: 100%, retention time = 13.05 min (Method A); 100%, retention time = 11.81 min (Method B).

*(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-3-{4-[2-(methoxycarbonyl)phenyl]phenyl}propanoic Acid (**3e**):* 87% yield (226 mg); off-white solids; ^{1}H NMR (500 MHz, methanol-d_4) δ 7.73 (d, J = 7.3 Hz, 2H), 7.69 (d, J = 7.7 Hz, 1H), 7.61−7.52 (m, 2H), 7.51−7.41 (m, 1H), 7.36 (t, J = 7.4 Hz, 1H), 7.32 (t, J = 7.2 Hz, 2H), 7.29−7.19 (m, 5H), 7.13 (d, J = 7.7 Hz, 2H), 4.47 (dd, J = 9.4, 4.7 Hz, 1H), 4.28 (dd, J = 10.7, 6.9 Hz, 1H), 4.21 (dd, J = 10.7, 6.8 Hz, 1H), 4.11 (q, J = 7.0, 6.4 Hz, 1H), 3.50 (d, J = 2.9 Hz, 3H), 3.23 (dd, J = 13.8, 4.6 Hz, 1H), 2.98 and 2.78 (dd, J = 13.8, 9.2 Hz, 1H); ^{13}C NMR (126 MHz, methanol-d_4) δ 171.12, 158.29, 145.19, 145.18, 143.26, 142.50, 140.96, 137.77, 132.38, 132.35, 131.58, 130.50, 130.17, 129.37, 128.73, 128.17, 128.12, 126.28, 126.21, 120.88, 120.86, 67.91, 56.79, 52.43, 48.32, 38.31; ESI-HRMS calcd for $C_{32}H_{28}NO_6$ $[M + H]^+$ 522.19111, found 522.19232, mass difference 2.319 ppm. Orthogonal HPLC purity: 92.0%, retention time = 11.55 min (Method A); 91.7%, retention time = 10.86 min (Method B).

*(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-3-[4-(2-methylphenyl)phenyl]propanoic Acid (**3f**):* 97% yield (231 mg); off-white solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.88 (s, 1H), 7.87 (d, J = 7.5 Hz, 2H), 7.70 (d, J = 8.5 Hz, 1H), 7.66 (d, J = 7.5 Hz, 1H), 7.63 (d, J = 7.5 Hz, 1H), 7.52 (d, J = 7.9 Hz, 2H), 7.39 (q, J = 7.6 Hz, 4H), 7.35−7.24 (m, 5H), 7.13 (d, J = 7.4 Hz, 1H), 4.31−3.97 (m, 4H), 3.12 and 3.05 (dd, J = 13.7, 4.4 Hz, total 1H), 2.91 and 2.79 (dd, J = 13.8, 10.4 Hz, 1H), 2.34 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.22, 155.86, 143.73, 143.69, 140.63, 140.61, 139.91, 138.26, 137.90, 137.19, 129.64, 128.70, 127.80, 127.55, 127.53, 127.11, 126.99, 126.37, 125.26, 125.19, 123.55, 120.03, 65.56, 55.48, 46.54, 36.14, 21.06; ESI-HRMS calcd for $C_{31}H_{28}NO_4$ $[M + H]^+$ 478.20128, found 478.20178, mass difference 1.035 ppm. Orthogonal HPLC purity: 98.9%, retention time = 12.31 min (Method A); 98.6%, retention time = 11.32 min (Method B).

*(2S)-3-(4-{3-[(tert-Butoxy)carbonyl]phenyl}phenyl)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)propanoic Acid (**3g**):* 85% yield (240 mg); off-white solids; mp 83−93 °C (amorphous); $[\alpha]_D^{20}$ −7.20 (c 1, DMF), $[\alpha]_D^{20}$ 11.46 (c 0.34, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (t, J = 1.8 Hz, 1H), 7.86 (dd, J = 7.7, 1.4 Hz, 3H), 7.83 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 7.7 Hz, 1H), 7.63 (d, J = 7.5 Hz, 1H), 7.58−7.48 (m, 3H), 7.41−7.35 (m, 2H), 7.31 (d, J = 7.8 Hz, 2H), 7.30−7.23 (m, 2H), 4.31−4.10 (m, 4H), 4.05 (td, J = 8.2, 4.5 Hz, 1H), 3.13 and 2.9 (dd, J = 13.6, 4.5 Hz, total 1H), 2.94 and 2.76 (dd, J = 13.6, 8.7 Hz, total 1H), 1.56 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.91, 164.84, 155.50, 143.83, 143.79, 140.64, 140.44, 138.55, 136.88, 131.95, 130.86, 130.03, 129.20, 127.65, 127.50, 126.97, 126.74, 126.28, 125.21, 125.14, 120.02, 80.84, 65.34, 56.03, 46.63, 36.52, 27.76; ESI-HRMS calcd for $C_{35}H_{37}N_2O_6$ $[M + NH_4]^+$ 581.26461, found at 581.26474, mass difference 0.218 ppm. Orthogonal HPLC purity: 100%, retention time = 13.10 min (Method A); 100%, retention time = 11.72 min (Method B).

