9.6 Hz, 1H; H-4), 4.66 (dd, ^3J ($H_{1''}$,$H_{2''}$) = 2.9 Hz, ^3J ($H_{2''}$,$H_{3''}$) = 3.9 Hz, 1H; H-2″), 4.58 (dd, ^3J ($H_{1'}$,$H_{2'}$) = 2.8 Hz, ^3J ($H_{2'}$,$H_{3'}$) = 4.0 Hz, 1H; H-2′), 4.24 – 4.08 (m, 5H; H-2, 6′, 6″), 4.01 (dd, ^3J (H_2,H_3) = 4.6 Hz, ^3J (H_3,H_4) = 9.6 Hz, 1H; H-3), 3.75 – 3.65 (m, 2H; H-5′, 5″), 3.60 (d, ^2J (H_{6a},H_{6b}) = 4.5 Hz, 2H; H-6), 3.55 – 3.50 (m, 1H, H-5), 2.12, 2.10, 2.07, 2.06, 2.06, 2.05, 2.04 (7s, 21H; 7 CH_3CO), 1.77, 1.71 (2s, 6H; 2 CH_3CO_3), 1.49 (d, ^3J (H,H) = 4.9 Hz, 3H; CH_3CH). **5**: m.p. 177 – 178 °C; $[\alpha]_D = +16.9$ ($c = 0.15$, trichloromethane); ^{1}H NMR: $\delta =$ 5.36 – 5.21 (m, 8H; H-1, 2′, 3′, 3″, 4, 4′, 4″, CH_3CH), 5.14 (dd, ^3J ($H_{1''}$,$H_{2''}$) = 1.9 Hz, ^3J ($H_{2''}$,$H_{3''}$) = 3.1 Hz, 1H; H-2″), 4.98 (d, ^3J ($H_{1''}$,$H_{2''}$) = 1.9 Hz, 1H; H-1″), 4.78 (d, ^3J ($H_{1'}$,$H_{2'}$) = 1.7 Hz, 1H; H-1′), 4.34 – 4.27 (m, 4H; H-2, 5′, 6″), 4.10 – 4.01 (m, 3H; H-5″, 6′), 3.91 (dd, ^3J (H_2,H_3) = 3.8 Hz, ^3J (H_3,H_4) = 9.7 Hz, 1H; H-3), 3.80 (dd, ^3J (H_5,H_{6a}) = 6.3 Hz, ^2J (H_{6a},H_{6b}) = 10.4 Hz, 1H; H-6_a), 3.62 – 3.56 (m, 2H; H-5, 6_b), 2.16, 2.15, 2.15, 2.11, 2.09, 2.06, 2.06, 2.00, 1.98 (9s, 27H; 9 CH_3CO), 1.53 (d, ^3J (H,H) = 5.0 Hz, 3H; CH_3CH). **6**: $[\alpha]_D = +2.4$ ($c =$ 0.15, trichloromethane); ^{1}H NMR: $\delta = 5.69$ (d, ^3J (H_{1B},H_{2B}) = 5.1 Hz, 1H; H-1B), 5.65 (d, ^3J ($H_{1B'}$,$H_{2B'}$) = 5.2 Hz, 1H; H-1B′), 5.50 (dd, ^3J (H_{2B},H_{3B}) = 2.4 Hz, ^3J (H_{3B},H_{4B}) = 1.2 Hz, 1H; H-3B), 5.42 (t, ^3J (H,H) = 1.9 Hz, 1H; H-3B′), 5.38 (2d, ^3J (H,H) = 3.3 Hz, 2H; H-4C, 4C′), 5.26 (d, ^3J (H_{1A},H_{2A}) = 1.6 Hz, 1H; H-1A), 5.26 (q, ^3J (H,H) = 5.0 Hz, 1H; CH_3CH), 5.16 (2 dd, ^3J ($H_{1C(C')}$,$H_{2C(C')}$) = 8.0 Hz, ^3J ($H_{2C(C')}$,$H_{3C(C')}$) = 9.6 Hz, 2H; H-2C, 2C′), 5.10 (t, ^3J (H,H) = 9.5 Hz, 1H; H-4A), 5.00 (2dd, 2H; H-3C, 3C′), 4.60, 4.55 (2d, ^3J ($H_{1C(C')}$,$H_{2C(C')}$) = 8.0 Hz, 2H; H-1C, 1C′), 4.41 (dd, 1H; H-2B), 4.35 (dd, 1H; H-2B′), 4.30 – 4.20 (m, 3H; H-2A, $6B_a$, $6B'_a$), 4.13 – 4.05 (m, 6H; H-$6B_b$, $6B'_b$, 6C, 6C′), 3.98 – 3.88 (m, 3H; H-3A, 5C, 5C′), 3.85 – 3.78 (m, 2H; H-5B, 5B′), 3.64 (2t, ^3J (H,H) = 9.5 Hz, 2H; H-4B, 4B′), 3.60 – 3.58 (m, 3H; H-5A, 6A), 2.17 – 1.98 (13s, 39H; 13 CH_3CO), 1.74, 1.68 (2s, 6H; 2 CH_3CO_3), 1.47 (d, ^3J (H,H) = 5.0 Hz, 3H; CH_3CH).

