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A concise synthesis of the *O*-glycosylated amino acid building block; using phenyl selenoglycoside as a glycosyl donor

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Abstract

A new glycosylation methodology for synthesizing a protected TF antigen is described. The key step is to use phenyl selenoglycoside as a glycosyl donor, thereby successfully establishing *O*-linked Fmoc-protected threoninyl monosaccharide in an excellent yield with high α selectivity. From protected D-galactal, a protected TF antigen building block is obtained in 40% total yield. © 2000 Elsevier Science Ltd. All rights reserved.

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The significance of *O*-glycopeptides has been receiving increasing attention with its biological functions. Mucine-type glycopeptides with *O*-linked carbohydrate structures are currently being investigated as vaccines for the immunotherapy of a variety of cancers of epithelial origin.[1] Additional roles are involved in numerous disease states as the modification of the τ protein in Alzheimer's disease,[2] and L-serinyl-β-D-glucoside enkephalin analogues are able to cross the blood–brain barrier.[3]

Despite numerous methods to synthesize glycopeptides in general being available, the synthesis of serine and threonine with specific α- and β-*O*-linked carbohydrate structures as building blocks has been an intriguing problem. Usually, their synthesis results in lower yields or involves circuitous/cumbersome procedures,[1c,4] especially in the synthesizing protected form of TN antigen **1** and TF antigen **2** (Fig. 1). The resulting glycoamino acids are used to construct glycopeptides either by solid phase or solution phase methodology.[1c] Here we report an alternative synthetic methodology for constructing the valuable building block **2a**. The advantages in this synthetic strategy are: (i) efficient glycosylation between phenyl selenoglycoside and Fmoc-protected threonine — this is the first example demonstrating the usefulness of this glycosylation method for the construction of 2-amino-2-deoxy-α-D-galactosyl-threonine; and (ii) a concise synthetic strategy and higher total yield.

The azidonitration of protected glycals was discovered by Lemieux and Ratcliffe in 1979.[5] The obtained 2-azido-1-nitrate adducts could be transformed into various glycosyl donors.[5] However, the problem of the hydrolysis was addressed and several solutions have been proposed.[6] Azido-phenylselenylation of

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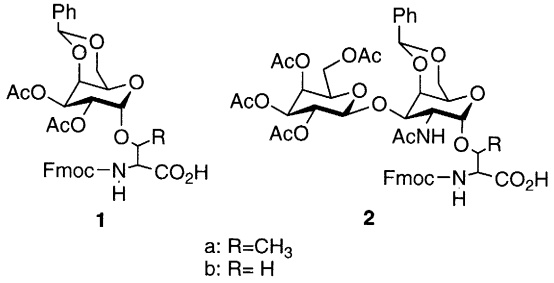
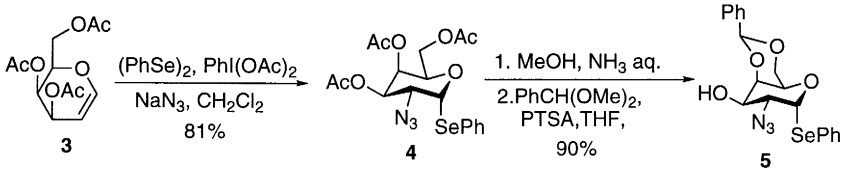


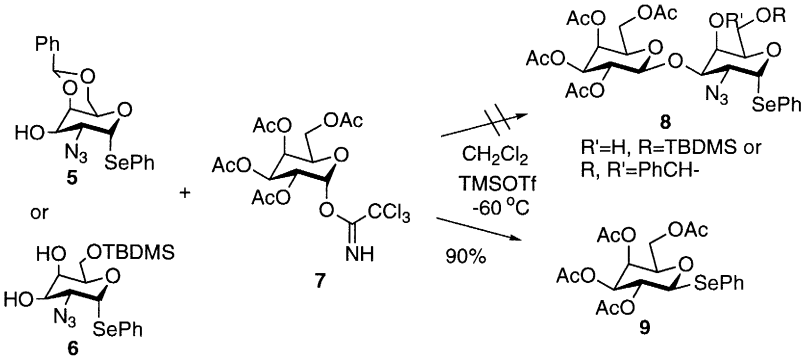
Fig. 1.

double bonds is a very versatile reaction because it allows the one-step introduction of two functionalities in a molecule,[7] and the phenyl selenoglycoside could be used as glycosyl donor directly.[8] Tri-*O*-acetyl-D-galactal **3** (1 equiv.) was treated with (diacetoxyiodo)benzene (1.4 equiv.) and sodium azide (2.4 equiv.) in the presence of diphenyldiselenide (0.7 equiv.) in CH_2Cl_2 (0.06 M) at rt for 48 h, and the sole product **4** was obtained in 81% yield.[7] The reaction must be performed at a lower concentration to prevent side reactions, especially in large-scale preparations. Subsequent *O*-deacetylation with NH_3 aq. in MeOH, followed by regioselective 4,6-*O*-benzylidenation with benzaldehyde dimethyl acetal/*p*-TsOH gave compound **5** in 90% total yield (Scheme 1). Mehta and Pinto[8] have previously described the selective activation of glycosyl bromide or trichloroacetimides over selenoglycoside. On the basis of the result, reaction of the selenoglycosidic acceptor **5** with the known glycosyl imidate donor **7** in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.05 equiv.) at −60°C[8] did not obtain the desired disaccharide **8**, but acquired monosaccharide **9** as a major product (90%). The same result was achieved in glycosylation of compound **6** with compound **7**. We proposed that selenoglycoside was selectively activated by TMSOTf over imidate, so phenyl selenide was first removed and proceeded nucleophilic reaction of glycosyl imidate in the presence of TMSOTf as a catalyst (Scheme 2). The phenomenon of the reactivity of both phenyl selenoglycosides (acceptors) and glycosyl trichloroacetimides (donors) was introduced by Mehta and Pinto. This was not a perfect statement because we achieved a reverse result in our case.



