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Orthogonality and compatibility between Tsc and Fmoc amino-protecting groups

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Abstract—New deprotection conditions that provide a complete orthogonality between Tsc and Fmoc amino-protecting groups are described. The potential of these orthogonal deprotection conditions was then demonstrated by the efficient solid-phase synthesis of branched peptides **20** and **21** using doubly protected amino acids such as Tsc-Lys(Fmoc)-OH **4c** and Fmoc-Lys(Tsc)-OH **4d**.

1. Introduction

Recently, we reported the 2-(4-trifluoromethylphenylsulfonyl)ethoxycarbonyl (Tsc) function as a novel base-labile amino-protecting group (Fig. 1).[1] The Tsc group differs from the 9-fluorenylmethoxycarbonyl (Fmoc) group regarding its less sensitivity to premature deprotection when installed on the exocyclic amino group of the heteroaromatic pyrrole (Py) and imidazole (Im) amino acids. The higher efficiency of Tsc compared to Fmoc in the solid-phase synthesis of pyrrole-imidazole polyamides envisions its promising use for protecting numerous amines.

The development of orthogonal amino-protecting groups or deprotection conditions allows new strategies for the solid- and solution-phase syntheses of more complex peptides and scaffolds.[2] Although a large number of orthogonal strategies are available, a combination of amino-protecting groups both orthogonal and compatible under basic deprotection conditions is rare.[3] Here, we report a dual Tsc/Fmoc strategy that provides a convenient procedure for both the orthogonal and compatible use of such amino-protecting groups under basic deprotection conditions. Our Tsc/Fmoc strategy was successfully applied to the synthesis of branched peptides by use of amino acids bearing the Tsc/Fmoc dyad.

2. Results and discussion

2.1. Synthesis of Tsc- and Fmoc-protected amino acids and esters

In order to develop orthogonal deprotection conditions required for the Tsc/Fmoc strategy, Tsc- and Fmoc-protected amino acids and esters were prepared (Fig. 1 and Scheme 1). Tsc- and Fmoc-protected pyrrole (**1a**, **1b**), imidazole (**2a**, **2b**), and phenylalanine (**3a**, **3b**) amino esters were synthesized by direct introduction of Tsc and Fmoc into the corresponding amino esters **13**, **14**, and **15**, respectively. Tsc-protected pyrrole **1c** and imidazole **2c** amino acids were prepared from esters **13** and **14**, respectively via base-resistant 2-(4-trifluoromethylphenylthio)ethoxycarbonyl (Ttc) protection.[1] Basic ester hydrolysis of Ttc-protected esters followed by subsequent oxidative conversion of Ttc into Tsc afforded the desired amino acids **1c** and **2c**. An alternative preparation of Tsc-Phe-OEt **3a** from Ttc-Phe-OEt was performed in a similar manner. Fmoc-protected pyrrole **1d** and imidazole **2d** amino acids were synthesized as described.[4] Lysine amino esters (**4a**, **4b**) and acids (**4c**, **4d**) with N^{α}-Tsc/N^{ε}-Fmoc or N^{α}-Fmoc/N^{ε}-Tsc protecting groups were prepared by direct introduction of Tsc into H-Lys(Fmoc)-OMe **16** and Fmoc-Lys-OMe **17b** amino esters and H-Lys(Fmoc)-OH and Fmoc-Lys-OH amino acids. It is noteworthy that Tsc-protected amino acids could be prepared using Tsc-Cl or convertible Ttc-Cl.

2.2. Chemical and thermal stability of Tsc and Fmoc in the presence of *N*,*N*-diisopropylethylamine

In order to implement the Tsc/Fmoc strategy, orthogonal deprotection conditions were required. We reasoned that this should be possible given the different deprotection rates

Keywords: Tsc; Fmoc; Amino-protecting group; Orthogonal deprotection; Branched peptide.
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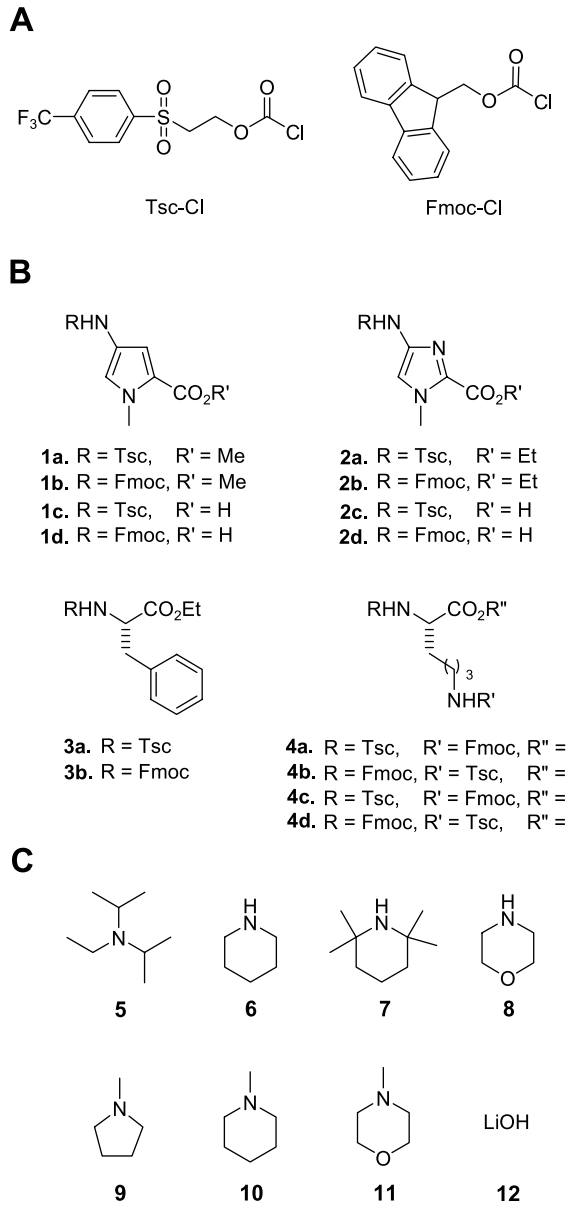
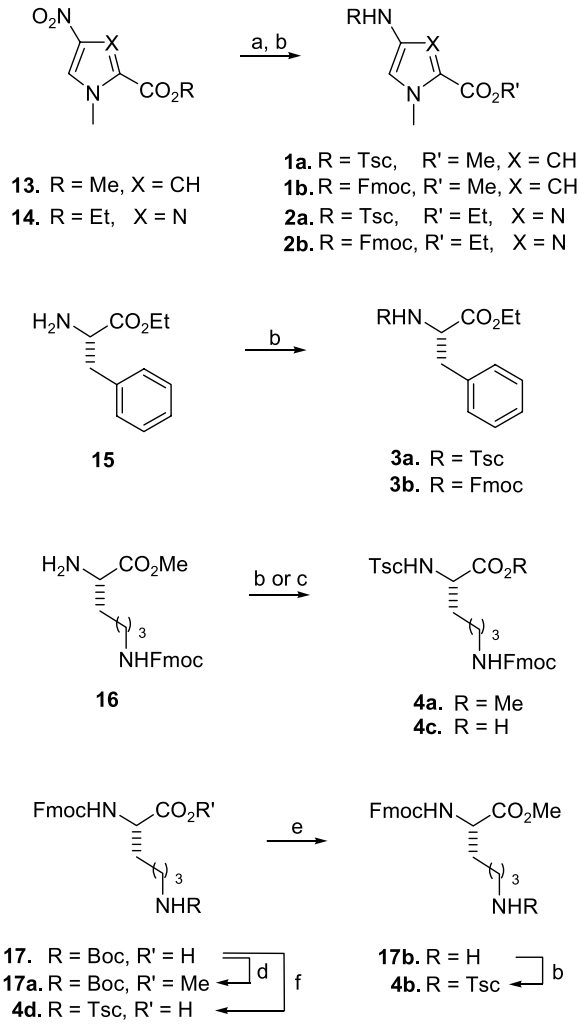


Figure 1. Structure of Tsc-Cl and Fmoc-Cl (A), Tsc- and Fmoc-protected amino acids and esters (B), and various bases tested for deprotecting Tsc and Fmoc groups (C).



Scheme 1. Reagents and conditions: (a) 10% Pd/C, 40 psi H_2, EtOAc (**13**) or DMF (**14**), rt; (b) Tsc-Cl (**1a**, 69%; **2a**, 82%; **3a**, 88%; **4a**, 92%; **4b**, 69%) or Fmoc-Cl (**1b**, 88%; **2b**, 83%; **3b**, 77%), DIEA, CH_2Cl_2, rt; (c) (i) 0.1 N LiOH, THF/H_2O (1:1), 0 °C, (ii) Tsc-Cl, $NaHCO_3$, 1,4-dioxane, rt, (**4c**, 85%); (d) CH_2N_2, CH_2Cl_2, rt, 74%; (e) 20% TFA, CH_2Cl_2, rt, (**17b** from **17a**, 88%); (f) (i) 9% TFA, CH_2Cl_2, rt, (ii) Tsc-Cl, $NaHCO_3$, 1,4-dioxane, rt, (**4d**, 38%).

of Tsc and Fmoc under basic conditions. To test such a possibility, we examined the premature deprotection of Tsc- and Fmoc-protected pyrrole and imidazole amino acids **1c**, **1d**, **2c**, and **2d** in the presence of *N*,*N*-diisopropylethylamine (DIEA, **5**) as a mild base (Fig. 2).[1,5] While both Tsc and Fmoc were completely cleaved within 5 min at room temperature by treatment with 20% (v/v) piperidine in DMF, their cleavage by 0.5 M DIEA in DMF proceeded much slower. Intriguingly, moreover, the difference in their deprotection rates is clearly significant. Careful HPLC monitoring of the decomposition of Tsc-Py-OH **1c** and Tsc-Im-OH **2c** in the presence of DIEA at 25 °C revealed that >98% of the Tsc group remained intact even after 4 h, whereas about 60 and 10% of the Fmoc groups were eliminated from Fmoc-Py-OH **1d** and Fmoc-Im-OH **2d**, respectively. Tsc-Py-OH and Tsc-Im-OH were also less prone to premature deprotection than their Fmoc analogs at higher temperature (>95% versus <50% intact after 2 h at 50 °C). Therefore, Tsc is superior to Fmoc in chemical and thermal stability when used to protect the exocyclic amino group of heteroaromatic amino acids. The higher stability of Tsc-Py-OH compared to Tsc-Im-OH is reversed in the case of Fmoc-Py-OH and Fmoc-Im-OH. Taken together, all these results indicate that the deprotection rates of Tsc and Fmoc are dependent on bases, temperature, and amino acids. Therefore, proper choice of deprotection conditions would permit the orthogonality between Tsc and Fmoc in addition to their compatibility affordable by piperidine treatment.

