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Solid-phase synthesis of C-terminal thio-linked glycopeptides

John P. Malkinson* and Robert A. Falconer

Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK

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Abstract—A solid-phase Mitsunobu reaction between a resin-bound 1-thiosugar and an *N*-Fmoc protected amino alcohol was successfully employed for thio-linked glycopeptide synthesis. Facile cleavage and deprotection in one step afforded the target glycopeptide in good yield and purity. © 2002 Elsevier Science Ltd. All rights reserved.

The carbohydrate substituents of native glycopeptides and glycoproteins are involved in many biologically important processes such as cell adhesion, recognition and differentiation, as well as influencing glycopeptide transport and uptake.[1,2] In addition, glycosylation plays an important role in the stabilisation of a glycoprotein's tertiary structure,[3,4] and in protection from hydrolysis by proteolytic enzymes.[5,6] The synthesis of modified glycopeptides may therefore represent a means to improve the absorption and distribution of poorly bioavailable therapeutic peptides. Such glycopeptides should gain not only increased resistance to enzymatic degradation, but also enhanced physicochemical properties and the potential to exploit various active transport uptake systems.[6]

There is, consequently, much current interest in the development of efficient methods for the synthesis of glycopeptides. Naturally occurring glycopeptides most commonly incorporate an *O*-glycosidic or an *N*-glycosidic (glycosyl amide) linkage between the carbohydrate moiety and the side-chain of an appropriate amino acid residue. Replacement of *O*-linked glycosyl amino acids with their *S*-linked (thioglycoside) isosteres results in glycopeptides that are more stable chemically as well as more resistant to the action of glycosidases.[7,8]

Of particular interest to us is the development of general synthetic protocols for glycopeptide assembly that are fully compatible with the well-established methodology for stepwise solid-phase peptide synthesis (SPPS).[9] A principal advantage of solid-phase synthesis is that reactions can be driven rapidly to completion by the use of large excesses of reactants, with subsequent isolation of the resin-bound product by simple filtration and washing. Such an approach also lends itself to the generation of libraries of compounds via combinatorial techniques.

The great majority of previously published work on the synthesis of *S*-glycosylated peptides employs a glycosyl amino acid building block approach. A suitably N^{α}-protected *S*-glycosyl cysteine (or homocysteine) is first prepared and then incorporated into the peptide sequence by stepwise synthesis or fragment condensation, either in solution or on the solid-phase.[8,10,11] Comparatively little work has been reported on *S*-glycopeptides glycosylated at either the N-terminus or the C-terminus of the peptide. Blocking either terminus by glycosylation is likely to result in inhibition of recognition by exopeptidases,[12] affording greater biological stability.

Previous work in our laboratory has employed the versatile Mitsunobu reaction for the solution-phase synthesis of simple thioglycosides and *S*-glycosyl amino acids.[13] The Mitsunobu reaction represents an efficient means by which thioglycosides can be prepared from 1-thiosugars and alcohols directly in high yield and under very mild conditions, without the need to synthesise and isolate halide or sulphonate intermediates. We were interested in transposing this reaction onto a solid-phase system.

There are many examples of Mitsunobu reactions on a solid-support[14] in the literature, but these are most commonly limited to *O*-alkylations (for example, the immobilisation of a phenol onto a hydroxymethyl derivatised resin)[15] and *N*-alkylations.[16] There are very

* Corresponding author. Tel.: +44-20-7753-5883; fax: +44-20-7753-5964; e-mail: john.malkinson@ulsop.ac.uk

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few examples of the synthesis of thioethers[17] via the solid-phase Mitsunobu reaction, and, to our knowledge, there are no other reports of thioglycoside synthesis using this method. The solid-phase approach here not only allows the reaction to be quickly driven to completion, but also permits the facile removal of the phosphine oxide by-product, so often problematic after the corresponding reaction in solution.[13] The covalent attachment of the starting 1-thiosugar to the solvated polymer network also eliminates the possibility[18] of disulphide formation.

The solid-phase synthesis of C-terminal *S*-linked glycopeptides necessitates the preparation of a suitably protected 1-thiosugar that can be immobilised onto the solid support either directly (via a free hydroxyl group) or, often more simply, following derivatisation with an appropriate linker. The nature of the solid support and that of the linker will determine the conditions necessary for removal of the completed glycopeptide from the resin, and can be tailored to meet the particular synthetic requirements of glycopeptide assembly.

