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# Trichloroethyl Group As a Protecting Group for Sulfonates and Its Application to the Synthesis of a Disulfonate Analog of the Tyrosine Sulfated PSGL-$1_{43-50}$ Peptide

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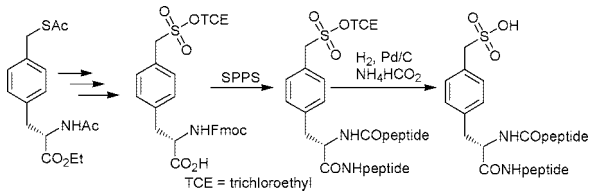
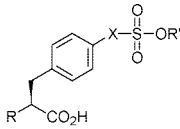



The trichloroethyl (TCE) group is shown to be a viable protecting group for sulfonates. TCE-protected sulfonates were found to be particularly stable to acid, a key characteristic that led to a straightforward enantioselective synthesis of L-FmocPhe(*p*-$CH_2SO_3TCE$)OH. This was used as a building block for the solid phase synthesis of an octapeptide corresponding to P-selectin glycoprotein ligand-1 residues 43−50 (PSGL-$1_{43-50}$) in which sulfotyrosine residues **46** and **48** were replaced with (sulfonomethyl)phenylalanine (SmP), an important hydrolytically stable sulfotyrosine mimic.

The sulfonic acid group has been used as an isostere for the phosphate and carboxylate groups in drug development[1a−e] and is a vital constituent of antagonists of follicle stimulating hormone[2] and P2 purinergic receptors.[3] Due to its highly polar nature, the sulfonic acid moiety is usually protected during multistep syntheses as this facilitates the handling and purification of the sulfonylated intermediates and minimizes side reactions. Sulfonic acids are most commonly protected as alkyl esters (mainly isopropyl,[2,4] isobutyl,[5] and neopentyl[1b,c,6,7]). As with any protecting group, these alkyl ester protecting groups have limitations.[5,6a,b] Due to the paucity of sulfonic acid protecting groups, the development of additional protecting groups, especially ones that exhibit chemical properties that are distinct from those already developed, is highly desirable.




**1** (Smp), R = $NH_2$, R' = H, X = $CH_2$
**2**, R = NHFmoc, R' = H, X = $CH_2$
**3**, R = NHFmoc, R' = nPt, X = $CF_2$
**4**, R = NHFmoc, R' = DCV, X = O
**5**, R = NHFmoc, R' = TCE, X = $CH_2$

**FIGURE 1.** Structure of compounds **1**−**5**.

Our interest in sulfonic acids and their protecting groups stems from our desire to construct peptides bearing (sulfonomethyl)phenylalanine (**1**, SmP, Figure 1). Over the last 20 years, there has been substantial interest in this amino acid as a stable replacement of sulfotyrosine (sTyr) and as a monoanionic phosphotyrosine mimic.[1b,8,9a−h]

In general, Smp is incorporated into peptides using solid phase peptide synthesis (SPPS) methodology and Fmoc-protected amino acid **2** in which the sulfonate group is unprotected (Figure 1).[9e−g] However, there are significant drawbacks to using **2** for the synthesis of Smp-bearing peptides. First, an efficient enantioselective synthesis of this amino acid has yet to be developed.[9e,g] It is commercially available but extremely expensive.[10] Most importantly, the synthesis of Smp-bearing peptides using amino acid **2** can sometimes be highly problematic and low yielding.[9g]

Several years ago we reported the synthesis of peptides bearing (difluoromethylsulfono)phenylananine ($F_2$Smp) using amino acid **3** as a building block (Figure 1). The sulfonate group in compound **3** is protected with a neopentyl group (nPt).[1c,6c] The desired peptides bearing $F_2$Smp were obtained in good yield upon removal of the neopentyl group from the sulfonate-protected peptides using aq. acid or LiBr in refluxing butanone. More recently, we have shown that sulfotyrosine-bearing peptides can be readily synthesized in good yield employing