*(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-3-[4-(3-methylphenyl)phenyl]propanoic Acid (**3h**):* 99% yield (237 mg); off-white solids; ^{1}H NMR (500 MHz, methanol-d_4) δ 7.74 (d, J = 7.5 Hz, 2H), 7.57 and 7.50 (dd, J = 7.8, 2.6 Hz, total 2H), 7.33 (t, J = 7.6 Hz, 2H), 7.27 (d, J = 7.9 Hz, 2H), 7.26−7.21 (m, 2H), 7.19−7.10 (m, 5H), 7.03 (d, J = 7.0 Hz, 1H), 4.49 (dd, J = 9.7, 4.7 Hz, 1H), 4.29 (dd, J = 10.5, 7.0 Hz, 1H), 4.17 (dd, J = 10.5, 7.2 Hz, 1H), 4.10 (t, J = 7.0 Hz, 1H), 3.26 and 3.03 (dd, J = 13.9, 4.7 Hz, total 1H), 2.97 and 2.81 (dd, J = 13.9, 9.7 Hz, total 1H), 2.14 and 2.11 (s, total 3H); ^{13}C NMR (126 MHz, methanol-d_4) δ 175.12, 158.35, 145.23, 145.15, 142.92, 142.52, 142.49, 141.77, 137.20, 136.24, 131.21, 130.62, 130.20, 130.11, 128.72, 128.18, 128.11, 126.72, 126.32, 126.21, 120.86, 120.84, 67.99, 56.77, 48.31, 38.37, 20.57; ESI-HRMS calcd for $C_{31}H_{28}NO_4$ $[M + H]^+$ 478.20128, found 478.20187, mass difference 1.224 ppm. Orthogonal HPLC purity: 96.9%, retention time = 12.22 min (Method A); 96.4%, retention time = 11.34 min (Method B).

*(2S)-3-[4-(3-Cyanophenyl)phenyl]-2-({[(9H-fluoren-9-yl)-methoxy]carbonyl}amino)propanoic Acid (**3i**):* 95% yield (232 mg); off-white solids; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.75 (d, J = 7.8 Hz, 4H), 7.61 (d, J = 7.7 Hz, 1H), 7.55 (d, J = 8.0 Hz, 2H), 7.51 (t, J = 8.2 Hz, 1H), 7.46 (d, J = 8.2 Hz, 2H), 7.41−7.29 (m, 4H), 7.24 (td, J = 7.5, 1.2 Hz, 1H), 7.22 (td, J = 7.7, 1.2 Hz, 1H), 4.56−4.43 (m, 1H), 4.28 (dd, J = 10.6, 6.9 Hz, 1H), 4.14 (dd, J = 10.5, 7.2 Hz, 1H), 4.04 (t, J = 7.1 Hz, 1H), 3.28 and 3.03 (d, J = 8.9 Hz, total 1H), 2.97 and 2.79 (dd, J = 13.9, 9.9 Hz, 1H); ^{13}C NMR (101 MHz, methanol-d_4) δ 175.03, 158.34, 145.28, 145.09, 143.41, 142.49, 139.14, 138.44, 132.46, 131.67, 131.26, 131.21, 130.90, 128.73, 128.11, 128.06, 126.36, 126.19, 120.91, 119.71, 113.86, 68.01, 56.62, 48.32, 38.38; ESI-HRMS calcd for $C_{31}H_{25}N_2O_4$ $[M + H]^+$ 489.18088, found 489.18145, mass difference 1.158 ppm. Orthogonal HPLC purity: 100%, retention time = 10.95 min (Method A); 100%, retention time = 10.54 min (Method B).