[9] a) J. Gass, M. Strobl, P. Kosma, *Carbohydr. Res.* **1993**, *244*, 69; b) M. L. Sznaidman, S. C. Johnson, C. Crasto, S. M. Hecht, *J. Org. Chem.* **1995**, *60*, 3942; c) T. Ogawa, K. Beppu, S. Nakabayashi, *Carbohydr. Res.* **1981**, *93*, C6; d) W. Wang, F. Kong, *Tetrahedron Lett.* **1998**, 1937.

[10] D. Crich, S. Sun, *J. Am. Chem. Soc.* **1998**, *120*, 435.

[11] J. Xia, Y. Hui, *Synth. Commun.* **1995**, *25*, 2235.

Chemoenzymatic Synthesis of a Characteristic Glycophosphopeptide from the Transactivation Domain of the Serum Response Factor**

Jörg Sander and Herbert Waldmann*

Phosphorylation[1] and O-glycosidic attachment of *N*-acetylglucosamine (GlcNAc)[2] to serine and threonine residues are key events in the cellular transduction of mitogenic signals. For instance, the serum response factor (SRF), a widely found transcription factor, is phosphorylated at various serine and threonine residues in response to extracellular stimuli. It then translocates to the nucleus, binds to the serum response element (SRE, which occurs in the promoters of numerous genes), and induces gene transcription.[3] In addition, SRF is glycosylated at four different sites,[4] where the major glycosylation site—most probably serine 383 in the transactivation domain—is flanked by several phosphorylation sites (Figure 1). These findings suggest that the correct orchestration of SRF phosphorylation and glycosylation as well as the controlled removal ot these covalent protein modifications appear to be paramount to the regulation of gene transcription under the control of SRF.

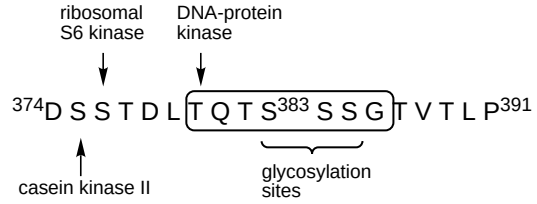


Figure 1. Phosphorylation and glycosylation sites in the transactivation domain of the serum response factor (underlined amino acid symbols). A kinase responsible for the phosphorylation of a certain amino acid is shown. The phosphorylated and glycosylated heptapeptide **17** synthesized in the context of this work is highlighted in the box.