2.3. Orthogonal deprotection conditions for Tsc and Fmoc groups

To develop orthogonal deprotection conditions for Tsc and Fmoc, a set of deprotection conditions were evaluated using

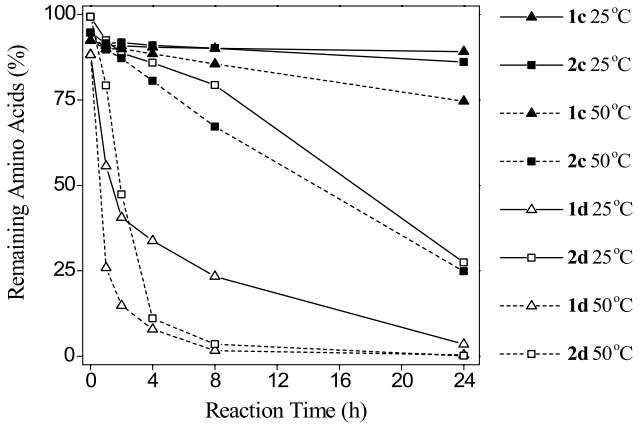
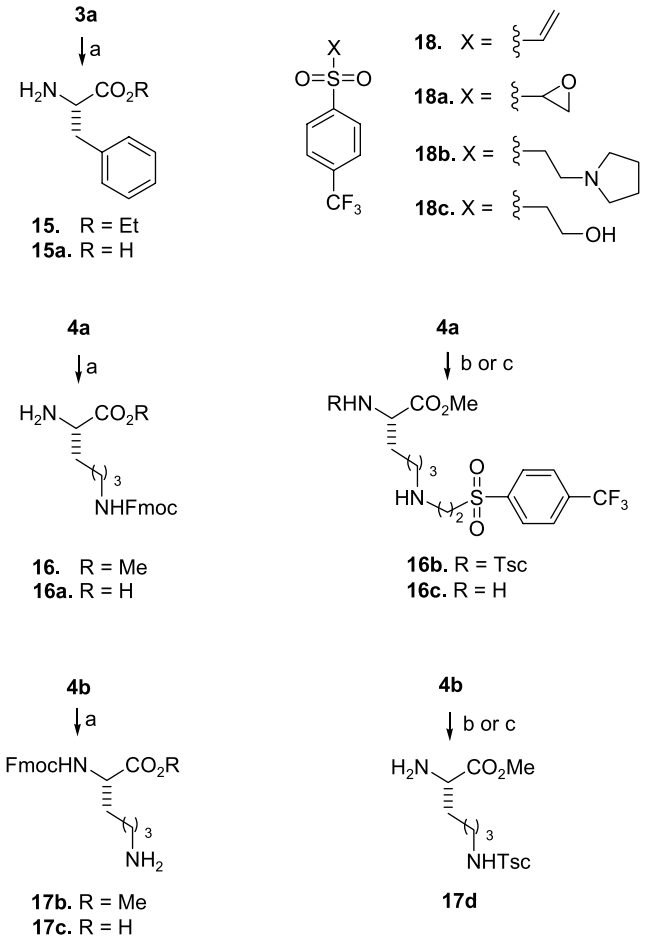


Figure 2. Effect of DIEA (**5**) on the chemical stability of amino acids at 25 or 50 °C. Time courses for the premature deprotection of amino-protecting groups in Tsc-Py-OH **1c** (▲), Tsc-Im-OH **2c** (■), Fmoc-Py-OH **1d** (△), and Fmoc-Im-OH **2d** (□) amino acids (0.1 M) were performed by their treatment with DIEA (0.5 M) in DMF at 25 °C (solid lines) or 50 °C (dashed lines). The amount of intact amino acids was determined by analytical HPLC on a Nova-Pak® C_{18} reverse-phase column (3.9×150 mm, 4 μm, Waters, Milford, MA) with UV monitoring at 280 nm under gradient conditions: 0–20 min, 5% MeCN/min, 1 mL/min flow rate.

the Tsc- (**1a**–**3a**) and Fmoc-protected (**1b**–**3b**) amino esters and basic reagents **6**–**12** (Table 1 and Scheme 2).[6] Among secondary and tertiary amines **6**–**11**, 1-methylpyrrolidine (**9**) showed a selective preference for the deprotection of Fmoc-protected amino esters **1b**–**3b** (Table 1). Complete and selective removal of Fmoc while maintaining Tsc in both heteroaromatic and aliphatic amino esters **1b**–**3b** was accomplished by using 50% 1-methylpyrrolidine in DMF for 1 h at 25 °C. We then attempted to develop conditions for the deprotection of Tsc without removing Fmoc. We found that LiOH is the reagent of choice for such purposes (Scheme 2). Fast cleavage of Tsc-protected amino ester **3a** was achieved in high yield (>99%) using 0.1 N aqueous



Scheme 2. Reagents and conditions: (a) 0.1 N LiOH, THF/H_2O (1:1), 0 °C, 5 min, (**15**, 85%, **15a**, 15%, **18a**, 77% from **3a**; **16**, 92%, **16a**, 8% from **4a**; **17b**, 75%, **17c**, 20% from **4b**); (b) 50% 1-methylpyrrolidine, DMF, 25 °C, 1 h, (**16b**, >95%; **17d**, >98%); (c) 0.5 M DIEA, DMF, 50 °C, 4 h, (**16c**, 82%; **17d**, 87%).

Table 1. Base sensitivities of the amino-protecting groups in Tsc-protected (**1a**, **2a**, and **3a**) and Fmoc-protected (**1b**, **2b**, and **3b**) amino esters[a]

		1a	2a	3a	1b	2b	3b
6	5%	H	H	H	H	H	H
	20%	H	H	H	H	H	H
	50%	H	H	H	H	H	H
7	5%	L	*M*	*L*	*M*	*M*	*L*
	20%	L	M	*L*	M	M	L
	50%	*M*	M	L	M	M	L
8	5%	*L*	*L*	*L*	*H*	*H*	M
	20%	L	L	L	H	*H*	*H*
	50%	L	L	M	H	H	*H*
9	5%	*L*	*L*	*L*	*H*	*H*	*L*
	20%	*L*	*L*	*L*	*H*	*H*	M
	50%	*L*	*L*	*L*	*H*	*H*	*H*
10	5%	*L*	*L*	*L*	M	M	M
	20%	*L*	*L*	*L*	M	M	M
	50%	*L*	*L*	*L*	M	M	M
11	5%	*L*	*L*	*L*	*L*	*L*	*L*
	20%	*L*	*L*	*L*	*L*	*L*	*L*
	50%	*L*	*L*	*L*	*L*	L	*L*

[a] Deprotection of amino esters **1a**–**3a** and **1b**–**3b** by various bases **6**–**11** was detected by HPLC monitoring of intact amino esters at 254 nm. Base sensitivity was classified based on the % amount of decomposed amino esters after their treatment (0.1 M) with bases (5, 20, and 50%) in DMF at room temperature for the indicated time: H (>90%, 5 min), *H* (>90%, 30 min), M (>90%, 2 h), *M* (>50%/<90%, 2 h), L (>10%/<50%, 2 h), *L* (<10%, 2 h).

LiOH/THF at 0 °C for 5 min wherein the occurrence of epimerization is negligible.[6a–d] Consequently, the best orthogonality between Tsc and Fmoc in terms of reactivity and selectivity in solution could therefore be achieved if Fmoc is deprotected with 50% 1-methylpyrrolidine in DMF at 25 °C for 1 h whereas Tsc is deprotected with 0.1 N LiOH in THF/H_2O (1:1) at 0 °C for 5 min.

The orthogonality of such conditions for Tsc/Fmoc deprotection was further demonstrated using doubly protected amino esters such as Tsc-Lys(Fmoc)-OMe **4a** and Fmoc-Lys(Tsc)-OMe **4b** (Scheme 2). Complete and selective cleavage of Tsc in the presence of Fmoc occurred in their treatment with LiOH/THF (Fig. 3). In addition, the selectivity of 1-methylpyrrolidine for complete Fmoc cleavage in the presence of Tsc proved to be excellent in both **4a** and **4b**. In the case of **4a**, however, **16b** rather than the desired Tsc-Lys-OMe was obtained as a major product. Presumably, the initially formed Tsc-Lys-OMe underwent the rapid conversion to **16b** by intermolecular Michael-like addition,[3j,7] which would occur more slowly in the solid-phase synthesis. Formation of free amines was accompanied by concomitant release of **18a** upon treatment of **3a** with LiOH. However, the negligible amount (<1%) of **18b** and **18c** was detected in the treatment of **4a** and **4b** with 1-methylpyrrolidine, respectively.[8]

The deprotection of Tsc with LiOH proceeded faster than ester hydrolysis. Thus, when **3a** was treated with 0.1 N LiOH, we obtained a product mixture of ethyl ester **15** (85%) and carboxylic acid **15a** (15%). The same thing happened with **4a** and **4b** that were treated with 0.1 N LiOH: 92% of ester **16** and 8% of acid **16a** from **4a**; 75% of ester **17b** and 20% of acid **17c** from **4b** (Scheme 2, and Fig. 3a and d). However, ester hydrolysis by LiOH is not expected to cause a problem in its usage for Tsc deprotection in the solid-phase peptide synthesis unless the Tsc/Fmoc/ester triad is needed due to ester groups in the side chain and/or resin linkage. In addition, it is unlikely that ester groups in the side chain limit the use of Tsc/Fmoc in tandem for preparing the Tsc/Fmoc/ester triad since base-labile Tsc could be introduced directly or via base-resistant Ttc.[9]