DOI: 10.1021/acs.joc.6b01965
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*(2S)-3-(4-{4-[(tert-Butoxy)carbonyl]phenyl}phenyl)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)propanoic Acid (**3j**):* 78% yield (439 mg); colorless solids; mp 115−125 (amorphous); $[\alpha]_D^{20}$ 2.48 (*c* 1, DMF), $[\alpha]_D^{20}$ 23.65 (*c* 0.42, MeOH); ^{1}H NMR (400 MHz, methanol-d_4) δ 7.94 (d, *J* = 8.3 Hz, 2H), 7.74 (d, *J* = 7.6 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 4H), 7.51 (d, *J* = 8.1 Hz, 2H), 7.38−7.28 (m, 4H), 7.28−7.17 (m, 2H), 4.56−4.38 (m, 1H), 4.29 (dd, *J* = 10.5, 7.0 Hz, 1H), 4.17 (dd, *J* = 10.5, 7.1 Hz, 1H), 4.08 (t, *J* = 7.0 Hz, 1H), 3.29−3.21 (m, 1H), 2.98 and 2.80 (dd, *J* = 13.8, 9.6 Hz, total 1H), 1.59 (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 175.16, 167.27, 158.31, 146.33, 145.20, 145.14, 142.50, 139.51, 138.88, 131.64, 131.03, 130.83, 128.71, 128.15, 128.10, 127.69, 126.30, 126.20, 120.85, 82.26, 67.96, 56.74, 48.32, 38.37, 28.46; ESI-HRMS calcd for $C_{35}H_{34}NO_6$ $[M + H]^+$ 564.23806, found 564.23896, mass difference 1.588 ppm. Orthogonal HPLC purity: 95.2%, retention time = 13.01 min (Method A); 94.1%, retention time = 11.63 min (Method B).

*(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-3-{4-[4-(trimethylsilyl)phenyl]phenyl}propanoic Acid (**3k**):* 97% yield (260 mg); off-white solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.77 (s, 1H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 1H), 7.65 (dd, *J* = 11.7, 7.6 Hz, 2H), 7.60−7.52 (m, 6H), 7.44−7.33 (m, 4H), 7.30 (td, *J* = 7.4, 1.1 Hz, 1H), 7.26 (td, *J* = 7.4, 1.1 Hz, 1H), 4.33−3.97 (m, 4H), 3.12 and 3.06 (dd, *J* = 13.8, 4.4 Hz, total 1H), 2.91 and 2.80 (dd, *J* = 13.8, 10.5 Hz, total 1H), 0.26 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.21, 155.91, 143.69, 140.63, 140.39, 138.40, 138.12, 137.37, 133.72, 129.69, 127.55, 127.54, 126.98, 126.40, 125.82, 125.22, 125.19, 120.03, 65.55, 55.40, 46.53, 39.50, 36.04, −1.15; ESI-HRMS calcd for $C_{33}H_{34}NO_4Si$ $[M + H]^+$ 536.22579, found 536.22578. Orthogonal HPLC purity: 100%, retention time = 11.26 min (Method A); 97.6%, retention time = 10.69 min (Method B).

*(2S)-3-[4-(4-Chlorophenyl)phenyl]-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)propanoic Acid (**3l**):* 97% yield (242 mg); off-white solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.87 (d, *J* = 7.6 Hz, 2H), 7.70−7.58 (m, 4H), 7.58−7.52 (m, 2H), 7.47 (d, *J* = 8.5 Hz, 2H), 7.40 (d, *J* = 6.4 Hz, 1H), 7.37 (d, *J* = 7.1 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.29 (td, *J* = 7.8, 1.4 Hz, 1H), 7.26 (td, *J* = 7.5, 1.2 Hz, 1H), 4.45−3.86 (m, 4H), 3.12 and 3.03 (dd, *J* = 13.8, 4.4 Hz, total 1H), 2.91 and 2.79 (dd, *J* = 13.8, 10.4 Hz, total 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.16, 155.84, 143.70, 140.61, 138.72, 137.79, 136.74, 132.03, 129.79, 128.77, 128.16, 127.55, 126.98, 126.31, 125.22, 125.18, 120.03, 65.53, 55.47, 46.54, 36.12; ESI-HRMS calcd for $C_{30}H_{25}ClNO_4$ $[M + H]^+$ 498.14666, found 498.14724, mass difference 1.159 ppm. Orthogonal HPLC purity: 97.1%, retention time = 12.60 min (Method A); 96.9%, retention time = 11.60 min (Method B).

*(2S)-3-{4-[4-(Dimethylcarbamoyl)phenyl]phenyl}-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)propanoic Acid (**3m**):* 94% yield (251 mg); off-white solids; ^{1}H NMR (500 MHz, methanol-d_4) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.66−7.54 (m, 4H), 7.51 (d, *J* = 8.2 Hz, 2H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.38−7.30 (m, 4H), 7.29−7.19 (m, 2H), 4.48 (dd, *J* = 9.6, 4.7 Hz, 1H), 4.31 (dd, *J* = 10.4, 6.9 Hz, 1H), 4.16 (dd, *J* = 10.5, 7.3 Hz, 1H), 4.08 (t, *J* = 7.0 Hz, 1H), 3.30−3.23 (m, 1H), 3.10 (s, 3H), 3.01 (s, 3H), 3.00−2.92 (m, 1H); ^{13}C NMR (126 MHz methanol-d_4) δ 175.30, 173.67, 158.32, 145.30, 145.17, 143.77, 142.54, 142.51, 139.69, 138.68, 135.81, 131.06, 128.73, 128.62, 128.13, 128.12, 128.05, 127.88, 126.38, 126.22, 120.86, 120.84, 67.97, 56.86, 40.10, 38.44, 35.70; ESI-HRMS calcd for $C_{33}H_{31}N_2O_5$ $[M + H]^+$ 535.22275, found 535.22214, mass difference −1.137 ppm. Orthogonal HPLC purity: 100%, retention time = 9.48 min (Method A); 100%, retention time = 9.32 min (Method B).