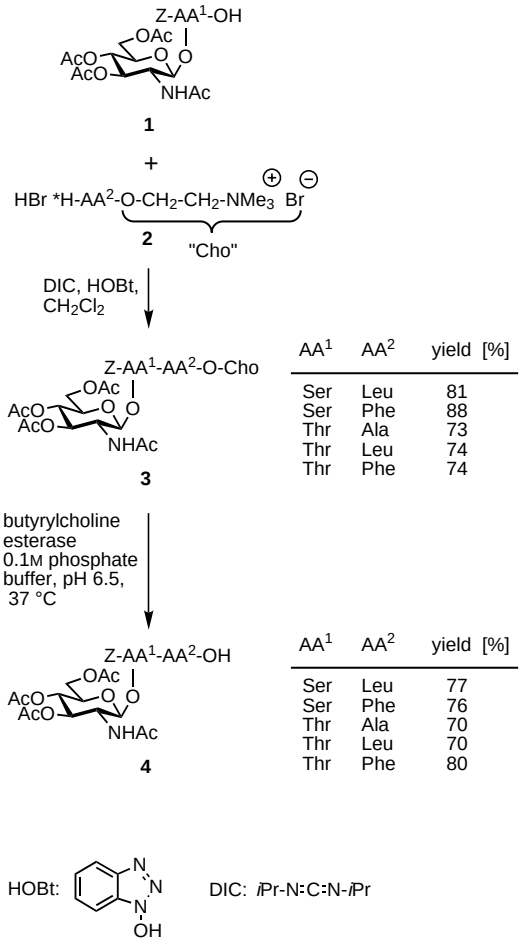
Peptide conjugates embodying the characteristic structural elements of the naturally occuring protein conjugates (namely, phosphates and O-glycosides) may be efficient tools for the study of such biological processes in molecular detail.[5, 6] However, glycopeptides are very sensitive to acid and base, and phosphopeptides are even more base labile, with carbohydrate and phosphotriester groups being lost in a β-elimination reaction at pH values greater than 8 – 9.[5] In the synthesis of glycophoshopeptides these problems potentiate each other and consequently protecting groups have to be used that can be removed with complete selectivity under the mildest conditions, preferably at pH 6 – 8 and room temperature.[5] We now report that such labile peptide conjugates can successfully be built up by employing the enzymatic removal of the choline ester and the *p*-phenylacetoxybenzyloxycarbonyl (PhacOZ) group as the key steps.

The choline ester was previously investigated as a possible blocking group for glycopeptide synthesis, but under the conditions required for its cleavage (pH 10 – 11) the sensitive O-glycosylated peptides were destroyed.[7] This problem can, however, be solved efficiently by employing the enzyme butyrylcholine esterase from horse serum for the removal of the protecting group. To investigate the suitability of the enzymatic choline ester cleavage for glycopeptide synthesis O-GlcNAc-modified serine/threonine building blocks **1**[8] were coupled with amino acid choline esters **2**[7] to give glycopeptides **3** in high yield. Glycodipeptide esters **3** were subsequently treated with butyrylcholine esterase in phosphate buffer at pH 6.5 (Scheme 1). In the ensuing enzymatic reaction the C-terminal choline esters were saponified exclusively. An attack on the acetate or the N-terminal urethane groups and an anomerization or β-elimination of the carbohydrate did not occur. The enzyme accepts different amino acid combinations and also tolerates sterically demanding residues such as phenylalanine at the C-terminus. The desired, selectively unmasked, glycodipeptides **4** were isolated in yields of 70 – 80 %. These results clearly indicate that the enzymatic removal of choline esters can be applied advantageously to glycopeptide chemistry. Particularly re-

[*] Prof. Dr. H. Waldmann, Dr. J. Sander
Institut für Organische Chemie der Universität
Richard-Willstätter-Allee 2, D-76128 Karlsruhe (Germany)
Fax: (+49) 721-608-4825
E-mail: waldmann@ochhades.chemie.uni-karlsruhe.de

[**] This research was supported by the Fonds der Chemischen Industrie.