(1) For some examples see: (a) Hellmuth, K.; Grosskopf, S.; Lum, C. T.; Wurtele, M.; Roder, N.; von Kries, J. P.; Rosario, M.; Rademann, J.; Birchmeirer, W. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 7275. (b) Hussain, M.; Ahmed, V.; Hill, B.; Ahmed, Z.; Taylor, S. D. *Bioorg. Med. Chem.* **2008**, *15*, 6764. (c) Leung, C.; Lee, J.; Meyer, N.; Jia, C.; Grzyb, J.; Liu, S.; Hum, G.; Taylor, S. D. *Bioorg. Med. Chem.* **2002**, *10*, 2309. (d) Bischoff, L.; David, C.; Roques, B.; Fournié-Zaluski, M.-C. *J. Org. Chem.* **1999**, *64*, 1420. (e) David, C.; Bischoff, L.; Meudal, H.; Mothe, A.; De Mota, N.; DaNascimento, S.; Llorens-Cortes, C.; Fournié-Zaluski, M.; Roques, B. *J. Med. Chem.* **1999**, *42*, 5197.

(2) Wrobel, J; Rogers, J.; Green, D.; Kao, W. L. *Synth. Commun.* **2002**, *32*, 2695.

(3) Yan, L.; Muller, C. E. *J. Med. Chem.* **2004**, *47*, 1031.

(4) Musicki, B.; Widlanski, T. S. *J. Org. Chem.* **1990**, *55*, 4231.

(5) Xie, M.; Widlanski, T. S. *Tetrahedron Lett.* **1996**, *37*, 4443.

(6) (a) Truce, W. E.; Vrencur, D. J. *J. Org. Chem.* **1970**, *35*, 1226. (b) Roberts, J. C.; Gao, H.; Gopalsamy, A.; Kongsjahju, A.; Patch, R. J. *Tetrahedron Lett.* **1997**, *38*, 335. (c) Liu, S.; Dockendorf, C.; Taylor, S. D. *Org. Lett.* **2001**, *3*, 1571–1574.

(7) Derivatives of the neopentyl ester protecting group, which are removed using the safety-catch principle, have also been developed See: (a) Seeberger, S.; Griffin, R. J.; Hardcastle, I. R.; Golding, B. T. *Org. Biomol. Chem.* **2007**, *5*, 132. (b) Reference 6b.

(8) Burke, T. R.; Lee, K. *Acc. Chem. Res.* **2003**, *36*, 426.

(9) (a) Marseigne, I.; Roques, B. P. *J. Org. Chem.* **1988**, *53*, 3621. (b) Marseigne, I.; Roy, P.; Dor, A.; Durieux, C.; Peleprat, D.; Reibaud, M.; Blanchard, J. C.; Roques, B. P. *J. Med. Chem.* **1989**, *32*, 445. (c) Gonzalez-Muniz, R.; Cornille, F.; Bergeron, F.; Ficheux, D.; Pother, J.; Durieux, C.; Roques, B. P. *Int. J. Peptide Protein Res.* **1991**, *37*, 331. (d) Dorville, A.; McCort,-Tranchepain, I.; Vichard, D.; Sather, W.; Maroun, R.; Ascher, P.; Roques, B. P. *J. Med. Chem.* **1992**, *35*, 2551. (e) Miranda, M. T. M.; Liddle, R. A.; Rivier, J. E. *J. Med. Chem.* **1993**, *36*, 1681. (f) Teresa, M.; Mirand, M.; Craig, A. G.; Miller, C.; Liddle, R. A.; Riveir, J. E. *J. Protein Chem.* **1993**, *12*, 533. (g) Herzner, H.; Kunz, H. *Tetrahedron* **2007**, *63*, 6423. (h) Lam, S. N.; Acharya, P.; Wyatt, R.; Kwong, P. D.; Bewley, C. A. *Bioorg. Med. Chem.* **2008**, *16*, 10113.

