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Design and Synthesis of Silyl Ether-Based Linker for Solid-Phase Synthesis of Glycopeptides

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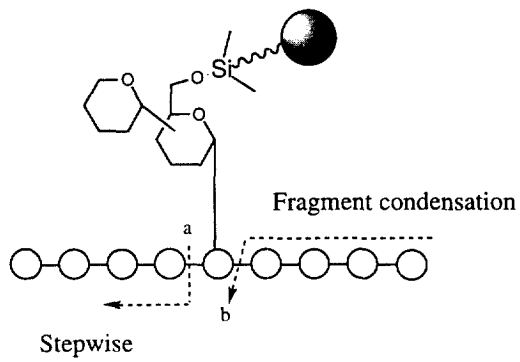
Abstract: A novel silyl linker was designed to facilitate the solid-phase synthesis of protected glycopeptide blocks. Alcohols (carbohydrate, serine, or threonine) were silylated with trialkylchlorosilane containing the p-nitrophenyl group. The nitro group was reduced and succinylated to give the succinanilic acids, which were attached to the glycine-preloaded resin via activation with HBTU/HOBt. After elongation of the peptide chain by segment condensation or Fmoc chemistry-based stepwise method, the synthesized glycopeptides in the protected form were split from the resin by fluoridolysis.

Solid-phase technologies are essential not only to rapidly assemble the long-chain peptides and nucleotides, but also to prepare the diverse molecules in a short time on the basis of combinatorial chemistry.[1] Attachment of the substrates (the first amino acid residue in peptide chemistry) to the resin supports has been achieved on a variety of anchoring groups, in which an acid- or a base-labile functionality is usually installed. Mildly or neutrally cleavable linkers have also been developed in order to retain the acid- and/or base-sensitive substituents. Amongst the latter, silyl linkers are of great promise because of their orthogonally cleavable property by fluoridolysis.[2] Several fluoridolyzable silyl linkers have been utilized for the syntheses of oligopeptides [2a, b] and oligosaccharides.[2c, i, j] In this paper we report a novel silyl linker useful for the synthesis of the protected glycopeptide blocks. The linker was designed so as to allow both the stepwise elaboration of N-terminus of the chain [a] and the segment condensation at the carboxylic acid end of the linked amino acid residue [b].



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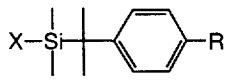
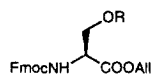
The known silyl chloride **1**, chloro(α,α-dimethylbenzyl)dimethylsilane,[3] was hydrolyzed with KOH in Et_2O-MeOH-H_2O to give silanol **2** (94%), which was converted into the p-nitro derivative **3** (61%) by nitration with NH_4NO_3 (1.2 eq) and $(CF_3CO)_2O$ (1.5 eq) in CH_3CN. Treatment of **3** with $(COCl)_2$ (1.2 eq) and DMF (cat) in CH_2Cl_2 afforded chloride **4** (93%) as colorless plates. Silylation of alcohols was facilitated using NaI and N-methylmorpholine (NMM) in DMF, as the conventional method using silyl chloride and imidazole in DMF resulted in elimination of p-nitrocumene to form the by-products.[4] The results of the silylation are summarized in Table 1.



1: R = H, X = Cl
2: R = OH, X = H
3: R = NO_2, X = OH
4: R = NO_2, X = Cl



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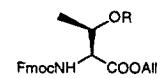

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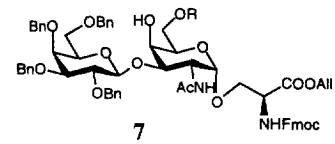

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Table 1. Yields of silyl ethers **5**, **6**, and **7** (R = $Si(Me)_2C_6H_4$-NO_2-p)

alcohol	yield (%) [a]	yield (%) [b]
5 (R = H)	97-100	37 (16) [c]
6 (R = H)	73	9 (73) [c]
7 (R = H)	86	30 (21) [c]

