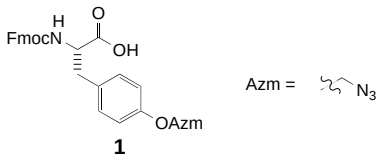
A Strategy for the Synthesis of Sulfated Peptides**

Travis Young and Laura L. Kiessling*

The sulfation of tyrosine residues is a posttranslational modification that occurs in all eukaryotes.[1] This ubiquitous modification has been identified on many secretory proteins with diverse biological activities. Tyrosine *O*-sulfate (Tyr-(SO_3H)) residues often are important for facilitating specific protein–protein interactions.[2] However, there is little information on the molecular basis for the importance of tyrosine sulfation.[3] Characterization of the molecular interactions of Tyr(SO_3H) has been hampered by lack of access to the necessary quantities of sulfated proteins and peptides. We report a method for the efficient production of sulfated peptides using a new protecting-group strategy.

Although several strategies for the synthesis of sulfated peptides have been reported,[4] a general, efficient approach is still being sought. The primary obstacle in the synthesis of sulfated peptides is the predilection of sulfotyrosine to undergo desulfation under acidic conditions. Sulfotyrosine-containing peptides generally decompose rapidly in the presence of trifluoroacetic acid, the standard reagent used for side-chain deprotection and cleavage from the solid supports employed in Fmoc-based (Fmoc = (9H-fluoren-9-ylmethoxy)carbonyl) solid-phase peptide synthesis (Fmoc-SPPS). The sulfate group counterion has been shown recently to have a dramatic influence on the stability of sulfated peptides toward acidic treatment.[5] Alternatively, acid-sensitive substrates can be released from the 2-chlorotrityl- (2-Clt-) derivatized resin without significant decomposition.[6] Indeed, sulfated peptides may be liberated from this support without significant accompanying desulfation.[7] Thus, the most attractive published method for sulfated peptide synthesis involves stepwise incorporation of FmocTyr(SO_3Na)OH into the growing peptide.[8] Unfortunately, couplings using this salt and subsequent reactions to elongate the resulting peptide can be sluggish.[9] Moreover, our attempts to synthesize peptides containing multiple sulfotyrosine residues were plagued by poor resin swelling and the need for extended coupling times.

Given the lack of general methods for the synthesis of sulfated peptides, we set out to develop a new approach. For the greatest flexibility, we employed a postsynthetic sulfation strategy. We envisioned that selective unmasking and sulfation of the desired tyrosine residues could be conducted on the solid support. Incorporation of orthogonally protected tyrosine derivatives at the peptide-assembly stage would allow the synthesis of multiply sulfated peptides with various sulfation patterns. Additionally, a solid-phase sulfation step would avoid the complicated purification of partially protected, sulfated peptide intermediates and, in the case of polysulfated peptides, mixtures with different sulfation patterns. Excess reagents could also be used to drive the sulfation reaction to completion. With this strategy, both sulfated and unmodified peptides could be produced from the same peptide synthesis. To implement this approach, we required a protecting group for the tyrosine phenol that is stable to the conditions used in Fmoc-SPPS, yet can be unmasked selectively and quantitatively under mild conditions. Strongly acidic or basic conditions for protecting-group removal must therefore be avoided. We also wished to circumvent the laborious process of cleavage and characterization of intermediates to confirm the success of the deprotection reaction. We therefore sought to design a protecting group that could be removed under mild conditions and that possesses a characteristic spectroscopic signal by which to monitor the deprotection step.



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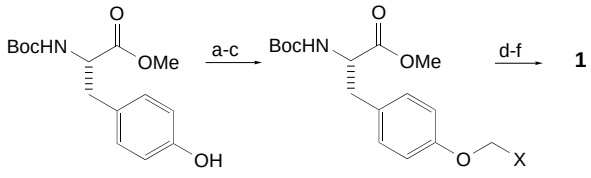
Scheme 1. Target tyrosine building block for the synthesis of sulfated peptides.