A number of approaches have been developed for the solid-phase immobilisation of carbohydrate derivatives. These derivatives usually act as glycosyl donors (commonly thioglycosides) or acceptors and serve as starting points in the solid-phase assembly of oligosaccharides. Anchoring to the solid support has been achieved by derivatisation with a variety of linkers including substituted benzoate, succinate and similar esters,[19] as well as trialkylsilyl, triphenylmethyl (trityl) and *p*-alkoxybenzyl ethers.[20] Pyranoses are typically anchored to the functionalised resin or linker through their 6-position primary hydroxyl groups, which are usually most accessible synthetically.

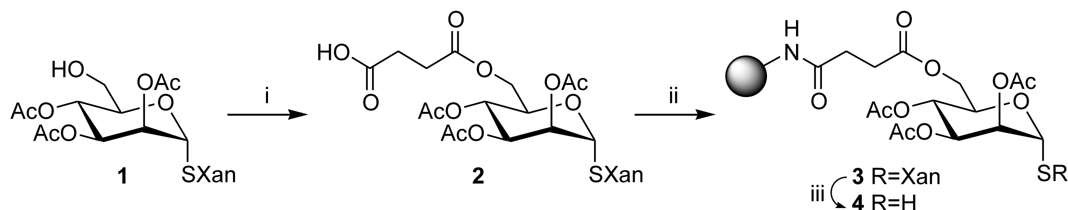
While there is a great deal of experience with selective deprotection of the thiol function of cysteine in SPPS, there is comparatively little published work on *S*-protected 1-thiosugars. Hindsgaul and co-workers[20b] employed anomeric thiosugars protected as their ethyl disulphide derivatives for the solid-phase synthesis of thio-oligosaccharides. We have previously used the highly acid labile *S*-9*H*-xanthen-9-yl (Xan) protecting group[21] for temporary thiol protection in solution-phase thioglycoside synthesis.[22] The *S*-Xan group can be selectively removed on the solid-phase by very mild acidolysis and is fully compatible with the majority of linkers and protecting groups employed in SPPS.

S-Xan protected thioglycoside building block **1** was prepared from 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside as previously described.[22] Initial attempts to immobilise **1** onto a variety of derivatised solid supports directly via its free 6-position hydroxyl group met with only limited success. Loading onto 4-(bromomethyl)phenoxymethyl, 2-chlorotrityl chloride or 3,4-dihydro-2*H*-pyran-2-ylmethoxymethyl derivatised polystyrene resins proved difficult to monitor by conventional means and failed to proceed to completion even after repeated attempts and protracted reaction times. Unfortunately, each of these linkers was too acid labile to allow selective removal of the *S*-Xan protecting group without some degree of undesired cleavage from the resin. Loading of **1** onto a carboxypolystyrene resin also proved difficult and inconvenient to monitor.

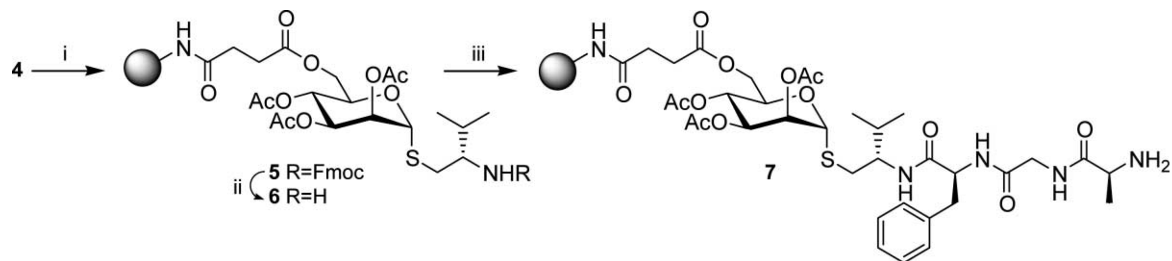
In order to overcome these problems, mannosyl building block **1** was modified with a succinate linker at its 6-position hydroxyl by reaction with succinic anhydride[22] (Scheme 1). The advantages of this approach are several-fold. The succinate linker contains a free carboxyl group allowing facile loading onto an amino-functionalised resin using classical solid-phase peptide coupling methodology, the completion of which can easily be monitored using the Kaiser test.[23] The extended succinate linker may help to overcome the steric problems associated with attempting to immobilise directly via the 6-position hydroxyl. The linker is also completely stable to the conditions of *S*-Xan deprotection and peptide chain extension, and can be cleaved simply with methanolic NaOMe or ammonia, with concomitant removal of *O*-acetyl protecting groups.