  1433-7851/99/3809-1250 $ 17.50+.50/0

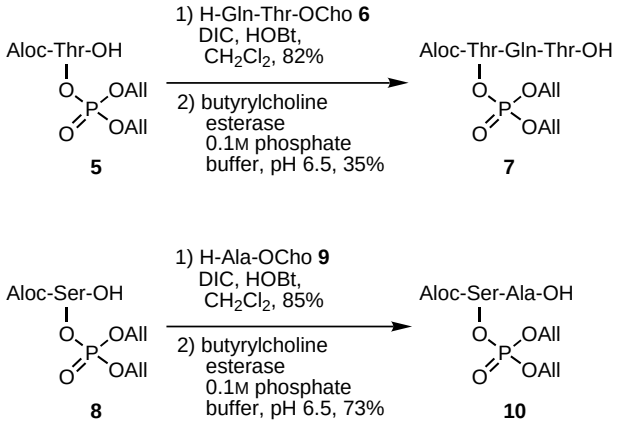


Scheme 1. Synthesis and selective enzymatic deprotection of glycopeptide choline esters by butyrylcholine esterase from horse serum. AA = amino acid, DIC = diisopropylcarbodiimide, HOBt = 1-hydroxy-1*H*-benzotriazole, HOCho = choline, Z = benzyloxycarbonyl.

warding is the high solubility of the charged substrates in aqueous buffer; a solubility enhancing cosolvent that might denature the enzyme is not required.

The suitability of the choline ester for phosphopeptide synthesis was investigated after coupling the O-phosphorylated threonine **5**[9] and the serine derivative **8** with the peptide choline ester **6** and the alanine choline ester **9**,[10] respectively (Scheme 2). The subsequent deprotections under the conditions described above proceeded once more without any undesired attack on the side-chain functionality and a β-elimination of the phosphate group could also not be observed.[11] Thus, the choline ester group can also be applied advantageously to the synthesis of very base-sensitive phosphopeptides.

To demonstrate the full capacity of the enzymatic protecting group technique a phosphorylated and glycosylated heptapeptide was built up. This peptide (see Figure 1) represents the central part of the multiphosphorylated and glycosylated substructure of the transactivation domain of the serum response factor. In planning the synthesis we intended to combine the enzyme-labile choline ester as a C-terminal blocking function with the enzymatically removable *p*-phenylacetoxybenzyloxycarbonyl urethane group. This urethane



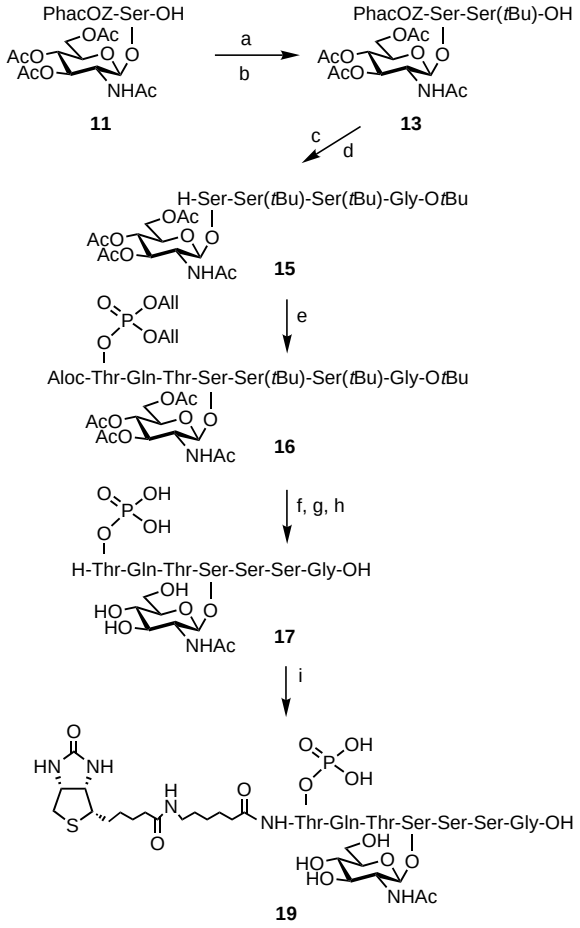
Scheme 2. Synthesis and selective enzymatic deprotection of phosphopeptide choline esters by butyrylcholine esterase from horse serum. All = allyl, Aloc = allyloxycarbonyl.