*(2S)-3-{4-[4-(Cyclopropylcarbamoyl)phenyl]phenyl}-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)propanoic Acid (**3n**):* 83% yield (228 mg); off-white solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.78 (s, 1H), 8.44 (d, *J* = 4.2 Hz, 1H), 7.87 (d, *J* = 8.5 Hz, 2H), 7.86 (d, *J* = 7.1 Hz, 2H), 7.75 (d, *J* = 8.6 Hz, 1H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.65 and 7.52 (d, *J* = 7.7 Hz, total 1H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.62 and 7.48 (d, *J* = 8.2 Hz, total 1H), 7.42−7.32 (m, 4H), 7.29 (td, *J* = 7.5, 1.2 Hz, 1H), 7.26 (td, *J* = 7.5, 1.1 Hz, 1H), 4.19 (tq, *J* = 13.6, 6.6 Hz, 4H), 3.13 and 3.07 (dd, *J* = 13.8, 4.4 Hz, total 1H), 2.92 and 2.79 (dd, *J* = 13.8, 10.6 Hz, total 1H), 2.85 (tt, *J* = 7.9, 3.9 Hz, 1H), 0.69 (td, *J* = 7.1, 4.7 Hz, 2H), 0.61−0.52 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.20, 167.04, 155.91, 143.70, 142.31, 140.62, 137.92, 137.16, 133.00, 129.75, 127.75, 127.56, 127.54, 126.98, 126.56, 126.10, 125.22, 125.18, 120.03, 65.56, 55.37, 46.53, 36.05, 23.03, 5.72; ESI-HRMS calcd for $C_{34}H_{31}N_2O_5$ $[M + H]^+$ 547.22274, found 547.22338, mass difference 1.154 ppm. Orthogonal HPLC purity: 96.8%, retention time = 9.59 min (Method A); 96.1%, retention time = 9.40 min (Method B).

*(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-3-[4-(4-phenoxyphenyl)phenyl]propanoic Acid (**3o**):* 98% yield (272 mg); off-white solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.80 (s, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.73 (d, *J* = 8.5 Hz, 1H), 7.65 (d, *J* = 7.7 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.7 Hz, 1H), 7.56−7.49 (m, 2H), 7.45−7.36 (m, 4H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.30 (td, *J* = 8.3, 7.9, 1.5 Hz, 1H), 7.26 (td, *J* = 7.2, 1.2 Hz, 1H), 7.16 (tt, *J* = 7.4, 1.2 Hz, 1H), 7.08−7.00 (m, 4H), 4.40−3.90 (m, 4H), 3.12 and 3.05 (dd, *J* = 13.8, 4.4 Hz, total 1H), 2.91 and 2.79 (dd, *J* = 13.8, 10.5 Hz, total 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.24, 156.45, 156.16, 155.90, 143.70, 140.62, 137.47, 136.96, 135.10, 130.05, 129.68, 128.02, 127.56, 126.99, 126.16, 125.23, 125.20, 123.57, 120.03, 118.78, 65.57, 55.42, 46.54, 36.06; ESI-HRMS calcd for $C_{36}H_{30}NO_5$ $[M + H]^+$ 556.21184, found 556.21212, mass difference 0.486 ppm. Orthogonal HPLC purity: 97.4%, retention time = 12.98 min (Method A); 96.3%, retention time = 11.94 min (Method B).