2.4. Synthesis of branched peptides using the Tsc/Fmoc strategy

The potential of these orthogonal deprotection conditions was then demonstrated by their use for the solid-phase synthesis of branched peptides (Scheme 3). First, the branched peptide **20** was synthesized on the Fmoc-Rink Amide MBHA resin through two methods A and B via **19a** and **19b**. Resin-bound peptides **19a** and **19b** can be branched off due to the presence of Tsc-Lys(Fmoc)-OH **4c** and Fmoc-Lys(Tsc)-OH **4d** at their N-terminus, respectively. Methods A and B differ in the order of applying Tsc and Fmoc deprotection conditions. Tsc deprotection

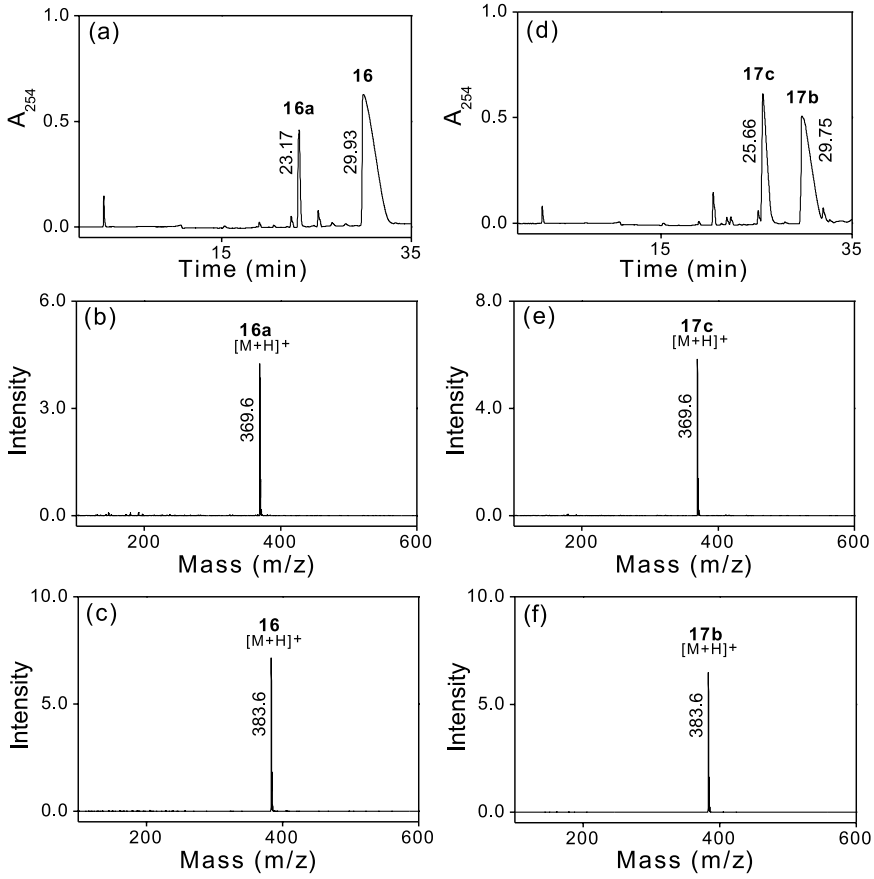
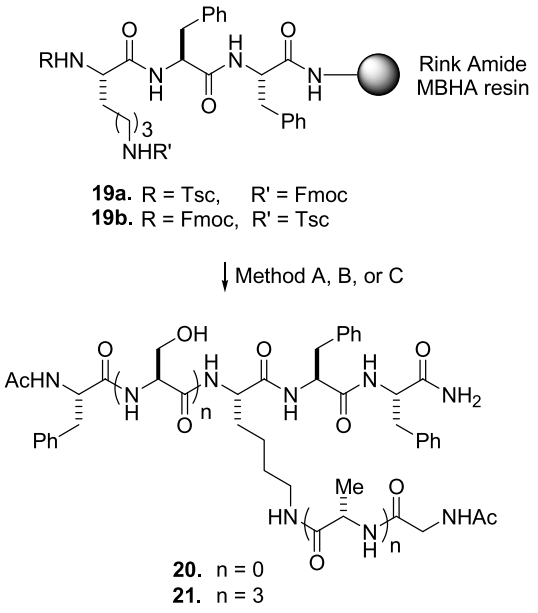


Figure 3. LC-MS analysis of a reaction mixture containing acid **16a** (23.17 min, b) and ester **16** (29.93 min, c) from **4a** (a) or acid **17c** (25.66 min, e) and ester **17b** (29.75 min, f) from **4b** (d). The reaction mixture was prepared by treating **4a** (a) or **4b** (d) with 0.1 N LiOH in THF/H_2O (1:1) at 0 °C for 5 min according to Scheme 2 and experimental 3.1.16 and 3.1.19.



Scheme 3. Reagents and conditions of method A: (a) 0.1 N LiOH, THF/H_2O (1:1), 0 °C, 10 min (LiOH); (b) Ac-Phe-OH for **19a** or Ac-Gly-OH for **19b**, PyBOP, DIEA, DMF, rt (coupling); (c) Ac_2O, DIEA, CH_2Cl_2, rt (capping); (d) 50% 1-methylpyrrolidine, DMF, rt, 1 h (MP); (e) Ac-Gly-OH for **19a** or Ac-Phe-OH for **19b**, coupling; (f) capping; (g) TFA, rt, (**20** via **19a**, 18%; **20** via **19b**, 15%). Method B: (a) MP; (b) Ac-Gly-OH for **19a** or Ac-Phe-OH for **19b**, coupling; (c) capping; (d) LiOH; (e) Ac-Phe-OH for **19a** or Ac-Gly-OH for **19b**, coupling; (f) capping; (g) TFA, rt, (**20** via **19a**, 29%; **20** via **19b**, 17%). Method C: (a) **19b**, MP; (b) Fmoc-Ser(But)-OH, coupling; (c) capping and then two repeats of (a)–(c); (d) MP; (e) Fmoc-Phe-OH, coupling; (f) capping; (g) MP; (h) capping; (i) LiOH×2 or 50% piperidine, rt, 10 min; (j) Tsc-Ala-OH, coupling; (k) capping and then two repeats of (i)–(k); (l) LiOH×2 or 50% piperidine, rt, 10 min; (m) Fmoc-Gly-OH, coupling; (n) capping; (o) 50% piperidine, rt, 10 min; (p) capping; (q) TFA, rt, (**21** via **19b**, 40% (LiOH) and 63% (piperidine)).

followed by Fmoc deprotection was carried out in method A whereas their deprotection order was reversed in method B. Peptides thus prepared were purified by reverse-phase HPLC. All the conditions afforded peptide **20** in >98% yield of each coupling step and overall recoveries between 15 and 29%. All observed molecular masses characterized by MALDI-TOF mass spectrometry agreed to within 0.1% of the calculated peptide mass. These results indicate that our Tsc/Fmoc orthogonal strategy is suitable for the preparation of branched peptides regardless of the position of Tsc and Fmoc on two amino groups of lysine and their deprotecting order.

HPLC and MALDI-TOF analyses showed that the cleaner preparation of the crude peptides was achieved using **4d** (via **19b**) compared to **4c** (via **19a**) at the branched position of **20** (Fig. 4, I, II versus III, IV). However, the crude peptide mixture prepared with **4d** (via **19b**) contained greater amounts of the Tsc-containing byproduct **20c** than the desired **20** (Fig. 4, III and IV). This result indicates that treatment of **4d** in **19b** with 0.1 N LiOH for 10 min at 0 °C is not efficient enough to give the complete deprotection of Tsc in the solid-phase peptide synthesis. Based on these results, Tsc deprotection twice with LiOH or once with piperidine was used in conjunction with **4d** for the efficient synthesis of the longer branched peptide **21**. As expected, such choices afforded peptide **21** in high isolated yield (up to 63%) (Scheme 3, Method C and Fig. 5).