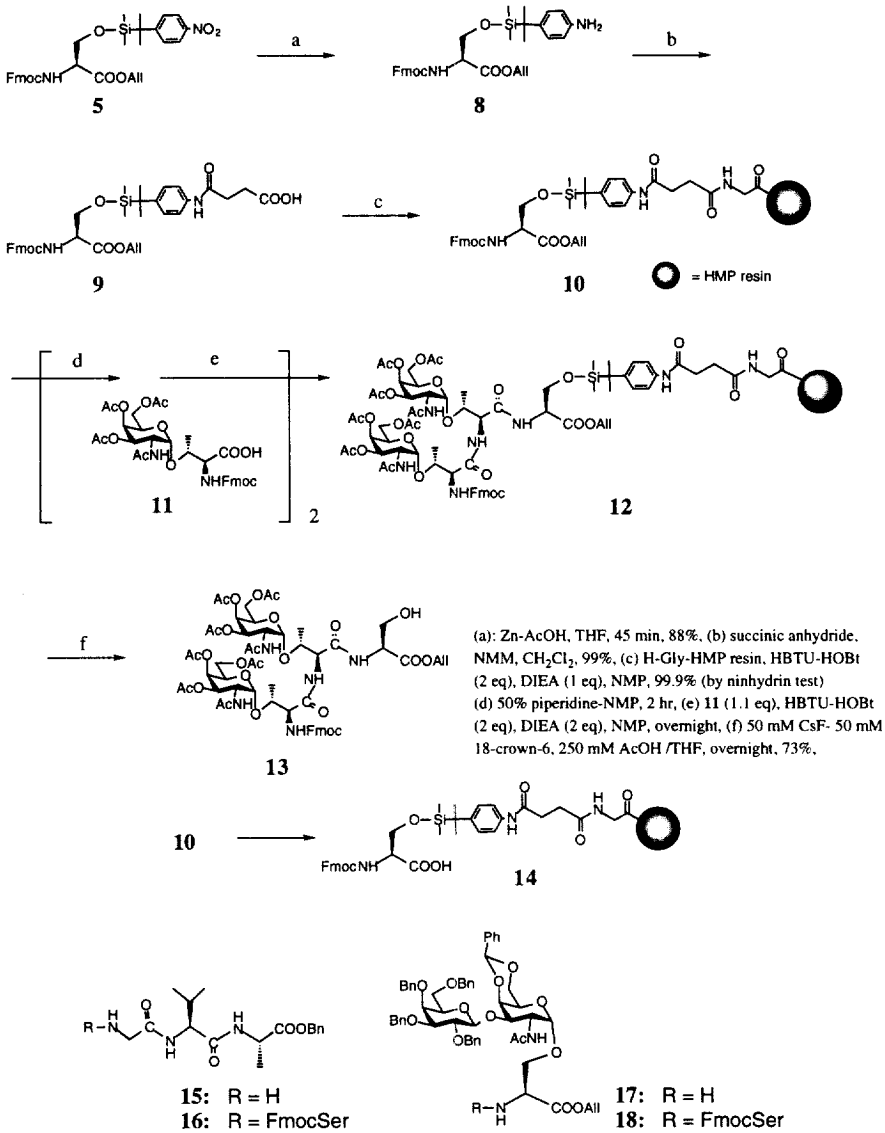
[a] **4** (1.2 eq), NaI (3 eq), NMM (1.2 eq) in DMF, room temp. 45 min - 2 h.

[b] **4** (1.2 - 2 eq), imidazole (3 - 4 eq) in DMF, room temp. 2-20h.

[c] Number in parenthesis indicates yield of the by-product (R = $Si(Me)_2OSi(Me)_2C_6H_4$-NO_2-p).

Versatility of the silyl linker in solid-phase synthesis was demonstrated by Fmoc serine allyl ester tethered to the linker. The silyl ether **5** (R = $Si(Me)_2C_6H_4$-NO_2-p) was reduced with Zn/AcOH (88%), and acylated with succinic anhydride to give succinanilic acid **9** (99%), which was then linked to the solid support. Commercially available FmocGly-preloaded Wang resin was N-deprotected by treatment with 50% piperidine in NMP (N-methylpyrrolidone) and the above succinanilic acid (1.2 eq) was attached by vortex-mixing with HBTU (O-benzotriazol-1-yl-N, N, N', N'-tetramethyluronium hexafluorophosphate, 2 eq), HOBt (1-hydroxybenzotriazole, 2 eq), and DIEA (diisopropylethylamine, 1 eq) in NMP at room temperature overnight utilizing the polypropylene test-tube. Since ninhydrin monitoring of the resultant resin exhibited 99.9% conversion and the serine-bound resin was obtained in reasonable quantity, the resin **10** was used further for synthesis of glycooligopeptides without any capping process.