*(2S)-3-[4-(2,6-Difluorophenyl)phenyl]-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)propanoic Acid (**3p**):* 58% yield (145 mg); off-white solids; ^{1}H NMR (500 MHz, methanol-d_4) δ 7.75 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 6.3 Hz, 7H), 7.29−7.10 (m, 2H), 7.01 (t, *J* = 7.9 Hz, 2H), 4.47 (dd, *J* = 9.4, 4.8 Hz, 1H), 4.31 (dd, *J* = 10.5, 7.1 Hz, 1H), 4.23 (dd, *J* = 10.6, 7.0 Hz, 1H), 4.15 (t, *J* = 7.2 Hz, 1H), 3.26 and 3.16 (dd, *J* = 13.9, 4.8 Hz, total 1H), 3.01 and 2.89 (dd, *J* = 13.9, 9.4 Hz, total 1H); ^{13}C NMR (126 MHz, methanol-d_4) δ 175.10, 161.43 (dd, *J* = 247.2, 7.1 Hz), 158.39, 145.23, 142.53, 138.95, 132.37 (d, *J* = 20.0 Hz), 131.36 (t, *J* = 2.0 Hz), 130.42 (t, *J* = 10.4 Hz), 130.28, 128.85, 128.70, 128.10, 126.29, 126.21, 120.84, 119.41 (t, *J* = 19.0 Hz), 112.67 (d, *J* = 26.9 Hz), 112.66 (d, *J* = 14.4 Hz), 67.96, 56.72, 48.35, 38.30; ESI-HRMS calcd for $C_{30}H_{24}F_2NO_4$ $[M + H]^+$ 500.16741, found 500.16735, mass difference 1.117 ppm. Orthogonal HPLC purity: 93.4%, retention time = 10.35 min (Method A); 93.5%, retention time = 9.82 min (Method B).

*(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-3-[4-(4-fluoro-2-methoxyphenyl)phenyl]propanoic Acid (**3q**):* 80% yield (204 mg); colorless solids; ^{1}H NMR (500 MHz, methanol-d_4) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.60 and 7.55 (d, *J* = 7.4 Hz, total 1H), 7.59 and 7.45 (d, *J* = 7.5 Hz, total 1H), 7.36 (d, *J* = 7.4 Hz, 1H), 7.34 (d, *J* = 7.4 Hz, 1H), 7.31−7.21 (m, 6H), 7.12 (dd, *J* = 8.4, 6.8 Hz, 1H), 6.78 (dd, *J* = 11.2, 2.5 Hz, 1H), 6.65 (td, *J* = 8.3, 2.5 Hz, 1H), 4.43−4.16 (m, 2H), 4.16−3.96 (m, 2H), 3.68 and 3.62 (s, total 3H), 3.24 and 3.04 (dd, *J* = 13.7, 4.7 Hz, 1H), 2.97 and 2.77 (dd, *J* = 13.7, 8.5 Hz, total 1H); ^{13}C NMR (126 MHz, methanol-d_4) δ 177.28, 164.36 (d, *J* = 243.9 Hz), 159.13 (d, *J* = 9.8 Hz), 158.04, 145.38, 145.29, 142.54, 142.52, 132.43 (d, *J* = 9.8 Hz), 130.34, 130.16, 128.70, 128.13 (d, *J* = 4.0 Hz), 126.45, 126.27, 120.83 (d, *J* = 3.3 Hz), 107.75 (d, *J* = 21.3 Hz), 100.42 (d, *J* = 25.9 Hz), 67.89, 58.13, 56.17, 48.40, 39.12; ESI-HRMS calcd for $C_{31}H_{27}FNO_5$ $[M + H]^+$ 512.18740, found 512.18647, mass difference −0.601 ppm. Orthogonal HPLC purity: 100%, retention time = 11.78 min (Method A); 98.2%, retention time = 11.08 min (Method B).

*(2S)-3-[4-(3-Cyanothiophen-2-yl)phenyl]-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)propanoic Acid (**3r**):* 85% yield (210 mg); colorless solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.94 (d, *J* = 3.9 Hz, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.60−7.57 (m, 2H), 7.44−7.35 (m, 2H), 7.34−7.15 (m, 5H), 4.30−4.11 (m, 3H), 4.07 and 3.94 (td, *J* = 8.2, 7.5, 4.2 Hz, total 1H), 3.13 (dd, *J* = 13.6, 4.5 Hz, 1H), 2.91 and 2.77 (dd, *J* = 13.6, 9.1 Hz, total 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.84, 155.52, 151.31, 143.80, 143.75, 140.63, 140.52, 140.17, 130.27, 129.51, 127.52, 126.97, 125.80, 125.20, 125.14, 124.07, 120.03, 120.01, 114.41, 106.15, 65.37, 55.87, 46.60, 36.63; ESI-HRMS calcd for $C_{29}H_{23}N_2O_4S$ $[M + H]^+$ 495.13730, found 495.13664, mass difference −1.342 ppm. Orthogonal HPLC purity: 97.9%, retention time = 11.78 min (Method A); 98.2%, retention time = 11.08 min (Method B).