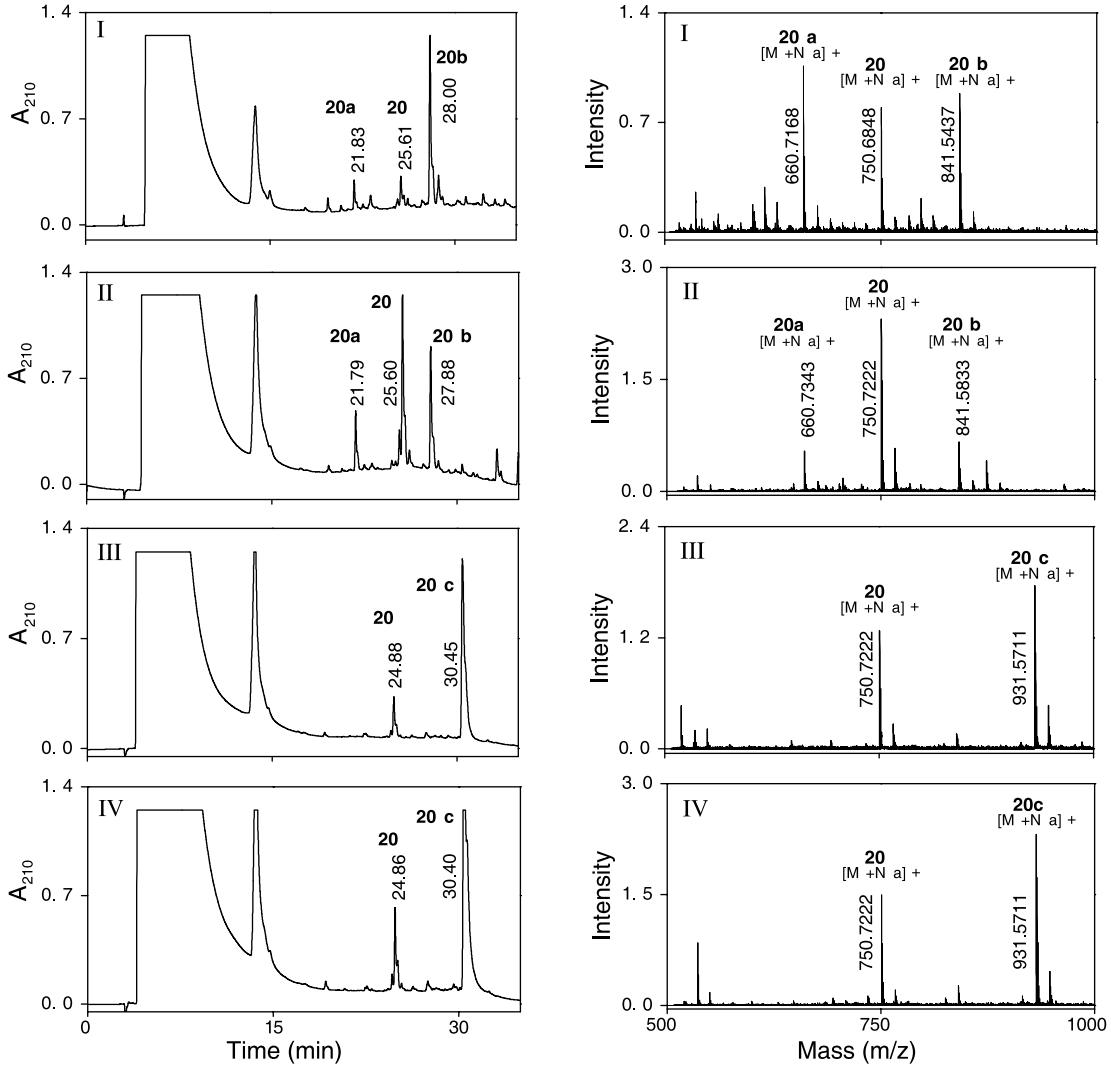
In conclusion, we have shown that 1-methylpyrrolidine and LiOH are the reagents of choice for mild and orthogonal deprotection of Fmoc and Tsc, respectively. The suitability of such conditions was demonstrated by their use for the efficient synthesis of branched peptides. Our new Tsc/Fmoc strategy should therefore greatly extend the scope of the Tsc group to dual protections under mild basic conditions. We anticipate that Tsc/Fmoc will be a potential alternative to standard Dde/Fmoc, particularly where use of the Dde amino-protecting group is not possible.[3d–f]

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 or a Bruker Avance 500 NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) with reference to tetramethylsilane or solvent and coupling constants (J) are reported in hertz (Hz). High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-AM505WA mass spectrometer using fast atom bombardment (FAB) or chemical ionization (CI) techniques. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded on an Applied Biosystems Voyager-DE™ STR mass spectrometer. HPLC analysis was performed on a Waters 600 HPLC system equipped with a 2487 dual λ absorbance detector. LC-MS analysis was performed on a Hewlett-Packard HP-1100 HPLC system and a Micromass QUATTRO LC triple quadrupole tandem mass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated plates (0.25 mm thickness, Merck). Flash chromatography was carried out on silica gel 60 (230–400 mesh, Merck). Reagent-grade chemicals were purchased from Aldrich, Fluka, Junsei, and TCI and used as received unless otherwise specified. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under N_2. *N*,*N*-Dimethylformamide (DMF) was distilled from calcium hydride in vacuo. Dichloromethane was distilled from calcium hydride under N_2.

3.1.1. Tsc-Py-OMe (1a). To a solution of **13** (3 g, 16.3 mmol) in EtOAc (40 mL) was added 10% Pd/C (50 mg). After stirring at room temperature for 10 h under 40 psi H_2, 10% Pd/C was removed by filtration through Celite 545 followed by washing with EtOAc and MeOH and then solvent evaporation. To a stirred solution of the resulting amine in dry CH_2Cl_2 (20 mL) was added *N*,*N*-diisopropylethylamine (DIEA, 4.11 mL, 23.6 mmol) and then Tsc-Cl[1] (5.67 g, 17.9 mmol) at 0 °C. After stirring at room temperature for 7 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:1) to give **1a** (4.90 g, 69%) as a white solid. TLC (EtOAc/*n*-hexane=1:1) R_f=0.33; ^1H NMR (300 MHz, DMSO-d_6) δ 9.30 (brs, 1H), 8.15 (d, J=8.1 Hz, 2H), 8.01 (d, J=8.4 Hz, 2H), 7.02 (d, J=2.1 Hz, 1H), 6.61 (d, J=1.8 Hz, 1H), 4.36 (t, J=5.6 Hz,



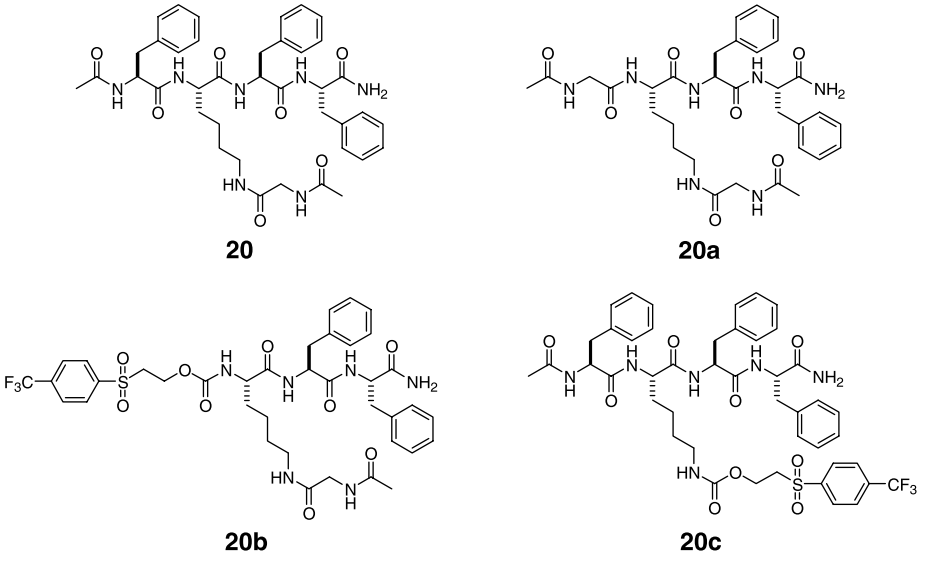


Figure 4. HPLC (left) and MALDI-TOF (right) analyses of a crude mixture containing peptide **20** (24.86–25.61 min in left) and byproducts **20a–c**. The crude mixture was prepared with **4c** (via **19a**) using method A (I) or B (II) or **4d** (via **19b**) using method A (III) or B (IV). HPLC analysis was performed on a C_{18} reverse-phase column (4.6×250 mm, 5 μm particle size, TP silica, Vydac, Hesperia, CA) with a linear H_2O/MeCN gradient containing 0.1% (v/v) TFA: 0–5 min 100% H_2O, 5–35 min 2.67% MeCN/min, 1 mL/min flow rate, 210 nm.

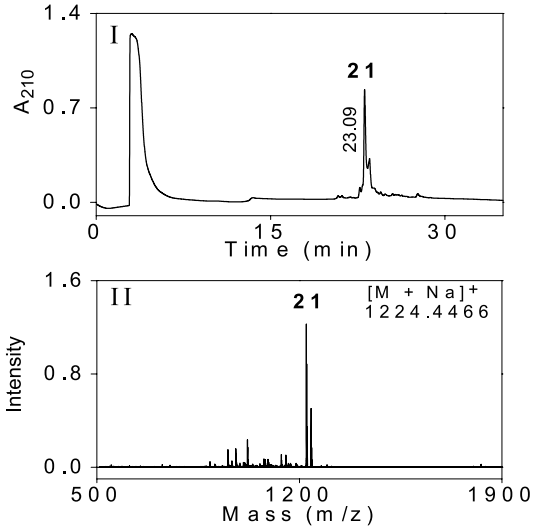


Figure 5. HPLC (I) and MALDI-TOF (II) analyses of a crude mixture containing peptide **21**. The crude mixture was prepared with **4d** (via **19b**) using method C (piperidine). The HPLC condition is identical to that of Fig. 4 except for using a linear gradient of 3.33% rather than 2.67% MeCN/min during 5–35 min.

2H), 3.86 (t, J=5.7 Hz, 2H), 3.80 (s, 3H), 3.72 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.68, 152.45, 143.23, 133.48 (q, J=32.3 Hz), 128.80, 126.61 (q, J=3.6 Hz), 123.35 (q, J=273.5 Hz), 122.32, 119.28, 118.82, 107.53, 57.52, 54.21, 50.92, 36.16; HRMS (FAB+) for $C_{17}H_{17}F_3N_2O_6S$ (M^+), calcd 434.0759, found 434.0765.

3.1.2. Fmoc-Py-OMe (1b). To a solution of **13** (3 g, 16.3 mmol) in EtOAc (40 mL) was added 10% Pd/C (50 mg). After stirring at room temperature for 10 h under 40 psi H_2, 10% Pd/C was removed by filtration through Celite 545 followed by washing with EtOAc and MeOH and then solvent evaporation. To a stirred solution of the resulting amine in dry CH_2Cl_2 (20 mL) was added *N*,*N*-diisopropylethylamine (4.11 mL, 23.6 mmol) and then Fmoc-Cl (4.64 g, 17.9 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:3) to give **1b** (5.41 g, 88%) as a white solid. TLC (EtOAc/*n*-hexane=1:1) R_f=0.62; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.51 (brs, 1H), 7.90 (d, J=7.5 Hz, 2H), 7.73 (d, J=7.5 Hz, 2H), 7.43 (td, J=7.2, 0.6 Hz, 2H), 7.35 (td, J=7.4, 1.1 Hz, 2H), 7.12 (d, J=1.5 Hz, 1H), 6.69 (d, J=1.8 Hz, 1H), 4.48 (d, J=6.6 Hz, 2H), 4.28 (t, J=6.3 Hz, 1H), 3.81 (s, 3H), 3.72 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.67, 153.28, 143.81, 140.81, 127.63, 127.10, 125.03, 122.71, 120.16, 119.28, 118.82, 107.45, 65.37, 50.91, 46.70, 36.17; HRMS (FAB+) for $C_{22}H_{20}N_2O_4$ (M^+), calcd 376.1423, found 376.1414.