We identified Loubinoux's little-used azidomethyl (Azm) phenol protecting group as an excellent candidate for tyrosine protection.[10] The mild conditions required for azide reduction were expected to release the phenol group without cleavage of the Clt-ester bond or modification of oxidation-sensitive amino acid residues such as cysteine and methionine.[11] We therefore pursued the synthesis of Fmoc-Tyr(OAzm)OH (Scheme 1).

The synthesis of target **1** proceeds from BocTyrOMe (**2**, Boc = *tert*-butyloxycarbonyl). Attempted alkylation of **2** with dibromomethane or bromochloromethane affords only the dimeric tyrosine methylene acetal, even when high concentrations of the electrophile are employed. Alkylation with an alternate, bifunctional methylene equivalent, chloromethyl methylsulfide, proceeds in good yield (Scheme 2). Initial



2 **3**: X = SMe; **4**: X = Cl; **5**: X = N_3

Scheme 2. Scheme for the synthesis of **1**. Conditions: a) KO*t*-Bu, NaI, CH_3SCH_2Cl, DMF, 82%; b) NCS, TMSCl, CH_2Cl_2; c) NaN_3, DMF, H_2O, 87% (over 2 steps); d) TMSOTf, CH_2Cl_2; e) FmocOSu, Et_3N, THF, 84% (over 2 steps); f) LiOH-H_2O, THF-H_2O, 0 °C, 88%. DMF = *N*,*N*-dimethylformamide, OSu = *N*-hydroxysuccinimide.

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screening of precedented[12] methods for the selective activation of the O,S-acetal **3** failed to yield satisfactory results.[13] For example, treatment of **3** with *N*-chlorosuccinimide (NCS) in CH_2Cl_2 afforded only 48 % yield of the desired compound, **4**.

Our unsuccessful attempts to transform the O,S-acetal prompted a search for more reactive electrophilic activators. We reasoned that Lewis acid activation of NCS would increase the consumption of starting material without compromising the stability of the product. Indeed, activation of the O,S-acetal with NCS in the presence of *tert*-butyldimethylsilyl chloride (TMSCl) provides the chloride **4** in good yield (Scheme 2).

The labile intermediate **4** can be purified by flash chromatography on deactivated silica gel, but not without some hydrolysis. A simple aqueous workup, however, provided **4** with little decomposition. The transformation of this material to the azide **5** proceeds in high yield (87 % for 2 steps).

The azidomethyl group is not impervious to acidic conditions, a feature that complicates the synthesis of **1**, as we were unable to remove the Boc carbamate selectively using protic acids. We therefore employed TMSOTf to remove the Boc group,[14] and protected the resulting amino group by reaction with Fmoc-*N*-hydroxysuccinimide ester.[15] Selective saponification of the methyl ester, the precursor to **1**, provides the target compound without loss of the Fmoc group[16] in excellent yield. The structure of the protected tyrosine derivative **1** was unequivocally established by X-ray crystallographic analysis.[17] The enantiopurity of this compound was confirmed by the synthesis of diastereomeric dipeptides.[18]

With FmocTyr(Azm)OH (**1**) in hand, we set out to demonstrate its use in sulfated peptide synthesis (Figure 1). Our approach involves standard Fmoc-based solid-phase peptide synthesis, followed by unmasking the desired phenols by removal of the Azm group using homogeneous conditions for azide reduction.[19] Complete removal of the Azm moiety is confirmed by inspection of the IR absorption spectrum for the strong, distinct band arising from the azide. The free phenolic hydroxy groups are then sulfated by the action of DMF·SO_3 in a pyridine/DMF mixture.[20] We opted for benzyl side-chain protecting groups for other tyrosine residues: serine, threonine, aspartic acid, and glutamic acid residues; this necessitated a two-step cleavage/deprotection procedure. Cleavage from the acid-labile 2-Clt resin under conditions compatible with the labile sulfate esters is followed by routine hydrogenolysis of the benzyl groups.