Solid-phase synthesis was first investigated for the N-terminal chain elongation according to the standard Fmoc protocol. The serine-linked resin was N-deprotected and coupled with the protected N-acetylgalactosaminyl threonine **11** (1.5 eq) using the aforementioned activating agents. The coupling reaction was performed overnight on the vortex mixer and completion of the reaction was confirmed by ninhydrin test (99.9 %). After N-deprotection, the coupling reaction with **11** was repeated (99.9 %). The glycotripeptide thus synthesized was cleaved from the resin by treatment of **12** with CsF and AcOH in THF in the presence of 18-crown-6. Chromatographic purification afforded **13** in 73% yield and none of the dipeptidic by-product was detected.



5 **8** **9** **10** **11** **12** **13** **14**

15: R = H
16: R = FmocSer

17: R = H
18: R = FmocSer

The allyl ester-linkage of the silyl-linked amino acid **10** was readily cleaved using Pd(0) catalyst in the presense of dimedone.[5] The liberated carboxylic acid on the resin (**14**) was condensed with tripeptide amine **15** and glycosyl serine **17** using HBTU, HOBt, and DIEA in NMP.

The oligopeptides thus prepared were released from the resin by fluoridolysis and chromatographically purified to afford the properly protected oligomer blocks **16** and **18** as the sole products in 76 and 73 % yields, respectively. While amino acid analysis has not been performed, any contamination of the racemized products was not observed in the ^{1}H-NMR spectra of the synthesized peptides. Recently, similar attempts on C-

terminal elongation via activation of the resin-bound carboxylic acids, which might lead to racemization, have been reported.[2i, 6]

In summary, silyl chloride **4**, which serves as a precursor of the useful linker for the solid-phase synthesis of protected glycopeptides, was prepared in 3 steps from **1**. Utilizing the linker, facile syntheses of the glycopeptides were performed by way of the N- or C-terminal elongation. Syntheses of the natural glycoprotein fragments based on this methodology are currently under investigation.

Acknowledgments
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References and Notes

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7. Selected physical data are given below. compound **4**: mp 114.5-117 ℃, δ_H: 7.96 (d, 2H), 7.23 (d, 2H), 1.31 (s, 6H), 0.11 (s, 6H). compound **9**: $[\alpha]_D$ +2.6° (c 1.0), δ_H: 7.74-7.13 (m, 12H), 5.87 (m, 1H), 5.56 (d, 1H), 5.31 (brd, 1H), 5.23 (brd, 1H), 4.64 (d, 2H), 4.40 (m, 1H), 4.39, 4.31, and 4.24 (3brt, 3H), 3.95 (dd, 1H), 3.69 (dd, 1H), 2.63 and 2.50 (2m, 4H), 1.29 and 1.30 (2s, 6H), -0.50 and -0.59 (2s, 6H). compound **13**: $[\alpha]_D$ +83.0° (c 1.0), δ_H: 7.78 (d, 2H), 7.64 (d, 2H), 7.43-7.32 (m, 4H), 7.02 (d, 1H), 6.75 (d, 1H), 5.92 (m, 1H), 5.84 (d, 1H), 5.37 (brd, 1H), 5.36 (brd, 2H). 5.29 (dd, 1H), 5.21 (dd, 1H), 5.17 (d, 1H), 5.10 (dd, 1H), 4.99 (d, 1H), 4.69 (brd, 2H), 2.16 (s, 3H), 2.15 (s, 3H), 2.04 (s, 3H), 2.00 (s, 9H), 1.99 (s, 6H), 1.28 (d, 3H), 1.24 (d, 3H).