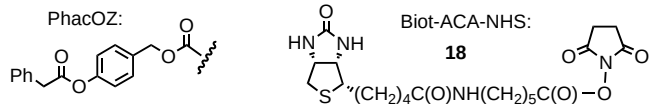
group can be cleaved under very mild conditions by a saponification of the built-in phenylacetate unit mediated by penicillin G acylase and subsequent spontaneous fragmentation of the phenolate liberated thereby.[12]

To this end, O-glycosylated PhacOZ-serine **11**[12] was condensed with serine choline ester **12** and the choline group was removed from the resulting PhacOZ-masked glycodipeptide ester in 88% yield (Scheme 3). Again, in this process no undesired side reaction was observed. The substrate specificity of the choline esterase and the mild reaction conditions guarantee that the glycoside and the phenol ester of phenylacetic acid incorporated into the N-terminal protecting group are not attacked. After elongation of the peptide chain with dipeptide **14** the PhacOZ urethane was selectively cleaved by fragmentation of the urethane blocking group by means of a reaction initiated by penicillin G acylase to yield selectively unmasked glycotetrapeptide **15** in high yield. The peptide chain was further elongated by coupling with phosphopeptide **7**, which had been synthesized by means of selective C-terminal removal of the choline ester protecting group (see Scheme 2). Glycophosphopeptide **16** thereby obtained in high yield was then completely deprotected. To this end, all allylic protecting groups were removed simultaneously by treatment with formic acid/*n*-butylamine in the presence of a Pd^0 catalyst at room temperature. Subsequently, the *tert*-butyl blocking functions were cleaved with trifluoroacetic acid, and the unmasking of the hydroxyl groups of the carbohydrate was achieved with hydrazine hydrate in methanol.

Finally, the glycophosphoheptapeptide **17** was equipped with a biotin label by selective N-acylation with biotinylaminocaproic acid-*N*-hydroxysuccinimide (**18**; Biot-ACA-NHS; Scheme 3). The biotinylated peptide conjugate **19** thus formed may serve as an efficient molecular probe. The biotin label can be traced by means of complex formation with the protein streptavidin, which is available in fluorescently labeled form or modified with colloidal gold and thus allows the study of a glycosylated and phosphorylated model protein in eukaryotic cells. For instance, such protein conjugates can be detected after microinjection by fluorescence microscopy and electron microscopy.[13]

  1433-7851/99/3809-1251 $ 17.50+.50/0





Scheme 3. Synthesis of a biotinylated glycophosphopeptide from the transactivation domain of the serum response factor. a) H-Ser(*t*Bu)-OCho (**12**), DIC, HOBt, CH_2Cl_2, 73%; b) butyrylcholine esterase, 0.01M phosphate buffer, pH 6.5, 37°C, 88%; c) H-Ser(*t*Bu)-Gly-O*t*Bu (**14**), DIC, HOBt, CH_2Cl_2, 78%; d) penicillin G acylase, 0.7M phosphate buffer/methanol (70/30, pH 7), NaI (500 equiv), room temperature (RT), 65%; e) Aloc-Thr(P(O)(OAll)$_2$)-Gln-Thr-OH (**7**), DIC, HOBt, CH_2Cl_2, 74%; f) $(PPh_3)_4Pd$, HCOOH/*n*BuNH$_2$ (10 equiv/6 equiv), RT; g) F_3CCOOH; h) hydrazine hydrate (3000 equiv), methanol, RT, 40% (three steps); i) Biot-ACA-NHS (**18**), DMF, 68%.

In conclusion we have devised a new and efficient strategy for the synthesis of glycosylated and phosphorylated peptides based on the combination of suitable enzyme-labile protecting groups. By means of this methodology acid- and base-sensitive biologically relevant labeled peptide conjugates can be built up, which may open up new avenues of research in biology and bioorganic chemistry. In particular, they should serve to unravel the chemical biology of the serum respone factor and the importance of its posttranslational modification in molecular detail.

