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Synthesis of Protected L-4-[Sulfono(difluoromethyl)]phenylalanine and Its Incorporation into a Peptide

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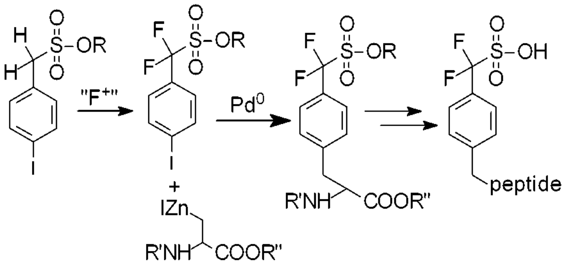
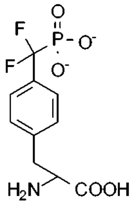
ABSTRACT



A protected form of L-4-[sulfono(difluoromethyl)]phenylalanine (F_2Smp), a novel non-hydrolyzable phospho- and sulfotyrosine mimetic, was synthesized via electrophilic fluorination of a benzylic sulfonate followed by a Pd-catalyzed cross-coupling reaction between the fluorinated sulfonate and the zincate of protected iodoalanine. F_2Smp was incorporated into a peptide using solid-phase peptide synthesis techniques.

The sulfation and phosphorylation−dephosphorylation of tyrosine residues in proteins are important post-translational events. Tyrosine sulfation is employed as a means of mediating protein−protein interactions, while tyrosine phosphorylation and dephosphorylation act as on−off switches in signal transduction pathways. Over the last several years there has been tremendous interest in developing inhibitors of proteins that are involved in these processes such as protein tyrosine kinases (PTKs),[1] protein tyrosine phosphatases (PTPases),[2] SH2 domain binding proteins,[3] and proteins that bind sulfotyrosine (sTyr).[4] Phosphono(difluoromethyl)phenylalanine (F_2Pmp, **1**) has been used extensively as a non-hydrolyzable phosphotyrosine mimetic for the development of inhibitors of enzymes that bind or hydrolyze phosphotyrosine (pTyr).[2] However, its sulfur analogue, L-[sulfono(difluoromethyl)]phenylalanine (F_2Smp, **2**) has never been reported. Recent studies with peptides bearing sulfotyrosine[5] and non-peptidyl compounds bearing the difluoromethylenesulfonic acid moiety[6] suggest that F_2Smp may be highly effective as a non-hydrolyzable, monoanionic, pTyr mimetic for obtaining inhibitors of therapeutically significant PTPases. It may also be useful as a pTyr mimetic for obtaining inhibitors of PTKs and SH2 domain binding proteins and as a sTyr mimetic for obtaining inhibitors of proteins that bind sTyr. In this letter, we describe an enantioselective synthesis of protected L-F_2Smp and the incorporation of L-F_2Smp into a peptide.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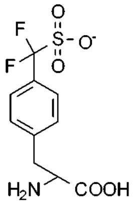
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The key steps in our approach to the synthesis of protected L-F_2Smp are outlined in Scheme 1. One is a palladium-

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(1) Al-Obeidi, F. A.; Lam, K. S. *Oncogene* **2000**, *19*, 5690−701.
(2) Ripka, W. C. *Ann. Rep. Med. Chem.* **2000**, *35*, 231−250.
(3) Cody, W. L.; Lin, Z.; Panek, R. L.; Rose, D. W.; Rubin, J. R. *Curr. Pharm. Des.* **2000**, *6*, 59.
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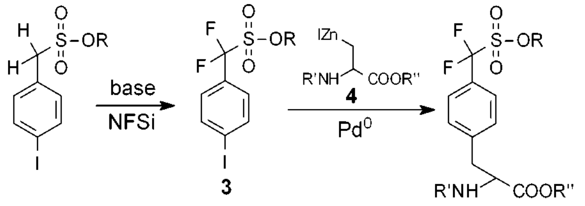
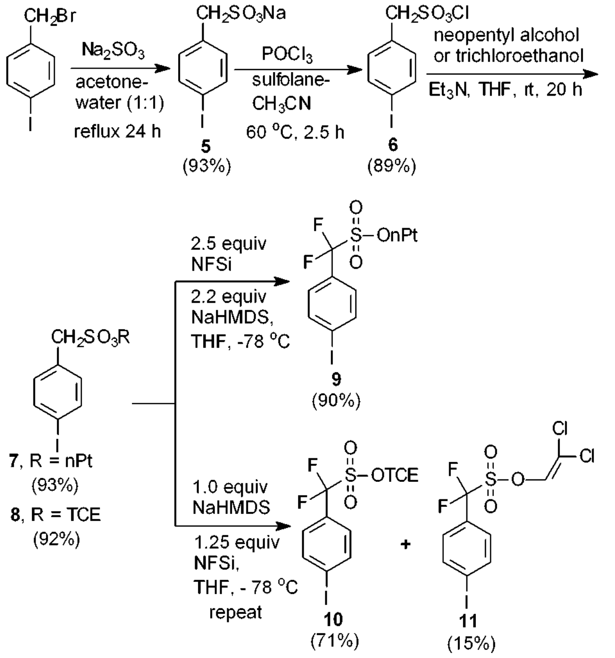
Scheme 1