3.1.3. Tsc-Im-OEt (2a). To a solution of **14** (2 g, 10.1 mmol) in DMF (20 mL) was added 10% Pd/C (45 mg). After stirring at room temperature for 10 h under 40 psi H_2, 10% Pd/C was removed by filtration through Celite 545 followed by washing with EtOAc and MeOH and then solvent evaporation. To a stirred solution of the resulting amine in dry CH_2Cl_2 (30 mL) was added *N*,*N*-diisopropylethylamine (1.93 mL, 11.1 mmol) and then Tsc-Cl (3.52 g, 11.1 mmol) at 0 °C. After stirring at room temperature for 7 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=3:1) to give **2a** (3.7 g, 82%) as a white solid. TLC (EtOAc/*n*-hexane=3:1) R_f=0.40; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.99 (brs, 1H), 8.12 (d, J=8.1 Hz, 2H), 7.97 (d, J=8.1 Hz, 2H), 7.20 (s, 1H), 4.34 (brt, 2H), 4.24 (q, J=7.2 Hz, 2H), 3.87 (brs, 5H), 1.27 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.37, 152.46, 143.20, 137.42, 133.40 (q, J=31.8 Hz), 131.01, 128.85, 126.54, 123.30 (q, J=272.3 Hz), 113.59, 60.46, 57.79, 54.14, 35.42, 14.08; HRMS (FAB+) for $C_{17}H_{19}F_3N_3O_6S$ (MH$^+$), calcd 450.0947, found 450.0957.

3.1.4. Fmoc-Im-OEt (2b). To a solution of **14** (2 g, 10.1 mmol) in DMF (20 mL) was added 10% Pd/C (45 mg). After stirring at room temperature for 10 h under 40 psi H_2, 10% Pd/C was removed by filtration through Celite 545 followed by washing with EtOAc and MeOH and then solvent evaporation. To a stirred solution of the resulting amine in dry CH_2Cl_2 (20 mL) was added *N*,*N*-diisopropylethylamine (1.93 mL, 11.1 mmol) and then Fmoc-Cl (2.87 g, 11.1 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:2) to give **2b** (3.27 g, 83%) as a white solid. TLC (EtOAc/*n*-hexane=1:1) R_f=0.39; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.41 (brs, 1H), 7.89 (d, J=7.5 Hz, 2H), 7.76 (d, J=6.6 Hz, 2H), 7.41 (t, J=7.4 Hz, 2H), 7.32 (t, J=7.4 Hz, 3H), 4.26 (m, 5H), 3.89 (s, 3H), 1.28 (t, J=6.8 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.38, 153.27, 143.70, 140.72, 137.78, 131.10, 127.70, 127.11, 125.44, 120.12, 113.62, 66.13, 60.51, 46.45, 35.43, 14.07; HRMS (FAB+) for $C_{22}H_{22}N_3O_4$ (MH$^+$), calcd 392.1610, found 392.1605.

3.1.5. Ttc-Phe-OEt. To a stirred solution of **15**-HCl (0.5 g, 2.18 mmol) in dry CH_2Cl_2 (15 mL) was added *N*,*N*-diisopropylethylamine (0.55 mL, 3.16 mmol) and then Ttc-Cl[1] (0.93 g, 3.27 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:1) to give the title compound (778 mg, 81%) as a white solid. TLC (EtOAc/*n*-hexane=1:2) R_f=0.52; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J=8.1 Hz, 2H), 7.41 (d, J=8.4 Hz, 2H), 7.27 (m, 3H), 7.13 (dd, J=7.8, 1.8 Hz, 2H), 5.20 (d, J=8.1 Hz, 1H), 4.61 (dt, J=8.0, 6.1 Hz, 1H), 4.23 (t, J=6.8 Hz, 2H), 4.17 (q, J=7.1 Hz, 2H), 3.17 (t, J=7.1 Hz, 2H), 3.13 (dd, J=13.5, 5.4 Hz, 1H), 3.07 (dd, J=13.8, 5.7 Hz, 1H), 1.24 (t, J=7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.37, 155.24, 140.86 (q, J=1.4 Hz), 135.65, 129.28, 128.54, 127.87, 127.79 (q, J=32.6 Hz), 127.11, 125.77 (q, J=3.8 Hz), 124.04 (q, J=271.8 Hz), 63.00, 61.54, 54.73, 38.26, 31.24, 14.07; HRMS (FAB+)

for $C_{21}H_{23}F_3NO_4S$ (MH^+), calcd 442.1300, found 442.1294.

3.1.6. Tsc-Phe-OEt (3a). To a stirred solution of **15**-HCl (1.5 g, 6.53 mmol) in dry CH_2Cl_2 (20 mL) was added *N*,*N*-diisopropylethylamine (1.69 mL, 9.70 mmol) and then Tsc-Cl (2.27 g, 7.18 mmol) at 0 °C. After stirring at room temperature for 7 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:1) to give **3a** (2.72 g, 88%) as a white solid. Alternatively, **3a** was synthesized by oxidation of Ttc-Phe-OEt with H_2O_2/$NaMoO_4$ in acetone.[1] To a solution of Ttc-Phe-OEt (1.2 g, 2.71 mmol) in acetone (30 mL) was added 0.3 M aqueous Na_2MoO_4 (0.8 mL, 0.24 mmol) and 30% aqueous H_2O_2 (1.5 mL). After stirring at room temperature for 18 h, the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:1) to give **3a** (1.18 g, 91%) as a white solid. TLC (EtOAc/*n*-hexane=1:1) R_f=0.51; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, J=8.4 Hz, 2H), 7.79 (d, J=8.4 Hz, 2H), 7.26 (m, 3H), 7.09 (dd, J=7.8, 1.5 Hz, 2H), 5.09 (d, J=8.1 Hz, 1H), 4.48 (dt, J=8.2, 6.2 Hz, 1H), 4.38 (t, J=5.9 Hz, 2H), 4.15 (q, J=7.1 Hz, 2H), 3.47 (td, J=5.9, 1.8 Hz, 2H), 3.08 (dd, J=14.1, 6.0 Hz, 1H), 3.00 (dd, J=13.7, 6.5 Hz, 1H), 1.22 (t, J=7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.01, 154.38, 142.86 (q, J=1.2 Hz), 135.50, 135.34 (q, J=33.2 Hz), 129.10, 128.72, 128.45, 127.04, 126.32 (q, J=3.7 Hz), 122.95 (q, J=273.2 Hz), 61.48, 58.08, 55.23, 54.59, 37.91, 13.92; HRMS (FAB+) for $C_{21}H_{23}F_3NO_6S$ (MH^+), calcd 474.1198, found 474.1213.

3.1.7. Fmoc-Phe-OEt (3b). To a stirred solution of **15**-HCl (1.5 g, 6.53 mmol) in dry CH_2Cl_2 (20 mL) was added *N*,*N*-diisopropylethylamine (1.69 mL, 9.70 mmol) and then Fmoc-Cl (1.86 g, 7.18 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:3) to give **3b** (2.1 g, 77%) as a white solid. TLC (EtOAc/*n*-hexane=1:2) R_f=0.45; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J=7.2 Hz, 2H), 7.55 (dd, J=6.8, 5.0 Hz, 2H), 7.38 (t, J=7.2 Hz, 2H), 7.29 (td, J=7.4, 1.2 Hz, 2H), 7.25 (m, 3H), 7.10 (dd, J=6.6, 1.2 Hz, 2H), 5.35 (d, J=7.8 Hz, 1H), 4.65 (dt, J=8.2, 6.0 Hz, 1H), 4.43 (dd, J=10.4, 7.1 Hz, 1H), 4.32 (dd, J=10.4, 6.8 Hz, 1H), 4.19 (t, J=6.6 Hz, 1H), 4.16 (q, J=7.2 Hz, 2H), 3.14 (dd, J=13.4, 5.6 Hz, 1H), 3.08 (dd, J=13.5, 5.7 Hz, 1H), 1.22 (t, J=7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.40, 155.46, 143.76, 143.65, 141.20, 135.71, 129.28, 128.45, 127.61, 127.00, 126.96, 125.04, 124.97, 119.90, 119.88, 66.81, 61.44, 54.72, 47.05, 38.18, 14.04; HRMS (FAB+) for $C_{22}H_{26}NO_4$ (MH^+), calcd 416.1862, found 416.1856.

3.1.8. Tsc-Lys(Fmoc)-OMe (4a). To a stirred solution of **16**-HCl (200 mg, 0.477 mmol, Novabiochem, La Jolla, CA) in dry CH_2Cl_2 (20 mL) was added triethylamine (73 μL, 0.525 mmol) and then Tsc-Cl (181 mg, 0.573 mmol) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:1) to give **4a** (290 mg, 92%) as a white solid. TLC (EtOAc/*n*-hexane=2:1) R_f=0.29; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.12 (d, J=8.0 Hz, 2H), 8.01 (d, J=8.0 Hz, 2H), 7.87 (d, J=7.5 Hz, 2H), 7.66 (d, J=7.5 Hz, 2H), 7.49 (d, J=7.5 Hz, 1H), 7.40 (t, J=7.3 Hz, 2H), 7.31 (t, J=7.3 Hz, 2H), 7.24 (t, J=5.3 Hz, 1H), 4.27 (m, 3H), 4.22 (dd, J=12.5, 6.0 Hz, 1H), 4.19 (t, J=6.8 Hz, 1H), 3.88 (td, J=8.0, 5.2 Hz, 1H), 3.77 (t, J=5.3 Hz, 2H), 3.59 (s, 3H), 2.93 (q, J=6.3 Hz, 2H), 1.64–1.47 (m, 2H), 1.38–1.30 (m, 2H), 1.25–1.18 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.58, 156.02, 155.22, 143.88, 143.26, 140.68, 133.41 (q, J=32.4 Hz), 128.79, 127.52, 126.97, 126.55 (q, J=3.4 Hz), 125.05, 123.34 (q, J=272.8 Hz), 120.05, 65.10, 57.53, 54.19, 53.74, 51.74, 46.73, 39.66, 30.18, 28.79, 22.54; HRMS (FAB+) for $C_{32}H_{34}F_3N_2O_8S$ (MH^+), calcd 663.1988, found 663.1979.