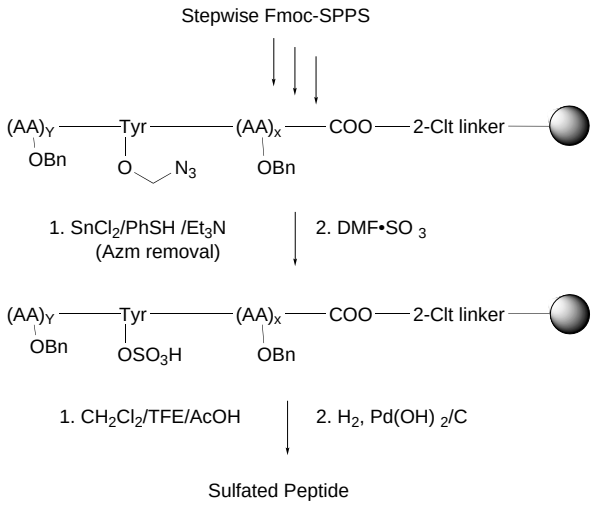

Figure 1. Overview of sulfated peptide synthesis.

To test our new method, we synthesized a sequence derived from PSGL-1, a sulfated glycoprotein. PSGL-1 is the relevant ligand for P-selectin.[21] Significantly, a sulfated and glycosylated epitope at the N-terminus of PSGL-1 is crucial for binding.[22] We synthesized peptides related to this epitope.[23] Access to such compounds could facilitate dissection of the contributions caused by the distinct posttranslational modifications.[24] We synthesized a sequence; the corresponding residues 5–12 of mature PSGL-1 that contains all three putative sulfated tyrosine residues. To demonstrate the flexibility of our strategy, we targeted the fully sulfated octapeptide and a monosulfated octapeptide (Figure 2).

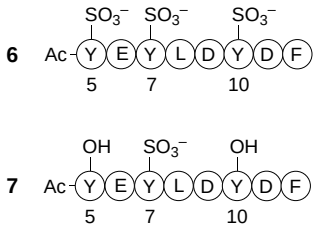

Figure 2. Sulfated octapeptide targets **6** and **7**.

Synthesis of the precursor to the trisulfated sequence **6** by automated continuous-flow Fmoc-based chemistry proceeds smoothly. Gratifyingly, amide-bond formation with our building block proceeds with a similar efficiency as with other amino acids; no special reagents or conditions are required. After the postsynthetic operations of Azm removal, sulfation, resin cleavage, and benzyl-group hydrogenolysis, the desired peptide **6** was isolated in 27 % yield after HPLC purification. Importantly, we observed no desulfated peptide in either the MALDI-TOF mass spectrum (prior to HPLC) or during HPLC purification. Synthesis of the monosulfated peptide **7** proceeds somewhat less efficiently, yielding several apparent deletion peptide products as well as the desired product. However, after HPLC purification, we isolated the target peptide in 5 % overall yield based on resin loading. Again, we were unable to detect the corresponding desulfated peptide. These achievements validate our strategy for the synthesis of sulfated peptides.

Protein tyrosine sulfation has been pushed to the forefront of research by its role in important physiological protein–protein recognition events. The ubiquity and emerging significance of this posttranslational modification provides an imperative for the development of efficient synthetic approaches to sulfated peptides. We anticipate the strategy outlined here will aid the study of tyrosine sulfation by making sulfated peptide ligands and their analogues readily available.