(10) Amino acid **2** is commercially available from several companies for $900−1000 USD for 1 g.

10.1021/jo900122c CCC: $40.75 © 2009 American Chemical Society
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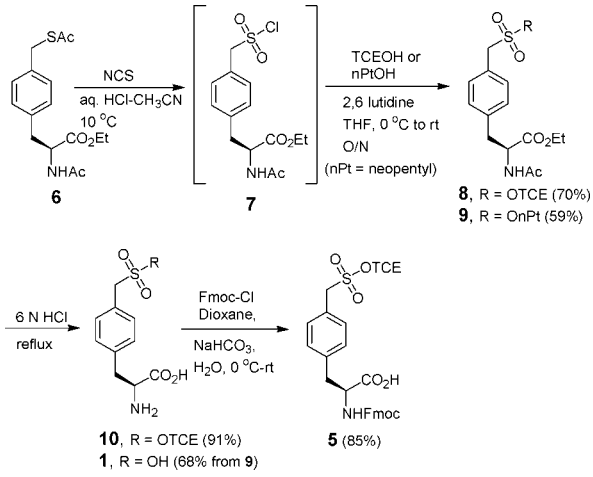
**SCHEME 1**



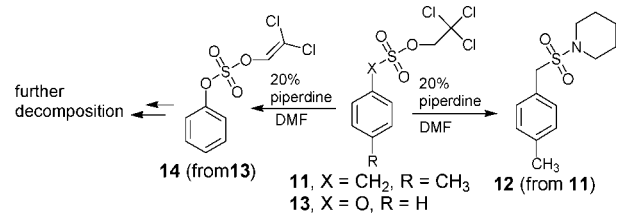

FmocTyr($SO_3$DCV)OH (**4**, Figure 1) (DCV = dichlorovinyl) as a building block. To avoid loss of the DCV group during Fmoc removal, 2-methylpiperidine (2-MP), a more sterically hindered base than piperidine, the most commonly used base for Fmoc removal, was used.[11] The DCV group was removed by hydrogenolysis after cleavage of the peptide from the support.[11]

These ease with which sulfonate- and sulfate-protected amino acids **3** and **4** were incorporated into peptides has prompted us to examine whether a similar approach could be used for the synthesis of peptides bearing Smp. Here we report an efficient synthesis of a derivative of amino acid **2**, in which the sulfonate group is protected with a trichloroethyl (TCE) group (compound **5**, Figure 1). We demonstrate that TCE group is a viable protecting group for sulfonates and such protected sulfonates are remarkably stable to acid, a key characteristic that led to a straightforward synthesis of compound **5**. We also show that TCE-protected sulfonates exhibit different reactivity patterns compared to TCE-protected sulfates when subjected to organic bases. Finally, we show that amino acid **5** is an effective building block for the solid phase synthesis of a disulfonate analog of the tyrosine sulfated PSGL-$1_{43-50}$ peptide.

Our synthesis of a sulfonate-protected version of amino acid **2** is shown in Scheme 1. It begins with the oxidative chlorination of readily prepared amino acid **6**.[12] We previously reported that thioester **6** can be converted into sulfonyl chloride **7** by oxidative chlorination using $Cl_2$ gas in $CH_2Cl_2-H_2O$.[12] Very recently, Nishiguchi et al. reported the synthesis of sulfonyl chlorides from thioesters in high yield using *N*-chlorosuccinimide (NCS) in aq. HCl-MeCN.[13] This procedure has the advantage of not requiring $Cl_2$ gas, a toxic and difficult substance to work with. We applied Nishiguchi et al.'s procedure to thioester **6**. $^1$H NMR of the crude reaction mixture, after a brine wash, indicated that sulfonyl chloride **7** was produced relatively cleanly with the main contaminant being succinimide and this material was used without purification for the next step.