DOI: 10.1021/acs.joc.6b01965
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*(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-3-[4-(6-methoxypyridin-3-yl)phenyl]propanoic Acid (**3s**):* 98% yield (242 mg); off-white solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.43 (d, J = 2.5 Hz, 1H), 7.94 (dd, J = 8.6, 2.6 Hz, 1H), 7.86 (d, J = 7.6 Hz, 2H), 7.65 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.54 (d, J = 8.0 Hz, 2H), 7.39 (t, J = 7.2 Hz, 1H), 7.38 (t, J = 7.2 Hz, 1H), 7.34 (d, J = 8.0 Hz, 2H), 7.29 (t, J = 7.5 Hz, 1H), 7.26 (t, J = 7.1 Hz, 1H), 6.87 (d, J = 8.6 Hz, 1H), 4.37−4.01 (m, 4H), 3.88 (s, 3H), 3.11 and 3.02 (dd, J = 13.7, 4.4 Hz, total 1H), 2.91 and 2.78 (dd, J = 13.8, 10.3 Hz, total 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.18, 162.87, 155.85, 144.39, 143.70, 140.61, 137.30, 134.93, 129.79, 129.06, 127.54, 126.98, 125.99, 125.22, 125.18, 120.03, 110.47, 65.53, 55.50, 53.19, 46.54, 36.08; ESI-HRMS calcd for $C_{30}H_{27}N_2O_5$ $[M + H]^+$ 495.19145, found 495.19231, mass difference 1.740 ppm. Orthogonal HPLC purity: 100%, retention time = 13.86 min (Method A); 99.0%, retention time = 12.36 min (Method B).

*(2S)-3-[4-(1-Benzofuran-2-yl)phenyl]-2-({[(9H-fluoren-9-yl)-methoxy]carbonyl}amino)propanoic Acid (**3t**):* 74% yield (187 mg); off-white solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (d, J = 7.5 Hz, 2H), 7.82−7.76 (m, 2H), 7.65−7.62 (m, 3H), 7.60 and 7.55 (dd, J = 8.1, 0.9 Hz, total 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.39−7.33 (m, 5H), 7.33−7.21 (m, 4H), 4.31−3.99 (m, 4H), 3.14 and 2.98 (dd, J = 13.7, 4.5 Hz, total 1H), 2.93 and 2.79 (dd, J = 13.7, 9.6 Hz, total 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.01, 155.69, 155.33, 154.11, 143.78, 143.71, 140.62, 139.33, 129.85, 128.85, 127.71, 127.52, 127.51, 126.97, 125.19, 125.15, 124.40, 124.35, 123.12, 120.98, 120.01, 110.99, 101.38, 65.47, 55.67, 46.57, 36.54; ESI-HRMS calcd for $C_{32}H_{25}NO_5$ $[M]^+$ 503.17272, found 503.17314, mass difference 0.83 ppm. Orthogonal HPLC purity: 100%, retention time = 12.44 min (Method A); 100%, retention time = 11.41 min (Method B).

*(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}amino)-3-{4-[(E)-2-phenylethenyl]phenyl}propanoic Acid (**3u**):* 98% yield (240 mg); off-white solids; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.86 (d, J = 7.4 Hz, 2H), 7.64 (d, J = 7.0 Hz, 1H), 7.63 (d, J = 7.0 Hz, 1H), 7.57 (d, J = 6.9 Hz, 2H), 7.47 (d, J = 8.1 Hz, 2H), 7.43−7.27 (m, 6H), 7.27−7.21 (m, 3H), 7.18 (d, J = 7.8 Hz, 2H), 4.28−4.14 (m, 3H), 4.09 (td, J = 8.5, 7.8, 4.6 Hz, 1H), 3.09 (dd, J = 13.7, 4.5 Hz, 1H), 2.96−2.82 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.99, 155.63, 143.79, 143.74, 140.62, 138.01, 137.10, 134.94, 129.55, 128.63, 128.28, 127.66, 127.53, 127.43, 127.01, 126.32, 126.16, 125.23, 125.17, 120.02, 65.46, 55.79, 46.58, 36.52; ESI-HRMS calcd for $C_{32}H_{28}NO_4$ $[M + H]^+$ 490.20128, found 490.20099, mass difference −0.601 ppm. Orthogonal HPLC purity: 100%, retention time = 12.33 min (Method A); 100%, retention time = 11.39 min (Method B).