3.1.9. Fmoc-Lys(Boc)-OMe (17a). To a stirred solution of **17** (500 mg, 1.07 mmol) in dry CH_2Cl_2 (15 mL) was added diazomethane (0.8 M in dry diethyl ether) at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was quenched with MeOH and extracted with CH_2Cl_2/H_2O. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=2:1) to give **17a** (380 mg, 74%) as a white solid. TLC (EtOAc/*n*-hexane=2:1) R_f=0.58; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.88 (d, J=7.5 Hz, 2H), 7.74 (d, J=7.5 Hz, 1H), 7.71 (dd, J=7.5, 3.0 Hz, 2H), 7.41 (t, J=7.5 Hz, 2H), 7.32 (t, J=7.5 Hz, 2H), 6.76 (t, J=5.5 Hz, 1H), 4.29 (m, 2H), 4.22 (t, J=7.0 Hz, 1H), 3.99 (td, J=8.5, 5.0 Hz, 1H), 3.61 (s, 3H), 2.89 (q, J=4.5 Hz, 2H), 1.70–1.56 (m, 2H), 1.36 (s, 9H), 1.32–1.23 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.89, 156.07, 155.53, 143.76, 143.72, 140.69, 127.58, 127.00, 125.18, 120.05, 77.30, 65.57, 53.82, 51.75, 46.63, 39.67, 30.32, 28.97, 28.22, 22.76; HRMS (FAB+) for $C_{27}H_{35}N_2O_6$ (MH^+), calcd 483.2495, found 483.2498.

3.1.10. Fmoc-Lys-OMe (17b). To a solution of **17a** (300 mg, 0.622 mmol) in CH_2Cl_2 (16 mL) was added trifluoroacetic acid (TFA, 4.0 mL) in one portion. After stirring at room temperature for 3 h, the reaction mixture was concentrated at 30 °C in vacuo to give **17b** (210 mg, 88%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89 (d, J=7.5 Hz, 2H), 7.74 (m, 3H), 7.70 (t, J=6.5 Hz, 2H), 7.41 (t, J=7.3 Hz, 2H), 7.33 (t, J=7.5 Hz, 2H), 4.34 (dd, J=10.5, 7.0 Hz, 1H), 4.29 (dd, J=10.5, 7.0 Hz, 1H), 4.22 (t, J=7.0 Hz, 1H), 4.00 (td, J=8.5, 5.0 Hz, 1H), 3.62 (s, 3H), 2.75 (q, J=5.5 Hz, 2H), 1.72–1.57 (m, 2H), 1.56–1.47 (m, 2H), 1.39–1.29 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.77, 156.10, 143.75, 143.72, 140.72, 127.61, 127.02, 125.16, 125.13, 120.10, 65.55, 53.60, 51.85, 46.63, 38.52, 30.04, 26.42, 22.36; HRMS (FAB+) for $C_{22}H_{27}N_2O_4$ (MH^+), calcd 383.1971, found 383.1976.

3.1.11. Fmoc-Lys(Tsc)-OMe (4b). To a stirred solution of **17b** (600 mg, 1.57 mmol) in dry CH_2Cl_2 (20 mL) was added *N*,*N*-diisopropylethylamine (272 μL, 1.57 mmol) and then

Tsc-Cl (596 mg, 1.88 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:1) to give **4b** (715 mg, 69%) as a white solid. TLC (EtOAc/*n*-hexane=2:1) R_f=0.29; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.11 (d, J=8.0 Hz, 2H), 8.01 (d, J=8.0 Hz, 2H), 7.88 (d, J=7.5 Hz, 2H), 7.73 (d, J=7.5 Hz, 1H), 7.70 (dd, J=6.8, 1.8 Hz, 2H), 7.41 (t, J=7.5 Hz, 2H), 7.32 (t, J=7.5 Hz, 2H), 6.93 (t, J=5.3 Hz, 1H), 4.32–4.20 (m, 5H), 3.97 (td, J=8.1, 5.0 Hz, 1H), 3.76 (t, J=5.5 Hz, 2H), 3.61 (s, 3H), 2.82 (q, J=6.0 Hz, 2H), 1.67–1.54 (m, 2H), 1.29–1.22 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.86, 156.05, 155.09, 143.75, 143.72, 143.38, 140.69, 133.32 (q, J=32.0 Hz), 128.75, 127.58, 126.99, 126.51 (q, J=3.7 Hz), 125.16, 123.36 (q, J=272.8 Hz), 120.06, 65.56, 57.16, 54.35, 53.76, 51.76, 46.61, 39.67, 30.21, 28.71, 22.62; HRMS (FAB+) for $C_{32}H_{34}F_3N_2O_8S$ (MH$^+$), calcd 663.1988, found 663.1998.

3.1.12. Tsc-Lys(Fmoc)-OH (4c).[10] To a cooled (0 °C) solution of **16**-HCl (302 mg, 0.72 mmol) in THF (7 mL) was added dropwise over 3 min a solution of LiOH (56 mg, 2.34 mmol) in H_2O (7 mL). After stirring at 0 °C for 50 min (the time for complete reaction as judged by TLC (MeOH/CH_2Cl_2=1:9)), the reaction mixture was adjusted to pH 7 by adding saturated aqueous NH_4Cl. To the resulting suspension was added 1,4-dioxane (20 mL), 1 M aqueous $NaHCO_3$ (0.86 mL, 0.86 mmol), and then Tsc-Cl (273.6 mg, 0.864 mmol) at 0 °C. After stirring at room temperature overnight, the reaction mixture was quenched with 1 N aqueous HCl and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/CH_2Cl_2=1:9) to give **4c** (397 mg, 85%) as a white solid. TLC (MeOH/CH_2Cl_2=1:8) R_f=0.31; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.12 (d, J=8.5 Hz, 2H), 8.01 (d, J=9.0 Hz, 2H), 7.87 (d, J=7.5 Hz, 2H), 7.66 (d, J=7.5 Hz, 2H), 7.40 (t, J=7.5 Hz, 2H), 7.31 (t, J=7.3 Hz, 2H), 7.27 (d, J=8.0 Hz, 1H), 7.25 (t, J=5.8 Hz, 1H), 4.27 (m, 3H), 4.21 (dd, J=12.5, 5.5 Hz, 1H), 4.18 (t, J=5.8 Hz, 1H), 3.78 (td, J=9.0, 5.3 Hz, 1H), 3.75 (t, J=6.0 Hz, 2H), 2.94 (q, J=6.3 Hz, 2H), 1.65–1.46 (m, 2H), 1.40–1.30 (m, 2H), 1.26–1.18 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.63, 156.05, 155.25, 143.91, 143.30, 140.71, 133.40 (q, J=32.4 Hz), 128.83, 127.56, 127.01, 126.58 (q, J=3.7 Hz), 125.10, 123.40 (q, J=273.0 Hz), 120.08, 65.15, 57.46, 54.27, 53.79, 46.76, 39.67, 30.35, 28.88, 22.70; HRMS (FAB+) for $C_{31}H_{32}F_3N_2O_8S$ (MH$^+$), calcd 649.1831, found 649.1844.

3.1.13. Fmoc-Lys(Tsc)-OH (4d).[10] To a solution of **17** (1.39 g, 2.96 mmol) in CH_2Cl_2 (40 mL) was added trifluoroacetic acid (4.0 mL) in one portion. After stirring at room temperature for 3 h, the reaction mixture was concentrated at 30 °C in vacuo to give Fmoc-Lys-OH (1.09 g, >99%) as a white solid. To a stirred solution of crude Fmoc-Lys-OH in 1,4-dioxane (40 mL) was added 1 M aqueous $NaHCO_3$ (3.55 mL, 3.55 mmol) and then Tsc-Cl (1.12 g, 3.55 mmol) at 0 °C. After stirring at room temperature for 8 h, the reaction mixture was quenched with 1 N aqueous HCl and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/CH_2Cl_2=1:9) to give **4d** (720 mg, 38%) as a white solid. TLC (MeOH/CH_2Cl_2=1:9) R_f=0.28; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.54 (brs, 1H), 8.11 (d, J=8.0 Hz, 2H), 8.01 (d, J=8.0 Hz, 2H), 7.88 (d, J=7.0 Hz, 2H), 7.71 (d, J=7.5 Hz, 2H), 7.57 (d, J=8.0 Hz, 1H), 7.40 (t, J=7.5 Hz, 2H), 7.31 (t, J=7.0 Hz, 2H), 6.93 (t, J=5.8 Hz, 1H), 4.27–4.19 (m, 5H), 3.88 (td, J=8.5, 4.0 Hz, 1H), 3.76 (t, J=5.5 Hz, 2H), 2.82 (q, J=6.0 Hz, 2H), 1.68–1.53 (m, 2H), 1.29–1.22 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.90, 156.11, 155.10, 143.80, 143.77, 143.41, 140.69, 140.67, 133.33 (q, J=32.1 Hz), 128.77, 127.60, 127.03, 126.53 (q, J=3.4 Hz), 125.24, 125.22, 123.37 (q, J=272.8 Hz), 120.06, 65.55, 57.17, 54.36, 53.76, 46.63, 39.67, 30.35, 28.79, 22.79; HRMS (FAB+) for $C_{31}H_{32}F_3N_2O_8S$ (MH$^+$), calcd 649.1831, found 649.1822.