We attempted to prepare derivatives of amino acid **2** with the sulfonate moiety protected with either a neopentyl or a TCE group. The neopentyl group is best removed under nucleophilic conditions such as tetramethylammonium chloride in DMF at

**SCHEME 2**




160 °C[6b] or LiBr in refluxing butanone.[6c] The TCE group has never been examined as a protecting group for sulfonate esters; however, we have found that this moiety is a highly effective protecting group for sulfate esters and is removed under very mild reducing conditions.[14] Reaction of crude **7** with 5.0 equiv of 2,2,2-trichloroethanol or with 5.0 equiv of neopentyl alcohol in the presence of 4.0 equiv of 2,6-lutidine gave esters **8** and **9** in 70 and 59% yield, respectively (two steps). A key and potentially problematic step in this synthesis is the required selective deprotection of the carboxylic acid and amino functionalities without loss of the sulfonate protecting group. We were pleased to find that this could be achieved with compound **8** by refluxing **8** in 6 N HCl for 12 h, which gave amino acid **10** in a 91% yield. This reaction demonstrates the remarkable stability of TCE-protected sulfonates to acid. However, subjecting neopentyl ester **9** to the same conditions resulted in hydrolysis of the neopentyl ester to give Smp (**1**) in 68% yield. We were unable to find conditions that would allow us to selectively remove the ethyl and acetyl groups without removing the neopentyl group. Amino acid **5** was obtained in an 85% yield from **10** by reacting **10** with Fmoc-Cl under standard Schotten-Baumann conditions.[15]

Before attempting to prepare peptides using amino acid **5**, we determined conditions for removing the TCE group and examined the stability of TCE sulfonate esters by subjecting model sulfonate **11** (Scheme 2) to a variety of conditions. The TCE group in **11** was readily removed using mild reducing conditions such as Zn/ammonium formate in MeOH, Zn in HOAc and hydrogenolysis ($H_2$ or ammonium formate with 10% Pd/C). Not surprisingly, compound **11** is stable to 100% TFA with no detectable reaction occurring even after several days and to TFA containing scavenging reagents such as triisopropylsilane (TIPS), anisole and thioanisole. It is also stable to mild reducing agents such as $NaBH_4$. It is stable to sterically hindered organic bases such as triethylamine but slowly decomposes in the presence of an excess of stronger or less sterically hindered organic bases. For example, $^1$H NMR studies in 20% piperidine/DMF-$d_7$ revealed that ester **11** undergoes nucleophilic attack by piperidine on the sulfur atom to give compound **12** (Scheme 2) which was characterized by mass spectrometry and $^1$H NMR. Compound **11** was completely consumed within 24 h. This is in contrast to aryl sulfate **13** which we have shown first undergoes a rapid elimination of HCl to give DCV ester **14** followed by a slower attack by piperidine on the sulfur atom and subsequent formation of decomposition products (Scheme 2).[11] We have also demonstrated that although 20% 2-MP in DMF also converts sulfate ester **13** into DCV sulfate ester **14**; no further reaction between 2-MP and ester **14** occurs even after

(11) Ali, A.; Taylor, S. D. *Angew. Chem., Int. Ed.* **2009**, *48*, 2024.
(12) Hill, B; Ahmed, V.; Bates, D.; Taylor, S. D. *J. Org. Chem.* **2006**, *71*, 8190.
(13) Nishiguchi, A.; Maeda, K.; Miki, S. *Synthesis* **2006**, 4131.
(14) (a) Liu, Y.; Lien, I-F.; Ruttgaizer, S.; Dove, P.; Taylor, S. D. *Org. Lett.* **2004**, *6*, 209–212. (b) Ingram, L.; Taylor, S. D. *Angew. Chem., Int. Ed.* **2006**, *45*, 3503–3506.
(15) The enantiopurity of **5** was found to be >97% as determined by the synthesis of diastereomeric peptides followed by HPLC analysis. See the Supporting Information.