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[1] C. Niehrs, R. Beisswanger, W. B. Huttner, *Chem.-Biol. Interact.* **1994**, *92*, 257 – 271.
[2] a) M. Farzan, T. Mirzabekov, P. Kolchinsky, R. Wyatt, M. Cayabyab, N. P. Gerard, C. Gerard, J. Sodroski, H. Choe, *Cell* **1999**, *96*, 667 – 676; b) A. Leppanen, S. P. White, J. Helin, R. P. McEver, R. D. Cummings, *J. Biol. Chem.* **2000**, *275*, 39569 – 39578; c) E. G. Cormier, D. N. H. Tran, L. Yukhayeva, W. C. Olson, T. Dragic, *J. Virol.* **2001**, *75*, 5541 – 5549; c) J. Dong, P. Ye, A. J. Schade, S. Gao, G. M. Romo, N. T. Turner, L. V. McIntire, J. A. Lopez, *J. Biol. Chem.* **2001**, *276*, 16690; e) S. Costagliola, V. Panneels, M. Bonomi, J. Koch, M. C. Many, G. Smits, G. Vassart, *EMBO J.* **2002**, *21*, 504.
[3] W. S. Somers, J. Tan, G. D. Shaw, R. T. Camphausen, *Cell* **2000**, *103*, 467 – 479. For a review, see: J. W. Kehoe, C. R. Bertozzi, *Chem. Biol.* **2000**, *7*, R57-R61.
[4] a) Y. Kurano, T. Kimura, S. Sakakibara, *J. Chem. Soc. Chem. Commun.* **1987**, 323 – 325; b) N. Fujii, S. Futaki, S. Funakoshi, K. Akaji, H. Morimoto, R. Doi, K. Inoue, M. Kogire, S. Sumi, M. Yun, T. Tobe, M. Aono, M. Matsuda, H. Narusawa, M. Moriga, H. Yajima, *Chem. Pharm. Bull.* **1988**, *36*, 3281 – 3291; c) K. Kitagawa, S. Futaki, T. Yagami, *J. Synth. Org. Chem. Jpn.* **1994**, *52*, 675 – 685.
[5] a) K. Barlos, D. Gatos, J. Kallitsis, G. Papaphotiu, P. Sotiriu, W. Q. Yao, W. Schafer, *Tetrahedron Lett.* **1989**, *30*, 3943 – 3946; b) S. V. Campos, L. P. Miranda, M. Meldal, *J. Chem. Soc. Perkin Trans. 1* **2002**, 682 – 686.
[6] K. Barlos, D. Gatos, S. Kapolos, G. Papaphotiu, W. Schafer, W. Q. Yao, *Tetrahedron Lett.* **1989**, *30*, 3947 – 3950.
[7] K. Kitagawa, C. Aida, H. Fujiwara, T. Yagami, S. Futaki, *Tetrahedron Lett.* **1997**, *38*, 599 – 602.
[8] K. Kitagawa, C. Aida, H. Fujiwara, T. Yagami, S. Futaki, M. Kogire, J. Ida, K. Inoue, *J. Org. Chem.* **2001**, *66*, 1 – 10.
[9] See reference [2b] and T. Young, Ph.D. thesis, University of Wisconsin (Madison), **2001**.
[10] B. Loubinoux, S. Tabbache, P. Gerardin, J. Miazimbakana, *Tetrahedron* **1988**, *44*, 6055 – 6064.
[11] E. F. V. Scriven, K. Turnbull, *Chem. Rev.* **1988**, *88*, 297 – 386.
[12] a) T. Benneche, K. Undheim, *Acta Chem. Scand. B* **1983**, *37*, 93 – 96; b) P. J. Garegg, *Adv. Carbohyd. Chem. Biochem.* **1997**, *52*, 179 – 205.
[13] For a detailed description of various conditions for activation of **3**, see the Supporting Information.
[14] a) M. Sakaitani, Y. Ohfune, *Tetrahedron Lett.* **1985**, *26*, 5543 – 5546; b) A. J. Zhang, D. H. Russell, J. P. Zhu, K. Burgess, *Tetrahedron Lett.* **1998**, *39*, 7439 – 7442.
[15] J. Paladino, C. Guyard, C. Thurieau, J. L. Fauchere, *Helv. Chim. Acta* **1993**, *76*, 2465 – 2472.
[16] a) T. R. Burke, M. S. Smyth, A. Otaka, P. P. Roller, *Tetrahedron Lett.* **1993**, *34*, 4125 – 4128; b) S. F. Liu, C. Dockendorff, S. D. Taylor, *Org. Lett.* **2001**, *3*, 1571 – 1574.
[17] See the Supporting Information for the structure. Crystal data for $C_{26}H_{24}N_4O_5$: Crystal size = $0.09 \times 0.02 \times 0.02$ mm^3, orthorhombic, $P2_12_12_1$, $a = 5.0195(5)$, $b = 19.2028(12)$, $c = 24.566(2)$ Å, $V = 2367.9(3)$ Å^3, $D_{calcl} = 1.325$ mg m^{-3}, $2\theta_{max} = 41.06°$, $Ag_{K\alpha}$ radiation ($\lambda = 0.5594$ Å), ω scans, $T = 173(1)$ K, 26122 independent and 2803 unique reflections. The data were corrected for Lorentz and polarization effects, the empirical absorption correction was performed with SADABS as described in R. H. Blessing, *Acta Crystallogr. Sect. A* **1995**, *51*, 33 – 38, $\mu = 0.058$ mm^{-1}, $T_{max}/T_{min} = 0.9988/0.9948$. The structure was solved and refined with the program package SHELXTL (version 5.1) program library (G. Sheldrick, Bruker Analytical X-Ray Systems, Madison, WI). All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were included in the structure-factor calculation at idealized positions. The final least-squares refinement of 317 parameters against 2803 data resulted in residual factors R (based on F^2 for $I \geq 2\sigma$) and wR (based on F^2 for all data) of 0.0667 and 0.2167, respectively. The final difference Fourier map was featureless. CCDC-188441 (**1**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
[18] For details, see the Supporting Information.
[19] M. Bartra, P. Romea, F. Urpi, J. Vilarrasa, *Tetrahedron* **1990**, *46*, 587 – 594.
[20] Y. Matsubayashi, H. Hanai, O. Hara, Y. Sakagami, *Biochem. Biophys. Res. Commun.* **1996**, *225*, 209 – 214.
[21] D. Sako, X. J. Chang, K. M. Barone, G. Vachino, H. M. White, G. Shaw, G. M. Veldman, K. M. Bean, T. J. Ahern, B. Furie, D. A. Cumming, G. R. Larsen, *Cell* **1993**, *75*, 1179 – 1186.
[22] a) D. Sako, K. M. Comess, K. M. Barone, R. T. Camphausen, D. A. Cumming, G. D. Shaw, *Cell* **1995**, *83*, 323 – 331; b) T. Pouyani, B. Seed, *Cell* **1995**, *83*, 333 – 343.
[23] For syntheses of other sulfated peptides derived from PSGL-1, see: a) A. Leppanen, P. Mehta, Y. B. Ouyang, T. Z. Ju, J. Helin, K. L. Moore, I. van Die, W. M. Canfield, R. P. McEver, R. D. Cummings, *J. Biol. Chem.* **1999**, *274*, 24838 – 24848; b) K. M. Koeller, M. E. B. Smith, R. F. Huang, C. H. Wong, *J. Am. Chem. Soc.* **2000**, 122, 4241 – 4242; c) P. Durieux, J. Fernandez-Carneado, G. Tuchscherer, *Tetrahedron Lett.* **2001**, *42*, 2297 – 2299.
[24] See reference [23] and C. R. Bertozzi, L. L. Kiessling, *Science* **2001**, *291*, 2357 – 2364.