*(2S)-4-(tert-Butoxy)-2-[(2S)-3-{1-[(tert-butoxy)carbonyl]-1H-indol-3-yl}-2-[(2S)-2-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-3-(4-phenylphenyl)propanamido]propanamido]-4-oxobutanoic Acid (**9a**):* ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.45 (d, J = 8.1 Hz, 1H), 8.19 (d, J = 8.2 Hz, 1H), 8.02 (d, J = 8.3 Hz, 1H), 7.87−7.80 (m, 2H), 7.70 (d, J = 7.7 Hz, 1H), 7.63−7.57 (m, 2H), 7.53 (dd, J = 15.0, 7.3 Hz, 4H), 7.46 (d, J = 7.9 Hz, 2H), 7.44−7.11 (m, 10H), 4.71 (q, J = 8.0, 7.0 Hz, 1H), 4.60 (dt, J = 8.0, 6.6 Hz, 1H), 4.30−4.23 (m, 1H), 4.20−4.13 (m, 1H), 4.09 (q, J = 7.5, 6.6 Hz, 2H), 3.15 (dd, J = 14.9, 4.7 Hz, 1H), 3.04−2.92 (m, 2H), 2.82−2.72 (m, 1H), 2.69 (dd, J = 16.1, 6.2 Hz, 1H), 2.60−2.51 (m, 1H), 1.54 (s, 9H), 1.36 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.89, 171.24, 170.69, 169.11, 162.27, 155.58, 148.97, 143.63, 140.61, 139.93, 137.23, 134.68, 130.31, 129.71, 128.76, 127.50, 127.11, 126.95, 126.36, 126.20, 125.17, 124.15, 122.32, 119.95, 119.31, 116.04, 114.53, 83.26, 80.35, 65.65, 56.24, 52.05, 48.77, 46.52, 37.22, 35.70, 30.73, 27.58; ESI-HRMS calcd for $C_{54}H_{57}N_4O_{10}$ $[M + H]^+$ 921.40692, found 921.40684, mass difference −0.087 ppm. Orthogonal HPLC purity: 95.7%, retention time = 13.34 min (Method A); 95.1%, retention time = 11.95 min (Method B).

*(2S)-6-{[(tert-Butoxy)carbonyl]amino}-2-[(2S)-2-{[(2S)-1-{[(9H-fluoren-9-yl)methoxy]carbonyl}pyrrolidin-2-yl]formamido}-3-(4-phenylphenyl)propanamido]hexanoic Acid (**9b**):* ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.61 (s, 1H), 8.28 and 8.25 (d, J = 8.5 Hz, total 1H), 8.09−7.97 (m, 1H), 7.88 and 7.85 (d, J = 7.6 Hz, total 2H), 7.65 and 7.62 and 7.58 (d, J = 7.6 Hz, total 2H), 7.54 and 7.50 (d, J = 7.8 Hz, total 2H), 7.44−7.37 (m, 3H), 7.35−7.23 (m, 4H), 7.22−7.16 (m, 3H), 7.13 (d, J = 7.8 Hz, 1H), 6.74 (t, J = 5.7 Hz, 1H), 4.66 and 4.55 (td, J = 8.9, 4.5 Hz, total 1H), 4.41 and 4.35 (dd, J = 8.6, 3.2 Hz, total 1H), 4.28−4.18 (m, 1H), 4.18−3.99 (m, 2H), 3.95−3.84 (m, 1H), 3.52−3.22 (m, 2H), 3.01 and 3.04 (dd, J = 14.1, 3.9 Hz, total 1H), 2.92−2.72 (m, 3H), 2.26−2.11 and 2.01−1.87 (m, total 1H), 1.85−1.49 (m, 6H), 1.42−1.16 (m, 13H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.44, 173.37, 172.13, 171.53, 171.40, 170.88, 155.55, 154.37, 153.84, 143.84, 143.73, 143.53, 140.74, 140.63, 140.55, 140.04, 139.64, 138.03, 137.81, 137.12, 137.01, 129.85, 129.66, 128.85, 128.59, 127.66, 127.61, 127.18, 127.15, 127.12, 126.91, 126.43, 126.19, 126.10, 125.90, 125.66, 125.29, 125.12, 125.06, 120.12, 120.00, 77.33, 66.89, 66.59, 59.90, 59.34, 53.56, 53.50, 51.92, 47.16, 46.65, 46.52, 46.45, 36.93, 36.60, 31.21, 30.85, 30.76, 29.53, 28.26, 23.69, 22.76, 22.66; ESI-HRMS calcd for $C_{32}H_{55}N_9O_{14}$ $[M + H]^+$ 789.38630, found 789.38655, mass difference −0.962 ppm. Orthogonal HPLC purity: 96.1%, retention time = 11.40 min (Method A); 98.6%, retention time = 10.35 min (Method B).