several days.[11] $^1$H NMR studies of ester **11** in 20% 2-MP/DMF-$d_7$ revealed that although attack of 2-MP on the sulfur atom still occurs, the reaction is considerably slower compared to when piperidine is used and even after 8 h only 8% of **11** had reacted. These results suggested to us that amino acid **5** could be used in SPPS if 2-MP was used as the base for Fmoc removal. To determine if this was possible we prepared an octapeptide, Ac-Ala-Thr-Glu-Phe(*p*-$CH_2SO_3^-$)-Glu-Phe(*p*-$CH_2SO_3^-$)-Leu-Asp-$NH_2$ (**15**), which corresponds to residues 43−50 of P-selectin glycoprotein ligand-1 (PSGL-1) in which the sTyr residues at positions 46 and 48 are replaced with Smp. This was chosen as a model peptide since Herzner and Kunz reported difficulties in preparing a protected version of **15** using sulfonate-unprotected amino acid **2**.[9g] Automated SPPS was performed using the Rink amide resin and HBTU/HOBt for the coupling reactions. After the coupling of each Fmoc amino acid the peptide was subjected to 3 × 10 min of 20% 2-MP/DMF as opposed to the standard 2 × 10 min protocol when piperidine is used.[16,17] The completed peptide was cleaved from the resin using 98% TFA/2% TIPS then precipitated in ether. The HPLC chromatogram of the crude peptide consisted of one major peak plus a few minor peaks.[18] The $^-$ESI mass spectrum of the crude peptide indicated that the major product in the crude mixture was desired TCE-protected peptide Ac-Ala-Thr-Glu-Phe(*p*-$CH_2SO_3$TCE)-Glu-Phe(*p*-$CH_2SO_3$TCE)-Leu-Asp-$NH_2$ (**16**) and no peaks corresponding to peptides that had undergone substitution of the TCE group with 2-MP were detected.[19] After subjecting crude peptide **16** to $H_2$ (balloon), 30 wt. % of 10% Pd/C, and 15 equiv of ammonium formate for 6 h, HPLC analysis of the crude reaction mixture revealed one major peak plus a variety of minor peaks. Purification by semipreparative RP-HPLC gave pure peptide **15** in a 60% yield in 95% purity. We also prepared peptide **15** using the identical procedure except sulfonate-unprotected amino acid **2** was used as a building block. Amino acid **2** was prepared in 94% yield from compound **5** as its free acid by subjecting **5** to Zn/ammonium formate in MeOH. HPLC analysis of the crude peptide showed many peaks[18] and after a challenging purification using semipreparative RP-HPLC pure peptide **16** was obtained in only a 19% yield.

In summary, an efficient synthesis of amino acid **5** was achieved in which the sulfonate group is protected with a trichloroethyl group. A key step in this synthesis was the selective hydrolysis of the acetamide and ethyl esters in the presence of the TCE-protected sulfonate using refluxing 6 N HCl which demonstrates the exceptional stability of TCE-protected sulfonates to strong acid. We also showed that TCE-protected sulfonates are stable to a variety of conditions but are not stable to an excess of organic bases such as piperidine. We demonstrated that amino acid **5** is an effective building block for the solid phase synthesis of Smp-bearing peptides when using 2-MP as base and that this approach provided the targeted peptide in higher yield compared to when sulfonate-unprotected amino acid **2** was used. We expect that the TCE group will be useful as a protecting group for sulfonates in general and will be especially effective in situations where stability to strongly acidic conditions is required.