Nanotubes of Group 4 Metal Disulfides**

Manashi Nath and C. N. R. Rao*

Since the discovery of the carbon nanotubes, there has been active interest in exploring whether other layered materials, especially metal disulfides such as MoS_2 and WS_2, form nanotubes and related structures. Tenne et al.[1,2] succeeded in preparing nanotubes of MoS_2 and WS_2 by first heating the metal oxide in a stream of forming gas (95 % N_2 + 5 % H_2) followed by reaction with H_2S at elevated temperatures (700–1000 °C). Thermal decomposition of the ammonium thiometallate $(NH_4)_2MS_4$ (M = Mo or W) in a H_2 atmosphere has been employed recently to obtain the disulfide nanotubes.[3] Metal trisulfides are formed as intermediates in the formation of the disulfide nanotubes in both of the above methods. Accordingly, MoS_2 and WS_2 nanotubes could be prepared directly from the decomposition of MoS_3 and WS_3 in a hydrogen atmosphere.[3] NbS_2 nanotubes have also been prepared by the thermal decomposition of NbS_3 in a hydrogen atmosphere at 1000 °C.[4] These results suggested that it may indeed be possible to prepare nanotubes of other layered disulfides by the thermal decomposition of appropriate trisulfide precursors. Since the disulfides of Group 4 metals, such as Ti, Zr, and Hf, possess layered hexagonal structures,[5,6] we considered it feasible to prepare the nanotubes of these materials starting from their trisulfides. Herein we

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