Received: November 24, 1998 [Z 12705 IE]
German version: *Angew. Chem.* **1999**, *111*, 1337 – 1339

Keywords: enzyme catalysis · glycopeptides · protecting groups · protein modifications · signal transduction

[1] *Protein Phosphorylation* (Ed.: F. Marks), WILEY-VCH, Weinheim, **1996**.
[2] Review: G. W. Hart, L. K. Kreppel, F. I. Comer, C. S. Arnold, D. M. Snow, Z. Ye, X. Cheng, D. DellaManna, D. S. Caine, B. J. Earles, Y. Akimoto, R. N. Cole, B. K. Hayes, *Glycobiology* **1996**, *6*, 711 – 716.
[3] a) C. Norman, M. Runswick, R. Pollock, R. Treisman, *Cell* **1988**, *55*, 989 – 1003; b) J. M. Almendral, D. Sommer, H. MacDonald-Bravo, J. Burckhardt, J. Perera, R. Bravo, *Mol. Cell Biol.* **1988**, *8*, 2140 – 2148.
[4] A. J. Reason, M. R. Morris, M. Panico, R. Marais, R. H. Treisman, R. S. Haltiwanger, *J. Biol. Chem.* **1992**, *267*, 16911 – 16921.
[5] K. Hinterding, D. Alonso-D′az, H. Waldmann, *Angew. Chem.* **1998**, *110*, 716 – 780; *Angew. Chem. Int. Ed.* **1998**, *37*, 688 – 749.
[6] For a very instructive example, see T. Tagawa, T. Kuroki, P. K. Vogt, K. Chida, *J. Cell Biol.* **1995**, *130*, 255 – 263.
[7] a) H. Kunz, M. Buchholz, *Chem. Ber.* **1979**, *112*, 2145 – 2157; b) M. Buchholz, H. Kunz, *Liebigs Ann. Chem.* **1983**, 1859 – 1885.
[8] G. Arsequell, L. Krippner, R. A. Dwek, S. Y. C. Wong, *J. Chem. Soc. Chem. Commun.* **1994**, 2383 – 2384.
[9] D. Sebastian, A. Heuser, S. Schulze, H. Waldmann, *Synthesis* **1997**, 1098 – 1108, and references therein.
[10] V. Jungmann, Dissertation, Universität Karlsruhe **1998**.
[11] The relatively low yield of **7** is a result of problems during work-up. The conversion of the phosphotripeptide choline ester obtained from **5** and **6** was essentially complete.
[12] T. Pohl, H. Waldmann, *J. Am. Chem. Soc.* **1997**, 119, 6702 – 6710.
[13] G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, San Diego, **1996**.

$[(TeMe_2)Mn(CO)_4(\mu_5\text{-}Te)(\mu_4\text{-}Te)Mn_4(CO)_{12}]^-$: A Pentacoordinate Bridging Tellurido Ligand in a Square-Pyramidal Geometry**

Minghuey Shieh,* Horng-Sun Chen, Huey-Yea Yang, and Chuen-Her Ueng

Chalcogen-containing transition metal carbonyl complexes have attracted much attention recently due to their versatile bonding modes and reactivity.[1] Nevertheless, Mn – Te – CO clusters have remained unknown despite considerable advances in the corresponding chemistry of Fe – Te – CO clusters.[2, 3] We have discovered a simple and efficient route to this new family of clusters that involves the direct thermal reaction of the common reagents $[Mn_2(CO)_{10}]$ and K_2TeO_3. In contrast to the well-studied Fe system, the new Mn – Te clusters are based on octahedra with μ_4-Te centers.

The parent cluster **1** (PPN = $[P(C_6H_5)_3]_2N^+$) was synthesized by the reaction of K_2TeO_3 with $[Mn_2(CO)_{10}]$ in methanol

[*] Prof. Dr. M. Shieh, H.-S. Chen, H.-Y. Yang, Prof. Dr. C.-H. Ueng
Department of Chemistry
National Taiwan Normal University
88, Sec. 4, Tingchow Rd., Taipei 116 (Taiwan)
Fax: (+886) 2-2932-4249
E-mail: chefv012@scc.ntnu.edu.tw

[**] This work was supported by the National Science Council of the Republic of China (grant no. NSC-87-2113-M-003-001).

  1433-7851/99/3809-1252 $ 17.50+.50/0