## Experimental Section

**AcPhe(p-$CH_2SO_3$TCE)OEt (8).** To a mixture of $CH_3CN$ and 2N HCl (30 mL, 4:1) was added *N*-chlorosuccinimide (NCS, 4.7 g, 35.1 mmol), and the mixture was cooled to 10 °C and stirred until most of the NCS dissolved. To this was added dropwise a solution of thioester **6**[12] (3.0 g, 9.3 mmol) in $CH_3CN$ (15 mL) over 10 min. The solution was stirred for 40 min at 10 °C, diluted with $Et_2O$ (350 mL) and washed with 12% NaCl (3 × 30 mL). The organic layer was dried ($Na_2SO_4$) and concentrated to give **7** as an off-white solid. The crude sulfonyl chloride was dissolved in dry THF (60 mL). To this was added 2,2,2-trichloroethanol (4.45 mL, 46.5 mmol) and the mixture was cooled to 0 °C (ice bath). A solution of 2,6-lutidine (8.09 mL, 37.2 mmol) in dry THF (60 mL) was added dropwise over 10 min. The ice bath was removed and the mixture was allowed to come to rt and stirred for 16 h. The mixture was diluted with EtOAc (300 mL) and washed with 1.0 N HCl, sat. brine, and the organic layer was dried ($Na_2SO_4$) and concentrated. The residue was subjected to flash chromatography (50% EtOAc−50% hexane) which gave pure **8** as a white solid (3.08 g, 70%). Mp 96−98 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.38 (2H, d, $J$ = 8.1 Hz), 7.18 (2H, d, $J$ = 8.1 Hz), 6.08 (1H, d, $J$ = 7.6 Hz), 4.86 (1H, q, $J$ = 6.0 Hz), 4.50 (2H, s), 4.48 (2H, s), 4.17 (2H, dq, $J$ = 7.1, 1.0 Hz), 3.14 (2H, dq, $J$ = 13.8, 6.0 Hz), 1.99 (3H, s), 1.25 (3H, t, $J$ = 7.1 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 171.3, 169.6, 137.6, 130.9, 129.9, 125.5, 93.4, 77.7, 61.5, 57.3, 53.0, 37.6, 23.0, 14.0; LREIMS *m*/*z* (relative intensity): 459 ($M^+$, 2), 461 ($M^+$, 2), 189 (100); HREIMS calculated for $C_{16}H_{20}Cl_3NO_6S$: 459.0077 found 459.0085

**Phe(p-$CH_2SO_3$TCE)OH (10).** Sulfonate **8** (2.70 g, 5.6 mmol) was refluxed in 6 N HCl (50 mL) for 12 h. The mixture was allowed to cool and concentrated by high vacuum rotary evaporation to dryness. EtOH (100 mL) was added followed by 11 mL propylene oxide. The mixture was stirred for 16 h and filtered to give pure **10** as a white solid (1.88 g). The filtrate was concentrated, and $CHCl_3$ was added. The mixture was stirred for 30 min then filtered, which yielded an additional 0.157 g of pure **10**. The total yield was 2.03 g (91%). Mp 207 °C (dec.); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.39 (2H, d, $J$ = 8.1 Hz), 7.31 (2H, d, $J$ = 8.1 Hz), 4.98 (2H, s), 4.90 (2H, s), 3.68−3.64 (1H, m), 3.14 (1H, dd, $J$ = 14.4, 4.9 Hz), 2.95 (1H, dd, $J$ = 14.3, 7.5 Hz); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 169.9, 137.6, 131.0, 129.7, 126.2, 94.0, 78.0, 55.2, 54.6, 36.3; LRESI$^+$MS *m*/*z* (relative intensity): 390 ([M+H]$^+$, 100), 392 ([M + H]$^+$, 95); HRESI$^+$MS calculated for $C_{12}H_{15}NO_5SCl_3$ (M + H)$^+$ 389.9737 found 389.9744