Additionally, the amino-functionalised resin onto which the succinate-modified glycosyl building block is loaded can be selected such that the resulting amide bond is either stable to (4-methylbenzhydrylamine (MBHA) resin) or labile to (Rink amide MBHA resin) treatment with TFA. If the amide bond is stable to TFA, the amino acid side-chain protecting groups can be removed without cleavage from the resin, or vice versa. If labile to TFA, the side-chain deprotected peptide can be cleaved from the resin while leaving all carbohydrate hydroxyl groups protected. This affords enormous synthetic flexibility.

Thioglycoside **2** was immobilised onto an MBHA derivatised polystyrene resin in good yield (Scheme 1).



Scheme 1. *Reagents and conditions:* (i) succinic anhydride, DMAP, pyridine, rt, 18 h, 72%; (ii) MBHA resin, HBTU, HOBt, *i*Pr_2NEt, DMF, rt, 2×0.5 h, 99%; (iii) CH_2Cl_2/TFA/Et_3SiH 97:2:1, rt, 1 h, then CH_2Cl_2/TFA/Et_3SiH 89:10:1, rt, 2×0.5 h.



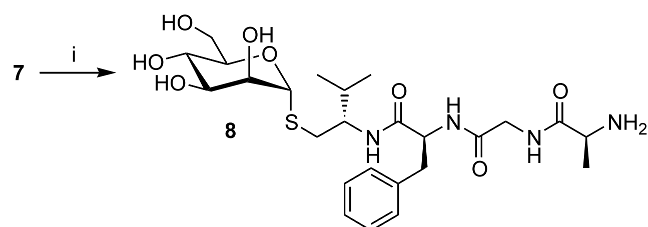
Scheme 2. *Reagents and conditions:* (i) Fmoc-Val-ol, PPh_3, DEAD, THF, rt, 3 h; (ii) 20% v/v piperidine, DMF, rt, 2×10 min; (iii) Fmoc-Phe-OH, HBTU, HOBt, $i$$Pr_2NEt$, DMF, rt, 2×0.5 h; 20% v/v piperidine, DMF, rt, 2×10 min; repeat for Fmoc-Gly-OH then Fmoc-Ala-OH.

The *S*-Xan protecting group was removed by mild acidolysis using 2–10% TFA in CH_2Cl_2 (employing triethylsilane as a scavenger) to generate resin-bound 1-thiosugar **4**.

The Mitsunobu reaction was performed in anhydrous THF, which promoted good solvation of the polystyrene polymer network. Diethyl azodicarboxylate (DEAD) was slowly added dropwise to a cooled solution of *N*-Fmoc protected amino alcohol and triphenylphosphine (the solution was cooled during addition of the DEAD in order to prevent potential decomposition and subsequent undesirable ethyl thioether formation). A twofold excess of alcohol over DEAD and phosphine was used to minimise the possibility of any remaining unreacted DEAD being alkylated by the thiol. After a short time to allow formation of the active oxyphosphonium species, the solution was added to the resin-bound 1-thiosugar and the Mitsunobu reaction allowed to proceed for 3 h (Scheme 2). After washing and *N*-Fmoc deprotection, the peptide chain was extended using standard N^{α}-Fmoc based SPPS.

The resin-bound model glycopeptide **7** was removed from the solid support (with simultaneous deprotection of the carbohydrate *O*-acetyl protecting groups) by nucleophilic cleavage with methanolic ammonia (Scheme 3). Freeze-drying afforded the crude product in 85% yield (based on resin loading) and 79% purity (determined by analytical HPLC[24]). This was confirmed by isolation of the pure (>97%) glycopeptide **8**[25] by preparative HPLC.[24]

The utility of the Mitsunobu reaction for the solid-phase assembly of C-terminal thioether-linked glycopeptides has been demonstrated for a simple model tetrapeptide containing no side-chain functionality. Current efforts are directed towards optimisation of the solvent, conditions and reagents used in the Mitsunobu reaction. We are also investigating the synthesis of glycopeptides incorporating amino acids with side-chain functionality, and how the nature of the amino-functionalised resin can be tailored to allow control of the order of cleavage, de-*O*-acetylation and side-chain deprotection.