Scheme 2



catalyzed cross-coupling reaction between suitably protected sulfonate **3** and the organozincate **4** to produce the fully protected L-F$_2$Smp. A similar approach has been used successfully for the enantioselective synthesis of protected L-F$_2$Pmp.[7] The other key step involves the synthesis of **3**. We anticipated that this could be accomplished using a procedure recently developed in our laboratory that involves reacting α-carbanions of sulfonate esters with *N*-fluorobenzenesulfonimide (NFSi), an electrophilic fluorinating agent.[8] Indeed, to our knowledge, this is the only method available for the preparation of benzylic α-fluorosulfonates.

During our earlier studies on electrophilic fluorination of benzylsulfonates it was found that the use of the neopentyl (nPt) protecting group was essential for obtaining good yields and that other protecting groups examined, ethyl and isopropyl, yielded only decomposition products.[8] The results with the ethyl and isopropyl esters were most likely due to deprotonation at the β-position of the protecting group followed by elimination of the sulfonate salt. However, at the time the present study was initiated, we were concerned that the nPt protecting group might be problematic since the conditions that had previously been used for its removal, tetramethylammonium chloride, DMF, 160 °C, 16 h[9] or LiBr in refluxing butanone for 48 h,[8] are quite harsh. Therefore, in addition to the nPt group, it was decided to also examine the trichloroethyl (TCE) group as a protecting group for the sulfonate moiety. The TCE group was chosen because it lacks β-protons that could interfere with the fluorination reaction, can be readily removed under mild conditions (e.g., Zn/HOAc, Cd/DMF) and has been used successfully as a phosphate and carboxylate protecting group in peptide synthesis.[10]

The synthesis of protected F$_2$Smp is outlined in Scheme 2. *p*-Iodobenzylbromide was transformed into the sulfonate salt **5**, which was then converted into the sulfonyl chloride **6** in overall high yield. Treatment of **6** with neopentyl alcohol or trichloroethanol in the presence of Et$_3$N in THF gave the nPt ester **7** and TCE ester **8** in excellent yields. Fluorination of the nPt ester was achieved in a single step by adding 3.0 equiv of NaHMDS in THF dropwise to a solution of 1.0 equiv of **7** and 2.5 equiv of NFSi in THF at −78 °C. After stirring at −78 °C for 2 h and room temperature for 2 h, the fluorinated product **9** was obtained in an excellent 90% yield. The fluorination of the TCE ester using similar conditions was less successful. In addition to the desired ester **10** (20−30% yield), an equivalent quantity of the chloride elimination product, **11**, was also obtained. None of the non-fluorinated or monofluorinated elimination products were formed. This suggested to us that the elimination reaction occurred only with the difluorinated product **10**. Therefore, we reasoned that the amount of byproduct could be reduced by performing the reaction in a stepwise manner by reacting **8** with 1.0 equiv of NaHMDS followed by the addition of a slight excess of NFSi and then repeating this process. When the reaction was performed in this manner, the yield of the desired ester **10** was increased to a respectable 71%, while the elimination product was obtained in a 15% yield.

Compounds **9** and **10** were coupled to Fmoc-3-iodo-L-alanine methylester using conditions similar to those developed by Burke and co-workers for the synthesis of protected L-F$_2$Pmp (Scheme 3).[7] Thus, a solution of 1 equiv of **9** or **10** in THF/DMAC (1:1) containing 5 mol % Cl$_2$Pd(PPh$_3$)$_2$ and 10 mol % DIBAL[11] in THF/DMAC was added to a solution of 2 equiv of the zincate **12**[7,12] in THF/DMAC (1:1), and the mixture was stirred at 65−70 °C for 6 h. This gave the nPt-protected and TCE-protected amino acids **13**

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(5) Desmarais, S.; Jia, Z.; Ramachandran, C. *Arch. Biochem. Biophys.* **1998**, *345*, 225.