**FmocPhe(p-$CH_2SO_3$TCE)OH (5).** To a solution of amino acid **10** (2.10 g, 5.38 mmol) in dioxane (30 mL) and 10% $Na_2CO_3$ (40 mL) was added Fmoc-Cl (2.18 g, 8.07 mmol) and the mixture was stirred for 20 h. The reaction was acidified to pH 2−3 using 1 N HCl and the mixture extracted with EtOAc, dried ($MgSO_4$) and concentrated. The crude material was subjected to gravity chromatography (1% MeOH-99% $CH_2Cl_2$ then 2.5% MeOH-97.5% $CH_2Cl_2$ then 5% MeOH-95% $CH_2Cl_2$ then 10% MeOH-90% $CH_2Cl_2$) which gave pure **5** as a white amorphous solid (2.81 g, 85%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.75 (2H, d, $J$ = 7.3 Hz); 7.53 (2H, d, $J$ = 6.8 Hz), 7.16−7.41 (10H, m), 5.19 (1H, d, $J$ = 8.4 Hz), 4.69 (1H, bs), 4.33−4.52 (6H, m), 4.17 (1H, app. t, $J$ = 7.3 Hz), 3.06−3.25 (2H, m); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 174.8, 158.1, 145.0, 142.4, 139.8, 132.1, 130.8, 128.7, 128.1, 127.3, 126.22, 126.17, 120.9, 95.0, 79.3, 67.9, 57.5, 56.5, 48.1, 38.2; LR$^+$ESIMS *m*/*z* (relative intensity): 614 (M + H, 100); HR$^+$ESIMS calculated for $C_{27}H_{25}NO_7SCl_3$ (M + H)$^+$ 612.0419, found 612.0417.

**FmocPhe(p-$CH_2SO_3$H)OH (2).** To a solution of amino acid **5** (1.0 g, 1.63 mmol) in 3 mL of HPLC grade methanol was added

(16) We have been able to obtain 2.5 kg of **2**-MP from a commercial supplier for $180.00 USD, and this could be used without further purification.

(17) Hachmann and Lebl have shown that Fmoc deprotection of FmocIle attached to chlorotrityl resin using **2**-MP occurred with a half-life that was 1.5 times greater than when piperidine was used. Hence, we have increased the Fmoc deprotection times by 1.5 times. See: Hachmann, J.; Lebl, M. *J. Comb. Chem* **2006**, *8*, 149.

(18) See the Supporting Information.

(19) Hari and Miller have shown that resin-supported (Wang) sulfonates exhibit superior stability to organic bases compared to their solution counterparts. This "resin protection" may be an explanation as to why we do not see any substitution of the TCE groups with 2-MP during SPPS. See: Hari, A.; Miller, B. L. *Org. Lett.* **1999**, *1*, 2109.

ammonium formate (0.61 g, 9.78 mmol) followed by Zn dust (0.64 g, 9.78 mmol). After overnight stirring, the reaction mixture was filtered through Celite and concentrated under *vacuum*. Compound **2** was purified by flash chromatography using $CH_2Cl_2$/MeOH/ AcOH/$H_2O$ (50:8:1:1), and the solvent system was shifted to ($CH_2Cl_2$/MeOH/ AcOH /$H_2O$ 7:3:0.3:0.6) when compound **2** started to come off the column. This gave pure **2** as a white solid (0.73 g, 94%). Mp = 182−185 °C; $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 7.76 (2H, d, $J$ = 7.5 Hz); 7.59 (2H, d, $J$ = 7.2 Hz), 7.38−7.25 (6H, m), 7.15 (2H, d, $J$ = 7.8 Hz), 4.35−4.27 (2H, m), 4.24−4.18 (1H, m), 4.15−4.11 (1H, m),3.97 (2H, s), 3.14 (1H, dd, $J_1$ = 9.3 Hz and $J_2$ = 4.8 Hz), 2.94 (1H, dd, $J_1$ = 8.4 Hz and $J_2$ = 5.4 Hz); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 174.08, 156.13, 144.33, 144.21, 141.08, 136.60, 133.56, 130.32, 128.83, 128.02, 127.50, 125.81, 125.69, 120.49, 65.98, 57.63, 56.52, 47.01, 36.89; LR$^-$ESIMS *m*/*z* (relative intensity): 480 ([M − H]$^-$, 100), 481 (M, 32); HR$^-$ESIMS calculated for $C_{25}H_{22}NO_7S$ (M − H)$^-$ 480.1117, found 480.1104