3.1.14. Tsc-Ala-OH. To a solution of H-Ala-OH (250 mg, 2.81 mmol) in 1 M aqueous $NaHCO_3$ (20 mL) was added 1,4-dioxane (20 mL) and then Tsc-Cl (1.07 g, 3.37 mmol) at 0°C. After stirring at room temperature for 2 h, the reaction mixture was quenched with 1 N aqueous HCl and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was recrystallized from diethyl ether and *n*-hexane to afford the title compound (515 mg, 50%) as a white solid. TLC (MeOH/CH_2Cl_2=1:9) R_f=0.34; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.52 (brs, 1H), 8.12 (d, J=8.5 Hz, 2H), 8.02 (d, J=8.0 Hz, 2H), 7.29 (d, J=7.5 Hz, 1H), 4.29 (m, 1H), 4.20 (m, 1H), 3.86 (m, 1H), 3.77 (m, 2H), 1.17 (d, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.03, 154.86, 143.36, 133.42 (q, J=32.4 Hz), 128.83, 126.58 (q, J=3.7 Hz), 123.39 (q, J=273.3 Hz), 57.48, 54.32, 49.07, 16.88; HRMS (FAB+) for $C_{13}H_{15}F_3NO_6S$ (MH$^+$), calcd 370.0572, found 370.0571.

3.1.15. Deprotection of Tsc-Phe-OEt with LiOH. To a cooled (0 °C) solution of **3a** (17.0 mg, 0.036 mmol) in THF (0.35 mL) was added dropwise over 1 min 0.2 N aqueous LiOH (0.35 mL, 0.070 mmol). After stirring at 0 °C for 10 min (the time for complete reaction as judged by TLC (EtOAc/*n*-hexane=1:5)), the reaction mixture was adjusted to pH 7 by adding saturated aqueous NH_4Cl and then extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/*n*-hexane=1:5) to give **18a** (7.0 mg, 77%) as a white solid. TLC (EtOAc/*n*-hexane=1:4) R_f=0.27; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, J=8.4 Hz, 2H), 7.89 (d, J=8.1 Hz, 2H), 4.17 (dd, J=3.8, 2.3 Hz, 1H), 3.49 (dd, J=5.4, 2.1 Hz, 1H), 3.19 (dd, J=5.4, 3.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.30 (q, J=1.2 Hz), 136.05 (q, J=33.2 Hz), 129.42, 126.53 (q, J=3.6 Hz), 122.97 (q, J=273.2 Hz), 63.05, 45.43; HRMS (CI+) for $C_9H_8F_3O_3S$ (MH$^+$), calcd 253.0146, found 253.0142.

3.1.16. Deprotection of Tsc-Lys(Fmoc)-OMe with LiOH. To a cooled (0 °C) solution of **4a** (5.9 mg, 9 μmol) in THF (87 μL) was added dropwise over 1 min 0.2 N aqueous LiOH (87 μL, 17.4 μmol). After stirring at 0 °C for 5 min, the reaction mixture (50 μL) was adjusted to pH 1 by adding

1 N aqueous HCl (100 μL) and then analyzed on a LC-MS using a C_{18} reverse-phase column (4.6×250 mm, 5 μm particle size, TP silica, Vydac, Hesperia, CA) with a linear H_2O/MeCN gradient containing 0.1% (v/v) AcOH: 0–5 min 100% H_2O, 5–35 min 2.67% MeCN/min, 1 mL/min flow rate, 254 nm, R_v=23.17 mL (**16a**, 8%) and 29.93 mL (**16**, 92%) (Fig. 3).

3.1.17. Deprotection of Tsc-Lys(Fmoc)-OMe with 1-methylpyrrolidine. To a stirred solution of **4a** (50.0 mg, 75.4 μmol) in dry DMF (189 μL) was added 1-methylpyrrolidine (189 μL, 1.82 mmol) at room temperature. After stirring at 25 °C for 1 h, the reaction mixture was directly purified by flash chromatography (MeOH/CH_2Cl_2=1:30 and then MeOH/CH_2Cl_2=1:9) to give **16b** (48.4 mg, 95%) and **18b**. **16b**: TLC (MeOH/CH_2Cl_2=1:9) R_f=0.43; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J=8.1 Hz, 2H), 8.07 (d, J=8.1 Hz, 2H), 7.87 (d, J=8.1 Hz, 2H), 7.86 (d, J=8.4 Hz, 2H), 5.11 (d, J=8.4 Hz, 1H), 4.43 (t, J=5.8 Hz, 2H), 4.21 (td, J=7.8, 5.3 Hz, 1H), 3.74 (s, 3H), 3.50 (t, J=5.9 Hz, 2H), 3.32 (t, J=6.3 Hz, 2H), 3.03 (t, J=6.3 Hz, 2H), 2.56 (t, J=6.9 Hz, 2H), 1.83–1.72 (m, 2H), 1.65–1.53 (m, 2H), 1.50–1.41 (m, 2H); HRMS (FAB+) for $C_{26}H_{31}F_6N_2O_8S_2$ (MH^+), calcd 677.1426, found 677.1425. **18b**: TLC (MeOH/CH_2Cl_2=1:9) R_f=0.33; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, J=8.7 Hz, 2H), 7.83 (d, J=8.4 Hz, 2H), 3.35 (t, J=7.5 Hz, 2H), 2.87 (t, J=7.4 Hz, 2H), 2.39 (m, 4H), 1.65 (m, 4H); HRMS (FAB+) for $C_{13}H_{16}F_3NO_2S$ (M^+), calcd 307.0854, found 307.0866.

3.1.18. Deprotection of Tsc-Lys(Fmoc)-OMe with DIEA. To a solution of **4a** (66.3 mg, 0.10 mmol) in dry DMF (1 mL) was added *N*,*N*-diisopropylethylamine (87.1 μL, 0.50 mmol) at room temperature. After heating at 50 °C for 4 h, the reaction mixture was quenched with H_2O and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/CH_2Cl_2=1:9) to give **16c** (32.5 mg, 82%) as a white solid. TLC (MeOH/CH_2Cl_2=1:9) R_f=0.27; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, J=8.1 Hz, 2H), 7.86 (d, J=8.7 Hz, 2H), 3.72 (s, 3H), 3.44 (dd, J=7.5, 5.4 Hz, 1H), 3.32 (t, J=6.5 Hz, 2H), 3.04 (t, J=6.5 Hz, 2H), 2.57 (t, J=6.9 Hz, 2H), 1.61–1.38 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.47, 142.90 (q, J=1.3 Hz), 135.53 (q, J=33.3 Hz), 128.65, 126.48 (q, J=3.7 Hz), 123.05 (q, J=273.5 Hz), 56.01, 54.26, 51.97, 49.21, 42.90, 34.59, 29.54, 23.24; HRMS (FAB+) for $C_{16}H_{24}F_3N_2O_4S$ (MH^+), calcd 397.1409, found 397.1395.

3.1.19. Deprotection of Fmoc-Lys(Tsc)-OMe with LiOH. To a cooled (0 °C) solution of **4b** (5.9 mg, 9 μmol) in THF (87 μL) was added dropwise over 1 min 0.2 N aqueous LiOH (87 μL, 17.4 μmol). After stirring at 0 °C for 5 min, the reaction mixture (50 μL) was adjusted to pH 1 by adding 1 N aqueous HCl (100 μL) and then analyzed on a LC-MS using a C_{18} reverse-phase column (4.6×250 mm, 5 μm particle size, TP silica, Vydac, Hesperia, CA) with a linear H_2O/MeCN gradient containing 0.1% (v/v) AcOH: 0–5 min 100% H_2O, 5–35 min 2.67% MeCN/min, 1 mL/min flow rate, 254 nm, R_v=25.66 mL (**17c**, 20%) and 29.75 mL (**17b**, 75%) (Fig. 3).

3.1.20. Deprotection of Fmoc-Lys(Tsc)-OMe with 1-methylpyrrolidine. To a stirred solution of **4b** (50.0 mg, 75.4 μmol) in dry DMF (189 μL) was added 1-methylpyrrolidine (189 μL, 1.82 mmol) at room temperature. After stirring at 25 °C for 1 h, the reaction mixture was directly purified by flash chromatography (EtOAc/*n*-hexane=2:1 and then MeOH/CH_2Cl_2=1:9) to give **17d** (32.5 mg, 98%) and **18c**. **17d**: TLC (MeOH/CH_2Cl_2=1:9) R_f=0.39; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J=8.1 Hz, 2H), 7.86 (d, J=8.4 Hz, 2H), 4.54 (t, J=5.7 Hz, 1H), 4.41 (t, J=5.7 Hz, 2H), 3.72 (s, 3H), 3.50 (t, J=5.9 Hz, 2H), 3.43 (dd, J=7.5, 5.3 Hz, 1H), 3.06 (q, J=6.5 Hz, 2H), 1.78–1.30 (m, 6H); HRMS (FAB+) for $C_{17}H_{24}F_3N_2O_6S$ (MH^+), calcd 441.1307, found 441.1321. **18c**: TLC (EtOAc/*n*-hexane=2:1) R_f=0.30; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, J=8.4 Hz, 2H), 7.88 (d, J=8.4 Hz, 2H), 4.06 (t, J=5.4 Hz, 2H), 3.40 (t, J=5.3 Hz, 2H); HRMS (FAB+) for $C_9H_{10}F_3O_3S$ (MH^+), calcd 255.0303, found 255.0303.