(6) Kotoris, C. C.; Chen, M.-J.; Taylor, S. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3275−3280.

(7) Smyth, M. S.; Burke, T. R. *Tetrahedron Lett.* **1994**, *35*, 551.

(8) Kotoris, C. C.; Chen, M.-J.; Taylor, S. D. *J. Org. Chem.* **1998**, *63*, 8052.

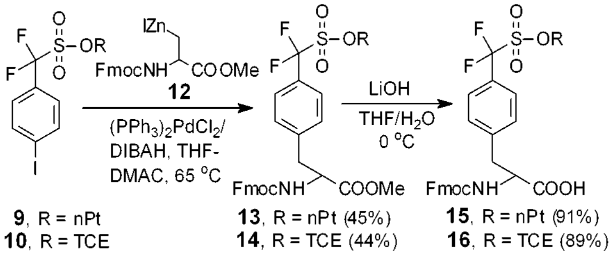
(9) Roberts, J. C.; Gao, H.; Gopalsamy, A.; Kongsjahju, A.; Patch, R. J. *Tetrahedron Lett.* **1997**, *38*, 335.

(10) For examples see: Paquet, A. *Int. J. Pept. Protein Res.* **1992**, *39*, 82. Mora, N.; Lacombe, J. M.; Pavia, A. A. *Int. J. Pept. Protein Res.* **1995**, *45*, 53.

(11) We found that the yields of the cross-coupling reactions could be slightly improved using Cl$_2$Pd(PPh$_3$)$_2$/DIBAL (Negishi method) instead of Cl$_2$Pd(PPh$_3$)$_2$ alone; see: Negishi, E. *Acc. Chem. Res.* **1982**, *15*, 340. For a recent example of this see: Amat, M.; Hadida, S.; Pshenichnyi, G.; Bosch, J. *J. Org. Chem.* **1997**, *62*, 3158−3171.

(12) Jackson, R. F. W.; James, K.; Wythes, M. J.; Wood, A. J. *J. Chem. Soc., Chem. Commun.* **1989**, 644.

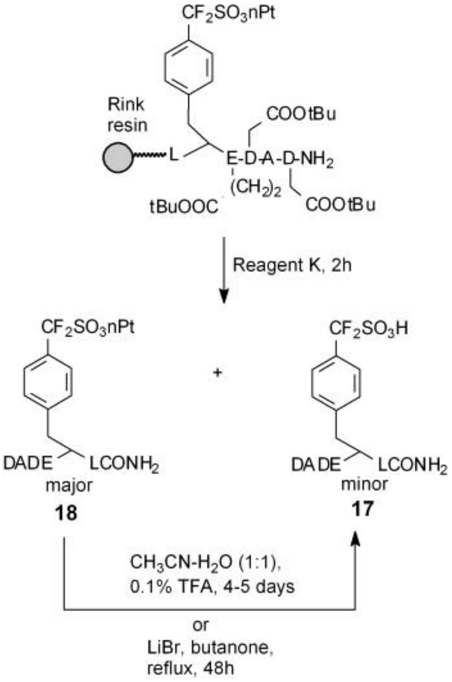
Scheme 3



and **14** in yields of 45% and 44%, respectively.[13] Selective hydrolysis of the carbon esters to give **15** and **16** was achieved in high yield using aqueous LiOH/THF.[14,15]