Scheme 3. *Reagents and conditions:* (i) $NH_3{\cdot}MeOH$/THF (3:1), rt, 18 h.

General procedure for *S*-Xan deprotection and Mitsunobu reaction

Reactions were performed using anhydrous solvents under an argon atmosphere. Resin-bound thioglycoside **3** (100 mg, 0.44 mmol g^{-1} loading, 44 μmol) was washed and swelled in CH_2Cl_2. The resin was treated with CH_2Cl_2/TFA/Et_3SiH, 97:2:1 (3 ml, 1 h) then CH_2Cl_2/TFA/Et_3SiH, 89:10:1 (3 ml, 2×0.5 h), washed with CH_2Cl_2 then washed and swelled in THF. DEAD (35 μl, 0.22 mmol) was added dropwise to a solution of Fmoc-Val-ol (143 mg, 0.44 mmol) and PPh_3 (58 mg, 0.22 mmol) in 2 ml THF at 0°C. After 1 h, this solution was added to the drained resin and the suspension mixed by gentle agitation for 3 h. The resin was drained and washed with THF, CH_2Cl_2, CH_2Cl_2/MeOH, 1:1, then washed and swelled in DMF.

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References

1. Varki, A. *Glycobiology* **1993**, *3*, 97–130.
2. Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1–27.
3. Imperiali, B.; Shannon, K. L.; Rickert, K. W. *J. Am. Chem. Soc.* **1992**, *114*, 7942–7944.