**Peptide Synthesis.** Peptide syntheses were performed on Rink resin (35 mg, 0.71 mmol/g, preswollen in DMF, 3 × 10min, before use) using a Quartet automated peptide synthesizer from Protein Technologies. The first amino acid was attached as a pentafluorophenyl ester by adding a solution of Fmoc-Leu-OPfp (4.0 equiv) and HOBt (4.0 equiv) in DMF manually to the resin and reacted for 1.5 h. Subsequent amino acids were coupled as their free acids (Fmoc-AA-COOH) using HOBt/HBTU/DIPEA (1 × 1.5 h). The side chains of Asp and Glu were protected as their t-butyl esters. The side chain of Thr was protected as a *t*-butyl ether. All Fmoc deprotections were accomplished using 20% 2-methylpiperidine/ DMF (3 × 10 min). The last amino acid was acetylated using a 2:1 mixture of acetic anhydride/pyridine. The peptides were cleaved from the resin by subjecting the resin supported peptides to TFA: triisopropylsilane (2 mL, 98:2) for 2.5 h after which the mixture was filtered and the resin was washed with the cleavage cocktail. The filtrate was concentrated by rotary evaporation to half the volume and cold t-butylmethyl ether was added to precipitate the peptides. The mixture was centrifuged, the supernatant discarded and the pellet suspended in ether and centrifuged again. The supernatant was discarded, the pellet was dried under vacuum for 1 h and suspended in water and lyophilized. This gave 34.5 mg of crude peptide **16** (when using building block **5**) and 22.3 mg of crude peptide **15** (when using building block **2**).

**Synthesis of Peptide 15 by Removal of the TCE Groups from Peptide 16.** To a solution of crude peptide **16** (30 mg) in 3 mL of HPLC grade methanol was added ammonium formate (19.4 mg, 15 equiv), followed by 10% Pd/C (9 mg, 30% wt.). The reaction was fitted with a balloon filled with $H_2$, stirred at rt and the reaction was monitored by HPLC for the disappearance of peptide **16** ($CH_3CN/H_2O$ (0.1% TFA) as eluent, linear gradient from 5 to 95 $CH_3CN$ in 60 min). After 6 h, the reaction mixture was transferred into an Eppendorf tube and centrifuged to pellet the Pd/C. The supernatant was removed and the pellet washed two more times with 3 mL of methanol. The combined supernatants were concentrated under reduce pressure. Peptide **15** was purified using semipreparative RP-HPLC with a UV detector set at 220 $\lambda$. A linear gradient of 1%$CH_3CN$/99% 100 mM ammonium acetate (pH = 9) to 10% $CH_3CN$/90% 100 mM ammonium acetate over 60 min was used as eluent ($t_R$ = 39.3 min). Fractions containing peptide **15** were pooled, concentrated by high vacuum rotary evaporation, and the residue was dissolved in water and repeatedly lyophilized until a constant weight was obtained. Peptide **15** was obtained as a flocculent white powder (16.7 mg, 60% yield based on resin loading). Peptide **15** was 95% pure as determined by analytical RP-HPLC.[18] HR$^-$ESIMS: calculated for $C_{49}H_{68}N_6O_{22}S_2$ (M − H)$^-$ 1198.3920, found 1198.3917.

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**Supporting Information Available:** $^1$H and $^{13}$C NMR spectra of compounds **2**, **5** and **8**−**10** and the $^1$H NMR spectrum of compound **12**. Analytical HPLC chromatogram of peptides. Experimental for the synthesis of dipeptides for determining the enantiomeric excess of amino acid **5** and their analytical HPLC chromatograms and $^1$H NMR's. This material is available free of charge via the Internet at http://pubs.acs.org.

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