3.1.21. Deprotection of Fmoc-Lys(Tsc)-OMe with DIEA. To a solution of **4b** (66.3 mg, 0.10 mmol) in dry DMF (1 mL) was added *N*,*N*-diisopropylethylamine (87.1 μL, 0.50 mmol) at room temperature. After heating at 50 °C for 4 h, the reaction mixture was quenched with H_2O and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/CH_2Cl_2=1:9) to give **17d** as a white solid (38.3 mg, 87%). TLC (MeOH/CH_2Cl_2=1:9) R_f=0.39; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J=8.1 Hz, 2H), 7.86 (d, J=8.4 Hz, 2H), 4.47 (t, J=5.4 Hz, 1H), 4.41 (t, J=5.9 Hz, 2H), 3.73 (s, 3H), 3.50 (t, J=5.9 Hz, 2H), 3.43 (dd, J=7.5, 5.4 Hz, 1H), 3.06 (q, J=6.5 Hz, 2H), 1.78–1.38 (m, 6H); HRMS (FAB+) for $C_{17}H_{24}F_3N_2O_6S$ (MH^+), calcd 441.1307, found 441.1305.

3.2. Peptide synthesis

Peptides **20** and **21** were synthesized on the Fmoc-Rink Amide MBHA resin in a stepwise fashion by a manual solid-phase method as described.[1,11] Fmoc-Rink Amide MBHA resin (loading 0.64 mmol/g resin, copolystyrene-1% DVB, 100–200 mesh, 31.3 mg, 20 μmol, Novabiochem, La Jolla, CA) was placed in a glass reaction vessel (5 mL, fitted with a glass frit (G3, 20–30 μm, Iwaki)) and swollen in CH_2Cl_2 for 5 min followed by drainage for use with standard Fmoc chemistry. Amino acids (50 μmol for **4c**, **4d**, and Tsc-Ala-OH or 60 μmol for other amino acids) were activated with PyBOP (31.2 mg, 60 μmol) in DMF (2 mL) and coupled in the presence of *N*,*N*-diisopropylethylamine (DIEA, 13.9 μL, 80 μmol) with shaking (wrist action shaker, Burrell) at room temperature for 3 h. Deprotection of the Fmoc group was performed using 50% (v/v) piperidine for 10 min or 50% (v/v) 1-methylpyrrolidine for 1 h in DMF with shaking at room temperature. Deprotection of the Tsc group was performed using 50% (v/v) piperidine for 10 min or 0.1 N LiOH in cooled (4 °C) THF/H_2O (1:1) for 10 min (or ×2) with shaking at room temperature. Unreacted free amines were capped by acetylation with acetic anhydride (9.4 μL, 100 μmol) and DIEA (17.4 μL, 100 μmol) in CH_2Cl_2 (2 mL) with shaking at room temperature for 15 min. All peptides were

acetylated at the N terminus and amidated at the C terminus. All couplings were monitored using a ninhydrin assay. A solvent wash (5 mL×3) with DMF, MeOH, CH_2Cl_2, and then DMF was performed right after deprotection, coupling, and capping. An additional CH_2Cl_2 wash was employed right before and after capping. Peptide resin cleavage and side-chain deprotection were performed in a single step using trifluoroacetic acid (TFA, 1 mL) at room temperature for 2 h. After evaporation of the solvent or precipitation with ether followed by centrifugation, crude peptides were dissolved in DMF (1 mL). HPLC purification was achieved using a C_{18} reverse-phase column (10×250 mm, 5 μm particle size, TP silica, Vydac, Hesperia, CA) with a linear H_2O/MeCN gradient containing 0.1% (v/v) TFA: 0–5 min 100% H_2O, 5–35 min 3.33% MeCN/min, 3 mL/min flow rate, 210/254 nm. Peptides were recovered upon lyophilization of the appropriate fractions as a solid (Scheme 3 and Figs. 4 and 5): **20** via **19a**, method A, 2.6 mg, 18% recovery; **20** via **19a**, method B, 4.2 mg, 29%; **20** via **19b**, method A, 2.3 mg, 15%; **20** via **19b**, method B, 2.5 mg, 17%; **21** via **19b**, method C, 9.6 mg, 40% (LiOH for Tsc deprotection) and 15.1 mg, 63% (piperidine for Tsc deprotection, Fig. 5). Purity of the peptide was determined to be >98% by analytical HPLC on a C_{18} reverse-phase column (4.6×250 mm, 5 μm particle size, TP silica, Vydac, Hesperia, CA) with a linear H_2O/MeCN gradient containing 0.1% (v/v) TFA: 0–5 min 100% H_2O, 5–35 min 3.33% MeCN/min, 1 mL/min flow rate, 210/254 nm, R_v=22.50 mL (**20**) and 23.09 mL (**21**). The molecular mass of each peptide was measured using MALDI-TOF mass spectrometry: **20**, $C_{39}H_{49}N_7O_7Na/C_{39}H_{49}N_7O_7K$ (MNa$^+$)/(MK$^+$), calcd 750.3586/766.3325, found 750.8125/766.7810; **21**, $C_{57}H_{79}N_{13}O_{16}Na/C_{57}H_{79}N_{13}O_{16}K$ (MNa$^+$/MK$^+$), calcd 1224.5660/1240.5399, found 1224.4465/1240.4049.

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References and notes

1. (a) Choi, J. S.; Lee, Y.; Kim, E.; Jeong, N.; Yu, H.; Han, H. *Tetrahedron Lett.* **2003**, *44*, 1607–1610. (b) Choi, J. S.; Lee, H.-S.; Lee, Y.; Jeong, N.; Kim, H.-J.; Kim, Y.-D.; Han, H. *Tetrahedron Lett.* **2002**, *43*, 4295–4299.
2. (a) Liebler, E. K.; Diederichsen, U. *Org. Lett.* **2004**, *6*, 2893–2896. (b) Bosques, C. J.; Imperiali, B. *J. Am. Chem. Soc.* **2003**, *125*, 7530–7531. (c) Stephenson, K. A.; Banerjee, S. R.; Besanger, T.; Sogbein, O. O.; Levadala, M. K.; McFarlane, N.; Lemon, J. A.; Boreham, D. R.; Maresca, K. P.; Brennan, J. D.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 8598–8599. (d) Opatz, T.; Liskamp, R. M. J. *Org. Lett.* **2001**, *3*, 3499–3502. (e) Yang, R.; Prorok, M.; Castellino, F. J.; Weliky, D. P. *J. Am. Chem. Soc.* **2004**, *126*, 14722–14723. (f) Hamzé, A.; Martinez, J.; Hernandez, J.-F. *J. Org. Chem.* **2004**, *69*, 8349–8402.
3. (a) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, NY, 1999. (b) Albericio, F. *Biopolymers* **2000**, *55*, 123–139. (c) Orain, D.; Ellard, J.; Bradley, M. *J. Comb. Chem.* **2002**, *4*, 1–16. (d) Díaz-Mochón, J. J.; Bialy, L.; Bradley, M. *Org. Lett.* **2004**, *6*, 1127–1129. (e) Bycroft, B. W.; Chan, W. C.; Chhabra, S. R.; Teesdale-Spittle, P. H.; Hardy, P. M. *J. Chem. Soc., Chem. Commun.* **1993**, 776–777. (f) Bycroft, B. W.; Chan, W. C.; Chhabra, S. R.; Hone, N. D. *J. Chem. Soc., Chem. Commun.* **1993**, 778–779. (g) Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294–308. (h) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1997**, *119*, 2301–2302. (i) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1998**, *120*, 2690–2691. (j) Carpino, L. A.; Mansour, E. M. E. *J. Org. Chem.* **1999**, *64*, 8399–8401. (k) Planas, M.; Bardají, E.; Jensen, K. J.; Barany, G. *J. Org. Chem.* **1999**, *64*, 7281–7289. (l) Kim, B. M.; Cho, J. H. *Tetrahedron Lett.* **1999**, *40*, 5333–5336.
4. Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6141–6146.
5. Bodanszky, M.; Deshmane, S. S.; Martinez, J. *J. Org. Chem.* **1979**, *44*, 1622–1625.
6. (a) Young, T.; Kiessling, L. L. *Angew. Chem., Int. Ed.* **2002**, *41*, 3449–3451. (b) Liu, S.; Dockendorff, C.; Taylor, S. D. *Org. Lett.* **2001**, *3*, 1571–1574. (c) Burke, T. R., Jr.; Symth, M. S.; Otaka, A.; Roller, P. P. *Tetrahedron Lett.* **1993**, *34*, 4125–4128. (d) Ye, B.; Akamatsu, M.; Shoelson, S. E.; Wolf, G.; Giorgetti-Peraldi, S.; Yan, X.; Roller, P. P.; Burke, T. R., Jr. *J. Med. Chem.* **1995**, *38*, 4270–4275. (e) Ueki, M.; Amemiya, M. *Tetrahedron Lett.* **1987**, *28*, 6617–6620. (f) Li, X.; Kawakami, T.; Aimoto, S. *Tetrahedron Lett.* **1998**, *39*, 8669–8672. (g) Bu, X.; Xie, G.; Law, C. W.; Guo, Z. *Tetrahedron Lett.* **2002**, *43*, 2419–2422. (h) Bu, X.; Wu, X.; Xie, G.; Guo, Z. *Org. Lett.* **2002**, *4*, 2893–2895. (i) Wu, X.; Bu, X.; Wong, K. M.; Yan, W.; Guo, Z. *Org. Lett.* **2003**, *5*, 1749–1752.
7. (a) Farrera-Sinfreu, J.; Royo, M.; Albericio, F. *Tetrahedron Lett.* **2002**, *43*, 7813–7815. (b) Intermolecular Michael-like addition is indicated by the absence of byproducts containing the 2-(4-trifluoromethylphenylsulfonyl)ethyl group on the ε-amino function of lysine when **4a** in **19a** was treated with 1-methylpyrrolidine in the solid-phase synthesis of peptide **20** (Fig. 4, II).
8. Smith, M. B.; March, J. *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 5th ed.; Wiley: New York, NY, 2001; pp 1331–1332.
9. Both Tsc and Ttc are also acid-resistant as indicated by the observation that Tsc-Phe-OEt **3a** and Ttc-Phe-OEt were intact even after their treatment (20 mM) with 90% TFA in CH_2Cl_2 at room temperature for 8 h.
10. Vedejs, E.; Kongkittingam, C. *J. Org. Chem.* **2000**, *65*, 2309–2318.
11. Kent, S. B. H. *Annu. Rev. Biochem.* **1988**, *57*, 957–989.