4. Dyson, H. J.; Rance, M.; Houghten, R. A.; Lerner, R. A.; Wright, P. E. *J. Mol. Biol.* **1988**, *201*, 161–200.
5. Fisher, J. F.; Harrison, A. W.; Bundy, G. L.; Wilkinson, K. F.; Rush, B. D.; Ruwart, M. J. *J. Med. Chem.* **1991**, *34*, 3140–3143.
6. Nomoto, M.; Yamaoa, K.; Haga, M.; Hayashi, M. *J. Pharm. Sci.* **1998**, *87*, 326–332.
7. Bertozzi, C.; Bednarski, M. In *Modern Methods in Carbohydrate Synthesis*; Khan, S. H.; O'Neill, R. A., Eds.; Harwood Academic GmBH: Amsterdam, 1996; pp. 317–318.
8. (a) Gerz, M.; Matter, H.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 269–271; (b) Michael, K.; Wittmann, V.; Konig, W.; Sandow, J.; Kessler, H. *Int. J. Peptide Protein Res.* **1996**, *48*, 59–70.
9. Malkinson, J. P.; Falconer, R. A.; Toth, I. *J. Org. Chem.* **2000**, *65*, 5249–5252.
10. Cohen, S. B.; Halcomb, R. L. *J. Am. Chem. Soc.* **2002**, *124*, 2534–2543.
11. Elofsson, M.; Walse, B.; Kihlberg, J. *Tetrahedron Lett.* **1991**, *32*, 7613–7616.
12. Oliyai, R.; Stella, V. J. *Ann. Rev. Pharmacol. Toxicol.* **1993**, *32*, 521–544.
13. Falconer, R. A.; Jablonkai, I.; Toth, I. *Tetrahedron Lett.* **1999**, *40*, 8663–8666.
14. Bunin, B. *The Combinatorial Index*; Academic Press: San Diego, 1998; pp. 106–112.
15. (a) Richter, L. S.; Gadek, T. R. *Tetrahedron Lett.* **1994**, *35*, 4705–4706; (b) Krchnak, V.; Flegelova, Z.; Weichsel, A. S.; Lebl, M. *Tetrahedron Lett.* **1995**, *36*, 6193–6196.
16. (a) Zaragoza, F.; Stephenson, H. *Tetrahedron Lett.* **2000**, *41*, 1841–1844; (b) Chhabra, S. R.; Khan, A. N.; Bycroft, B. W. *Tetrahedron Lett.* **2000**, *41*, 1099–1102.
17. Poupart, M.-A.; Cameron, D. R.; Chabot, C.; Ghiro, E.; Goudreau, N.; Goulet, S.; Poirier, M.; Tsantrizos, Y. S. *J. Org. Chem.* **2001**, *66*, 4743–4751.
18. Camp, D.; Jenkins, I. D. *Aust. J. Chem.* **1990**, *43*, 161–168.
19. (a) Nicolaou, K. C.; Watanabe, N.; Li, J.; Joaquin, P.; Winssinger, N. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1559–1561; (b) Kononov, L. O.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1997**, *38*, 1599–1602; (c) Oertel, K.; Zech, G.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 1431–1433.
20. (a) Doi, T.; Sugiki, M.; Yamada, H.; Takahashi, T. *Tetrahedron Lett.* **1999**, *40*, 2141–2144; (b) Hummel, G.; Hindsgaul, O. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1782–1784; (c) Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1997**, *119*, 5562–5566.
21. Hin, Y.; Barany, G. *J. Org. Chem.* **1997**, *62*, 3841–3848.
22. Falconer, R. A. *Tetrahedron Lett.* **2002**, *43*, 8503–8505.
23. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. *Anal. Biochem.* **1970**, *34*, 595–598.
24. Analytical separation was achieved on a Vydac 250 mm× 4.6 mm C_4 column; flow rate 1.2 mL min^{-1}; A (0.1% v/v TFA), B (0.1% v/v TFA in 90% v/v MeCN), 0% B to 70% B over 20 min then 70% B to 0% B over 5 min; monitored at 214 nm; retention time for **8** = 7.73 min; preparative separation was achieved on a TSK-GEL 250 mm×21.0 mm C_6 column; flow rate 8.0 mL min^{-1}; 0% B to 100% B over 90 min; monitored at 214 nm.
25. ^{1}H NMR for **8** (500 MHz; 10% D_2O, 90% H_2O): δ 0.74, 0.77 (2d, J = 5.4 Hz, 6H, 2×CH_3Val), 1.41 (d, J = 5.6 Hz, 3H, βCH_3Ala), 1.69 (m, J = 5.4 Hz, 1H, C*H*Me_2Val), 2.40 (dd, J = 10.8, 7.7 Hz, 1H, SCH_2), 2.64 (dd, J = 10.8, 3.3 Hz, 1H, SCH_2), 2.92 (dd, J = 11.1, 5.9 Hz, 1H, βCH_2Phe), 3.04 (dd, J = 11.0, 6.4 Hz, 1H, βCH_2Phe), 3.56 (t, J = 7.7 Hz, 1H, *H*-4), 3.61–3.68 (m, 3H, *H*-3, *H*-6 and C*H*NHVal), 3.75–3.86 (m, 5H, *H*-6′, *H*-5, αCH_2Gly and *H*-2), 4.00 (q, J = 5.6 Hz, 1H, αC*H*Ala), 4.53 (q, J = 5.9 Hz, 1H, αC*H*Phe), 5.07 (s, 1H, *H*-1), 7.20–7.24 (m, 3H, C*H*ArPhe), 7.27–7.30 (m, 2H, C*H*ArPhe), 7.94 (d, J = 7.6 Hz, 1H, N*H*Val), 8.14 (d, J = 5.8 Hz, 1H, N*H*Phe), 8.44 (t, J = 4.6 Hz, 1H, N*H*Gly); ^{13}C NMR (125 MHz; 10% D_2O, 90% H_2O): δ 17.5 (β*C*H_3Ala), 18.4, 19.7 (2× *C*H_3Val), 32.3 (*C*HMe_2Val), 33.7 (S*C*H_2), 38.4 (β*C*H_2Phe), 43.3 (α*C*H_2Gly), 50.3 (α*C*HAla), 55.2 (*C*HNHVal), 56.4 (α*C*HPhe), 62.0 (*C*-6), 68.3 (*C*-4), 72.3 (*C*-3), 72.8 (*C*-2), 74.3 (*C*-5), 85.2 (*C*-1), 128.3, 129.9, 130.4, 137.4 (*C*HArPhe), 171.5 (*C*OGly), 172.4 (*C*OAla), 173.9 (*C*OPhe); FAB MS ($C_{25}H_{40}N_4O_8S$) 556.2567 m/z (%): 557 $[M+H]^+$ (22), 579 $[M+Na]^+$.