For peptide synthesis, the hexapeptide DADE-F$_2$Smp-LNH$_2$, **17**, was chosen as a model system. The DADE-X-LNH$_2$ sequence has been used extensively for examining various moieties as phosphotyrosine mimetics[16] for obtaining inhibitors of PTP1B, a therapeutically significant PTPase.[17] Manual solid-phase peptide synthesis was performed using Fmoc-protected amino acids, the Rink amide AM resin, and HATU/HOAT as coupling agents. The peptide was cleaved from the support by subjecting the resin-bound material to Reagent K (82.5% TFA, 2.5% EDT, 5% H$_2$O, 5% thioanisole, and 5% phenol) for 2 h. When the TCE-protected amino acid **16** was used, we were surprised to find that the HPLC chromatogram of the crude peptide consisted of numerous peaks and, therefore, no attempt was made to obtain the pure peptide from the mixture.[18] However, when the nPt-protected amino acid **15** was used, the HPLC chromatogram of the crude peptide consisted mainly of two peaks. On the basis of electrospray mass spectral analysis, these peaks were attributed to the nPt-protected peptide **18** (major product) and the completely deprotected peptide **17** (minor product) (Scheme 4). Further studies with the resin-bound peptide revealed that the loss of the nPt group was occurring during the treatment with Reagent K. Although complete removal of the nPt group could be achieved by prolonged treatment with Reagent K, this resulted in an increase in the formation of impurities. Nevertheless, it was found that the nPt group could be removed cleanly under very mild conditions by stirring a solution of the crude peptide in 1:1 acetonitrile/water containing 0.1% TFA for 4−5 days. Pure peptide was obtained by preparative HPLC of the crude reaction mixture (32% yield). It was also found that the nPt group could be removed by refluxing the crude peptide in a solution of butanone containing 2 equiv of LiBr for 48 h. However, this reaction did not proceed as cleanly as the above procedure.

Scheme 4



In summary, we have described the first synthesis of protected L-F$_2$Smp and developed a straightforward procedure for incorporating F$_2$Smp into peptides, which should be readily adaptable to automated solid-phase peptide synthesis. Inhibition studies with PTP1B and peptide **17** indicate that it is almost 2 orders of magnitude more potent than the analogous peptides bearing other[16] monoanionic pTyr mimetics.[19] F$_2$Smp may also be useful as a monoanionic pTyr mimetic for the development of inhibitors and probes of other enzymes that bind phosphotyrosine such as PTKs and SH2 domain binding proteins. It has recently been shown that tyrosine sulfation on CCR5 is important for efficient adhesion of HIV and sulfated peptides that mimic the sulfated tyrosine sequences inhibit HIV infection of macrophages.[20,21] F$_2$Smp may also prove to be useful in the development of hydrolytically stable forms of such peptides.

Acknowledgment. This work was supported by the Natural Sciences and Engineering Research Council (NSERC)

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[13] The coupling reaction was also attempted using the Boc-protected zincate. However, separation of Boc-alanine methylester, a byproduct of the reaction, from the coupled product proved to be quite difficult and, as a result, provided considerably lower yields than when the Fmoc derivative was used.

[14] (a) Burke, T. R.; Smyth, M. S.; Otaka, A.; Roller, P. P. *Tetrahedron Lett.* **1993**, *34*, 4125. (b) Ye, B.; Akamatsu, M.; Shoelson, S. E.; Wolf, G.; Giorgeti-Peraldi, S.; Yan, X.; Roller, P. P.; Burke, T. R. *J. Med. Chem.* **1995**, *38*, 4270.

[15] Only the ee of compound **15** was determined. This was achieved by preparing two dipeptides, F$_2$Smp-L-Leucine and F$_2$Smp-DL-leucine, followed by analytical HPLC analysis of the dipeptides. The ee of **15** was found to be >97%. Since the procedure used to prepare **16** from **10** was identical to that used for preparing **15** from **9**, it is reasonable to assume that **16** was obtained with an ee comparable to that of **15**.

[16] See: Gao, Y.; Wu, L.; Luo, J. H.; Guo, R.; Yang, D.; Zhang, Z. Y.; Burke, T. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 923 and references therein.

[17] (a) Kennedy, B. P.; Ramachandran, C. *Biochem. Pharmacol.* **2000**, *60*, 877−883. (b) Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B P. *Science* **1999**, *283*, 1544−48.

[18] Studies with **16** in 20% piperidine in DMF suggested that some loss of the TCE group may have occurred during peptide synthesis, which may have resulted in an increase in the formation of impurities.

[19] Details of the kinetic studies will be reported elsewhere.

[20] Farzan, M.; Mirzabekov, T.; Kolchinsky, P.; Wyatt, R.; Cayabyab, M.; Gerard, N. P.; Gerard, C.; Sodroski, J.; Choe, H. *Cell* **1999**, *96*, 667.

[21] Farzan, M.; Vasilieva, N.; Schnitzler, C. E.; Chung, S.; Robinson, J.; Gerard, N. P.; Gerard, C.; Choe, H.; Sodroski, J. *J. Biol. Chem.* **2000**, *275*, 33516.

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Supporting Information Available: Preparation procedures and characterization data for **5**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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