*(2S)-2-[(2S)-2-[(2S)-2-({[(9H-Fluoren-9-yl)methoxy]carbonyl}-amino)-3-methylbutanamido]-3-[(triphenylmethyl)carbamoyl]-propanamido]-3-(4-phenylphenyl)propanoic Acid (**9c**):* ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62 (s, 1H), 8.31 (d, J = 8.2 Hz, 1H), 7.87 (d, J = 7.6 Hz, 2H), 7.83 (d, J = 7.5 Hz, 1H), 7.74 (d, J = 7.5 Hz, 1H), 7.70 (d, J = 7.5 Hz, 1H), 7.61−7.55 (m, 2H), 7.54−7.48 (m, 1H), 7.47 (d, J = 7.9 Hz, 2H), 7.44−7.36 (m, 4H), 7.36−7.27 (m, 4H), 7.21 (t, J = 7.7 Hz, 7H), 7.18−7.12 (m, 7H), 7.09 (t, J = 7.3 Hz, 2H), 4.64 and 4.54 (td, J = 8.7, 5.1 Hz, total 1H), 4.33−4.10 (m, 3H), 4.04 and 3.95 (dd, J = 9.2, 6.1 Hz, total 1H), 3.22−2.87 (m, 3H), 2.72−2.49 (m, 2H), 2.08−1.95 and 1.97−1.92 (m, total 1H), 0.84 and 0.82 (d, J = 6.7 Hz, total 3H), 0.78 and 0.72 (d, J = 6.7 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.56, 172.54, 172.11, 170.91, 170.16, 170.06, 169.07, 168.89, 156.07, 144.79, 144.72, 143.92, 143.68, 142.54, 140.64, 140.23, 139.39, 137.74, 137.66, 137.40, 130.11, 130.07, 128.90, 128.81, 128.54, 127.59, 127.41, 127.37, 127.26, 127.07, 127.03, 126.38, 126.27, 126.19, 126.05, 125.38, 121.35, 120.01, 109.71, 69.36, 69.32, 65.78, 59.56, 54.79, 50.24, 50.13, 46.63, 36.59, 30.99, 21.24, 19.54, 19.41, 17.67, 16.75; ESI-HRMS calcd for $C_{55}H_{57}N_3O_{10}$ $[M + H]^+$ 919.40385, found 919.40279, mass difference −4.064 ppm. Orthogonal HPLC purity: 98.3%, retention time = 13.05 min (Method A); 96.9%, retention time = 11.85 min (Method B).

*H-Bip-Tyr(tBu)-Leu-Dap(Boc)-Gly-NH$_2$ (**11b**):* ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 and 8.14 (d, J = 8.1 Hz, total 1H), 8.16 and 7.92 (t, J = 5.5 Hz, total 1H), 8.07 (d, J = 7.2 Hz, 1H), 8.05−7.99 (m, 1H), 7.66−7.59 (m, 2H), 7.54 (d, J = 7.9 Hz, 2H), 7.49 (s, 1H), 7.44 (t, J = 7.7 Hz, 2H), 7.33 (t, J = 7.4 Hz, 1H), 7.24 (d, J = 7.9 Hz, 2H), 7.09 and 7.05 (s, 1H), 6.88 (d, J = 8.4 Hz, 2H), 6.58 (d, J = 8.4 Hz, 2H), 4.56−4.44 (m, 1H), 4.33 (q, J = 7.7 Hz, 1H), 4.24 and 4.18 (dd, J = 12.2, 5.8 Hz, total 1H), 3.74−3.51 (m, 2H), 3.39 and 3.27 (dd, J = 8.3, 4.5 Hz, total 1H), 2.94−2.78 (m, 3H), 2.73 (dt, J = 14.0, 6.9 Hz, 2H), 2.57 (dd, J = 13.5, 8.4 Hz, 1H), 1.57 (td, J = 12.9, 12.0, 6.1 Hz, 1H), 1.53−1.40 (m, 2H), 0.90−0.79 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.81, 172.51, 170.98, 170.88, 170.76, 155.78, 140.08, 138.02, 137.89, 130.23, 129.93, 128.84, 127.26, 127.13, 126.45, 126.36, 114.73, 55.95, 55.29, 53.26, 51.01, 43.75, 41.93, 41.76, 40.52, 40.15, 36.97, 23.09, 22.88, 21.78, 21.68, 21.53; ESI-HRMS calcd for $C_{35}H_{46}N_7O_6$ $[M + H]^+$ 660.35041, found 660.35031, mass difference −0.149 ppm. Orthogonal HPLC purity: 98.8%, retention time = 4.55 min (Method A); 97.6%, retention time = 4.46 min (Method B).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01965.

Experimental procedures for the HTE ligand screen and NMR spectra for all compounds (PDF)

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DOI: 10.1021/acs.joc.6b01965
J. Org. Chem. 2016, 81, 9499−9506

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Sergey Melnikov for reversed-phase HPLC purifications, Christina Grosanu for silica gel chromatography purifications, Brian Redding for HRMS measurements, Gottfried Wenke for optical rotation measurements, and Dr. Yingru Zhang and the BDAS group for their support. We also thank Dr. Simon Berritt from the UPenn High Throughput Experimentation Laboratory for help in screen execution.

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DOI: 10.1021/acs.joc.6b01965
J. Org. Chem. 2016, 81, 9499−9506