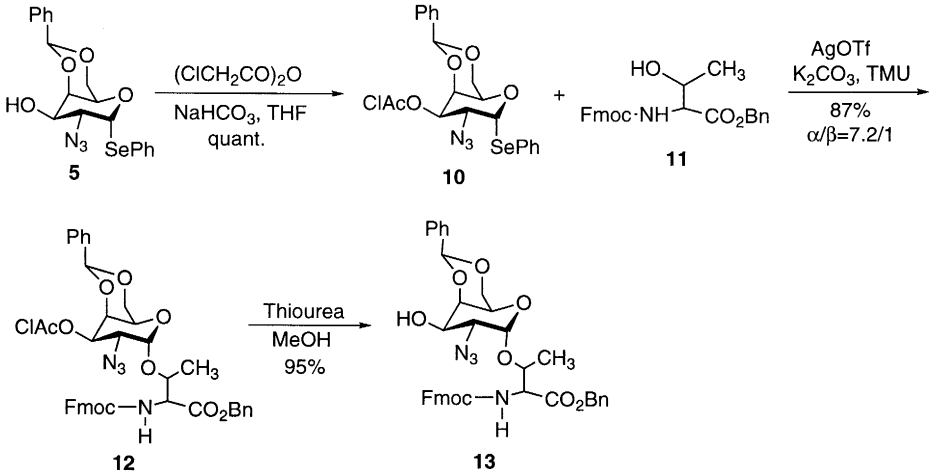
Scheme 1.

In order to circumvent the problem described above, we practiced an alternative strategy; *O*-threoninyl α-glycoside was first established, followed by the formation of disaccharide. The reaction of 3-*O*-unprotected selenoglycoside **5** with chloroacetic anhydride and $NaHCO_3$ in THF, started from −30°C to rt gave **10** quantitatively (Scheme 3).[9] The α-phenylselenide **10** was coupled with L-threonine derivative **11** (1.5 equiv.), and the reactivity was influenced by conducting the reaction in the varying conditions. Selenoglycoside **10** was unreactive under TMSOTf (0.05 equiv.) as a catalyst, because the chloroacetyl group in **10** reduced the reactivity of the anomeric center and thus prevented glycosylation proceeding. Glycosylation of acceptor **11** with selenoglycoside **10** in the presence of AgOTf (3 equiv.) and K_2CO_3 (5 equiv.) in dichloromethane at 25°C afforded the anomeric mixture **12** in 93% yield (α:β=1:1); even stirring at −20°C gave an unsatisfactory ratio (α:β=1.3:1). When ether or ether–dichloromethane was used as the solvent, instead of dichloromethane, glycosylations proceeded at a sluggish rate. Mehta



Scheme 2.

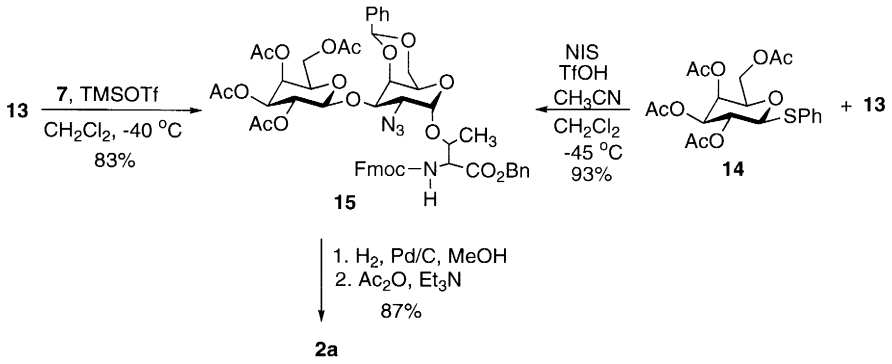
and Pinto[8] reported that selenoglycosides were rendered unreactive in the presence of an organic base such as collidine or 1,1,3,3-tetramethylurea (TMU), but an inorganic base such as K_2CO_3 did not quench the reaction. However, under the same conditions, adding TMU (2 equiv.) did not quench the reaction in our case, and an elevated α:β ratio of 3.8:1 (89% yield) under 25°C was obtained. When the condition was performed under lower temperatures (stirred at −10, 4 and 25°C for 16, 12 and 8 h, respectively), the better α:β ratio was improved to 7.2:1 (87% yield). A reverse anomeric effect[10] could explain the stereoselectivity; the formation of onium salt (by adding TMU) preferred the anomeric configuration being β, the nucleophile attacked from α orientation.[11] The chloroacetylated α-glycoside **12** was deblocked with thiourea to give **13** in 95% yield.[9]



Scheme 3.

Glycosylation of the threoninyl α-glycoside **13** with the glycosyl imidate donor **7** or thioglycoside donor **14** in the presence of either TMSOTf (0.5 equiv.) at −40°C or NIS (1.3 equiv.) and TfOH (0.4 equiv.) at −45°C gave the desired disaccharide **15** in 83 or 93% yield, respectively (Scheme 4). A large amount of TMSOTf or TfOH was used in order to avoid formation of the orthoester.[12] In the last step, the azido moiety and the benzyl ester of **15** were reduced with 5% Pd/C under H_2 in MeOH to provide the amino glycoside. The amine was converted to the acetamido derivative by acetic anhydride in the presence of Et_3N to afford the building block **2a** (exists as a mixture of rotamers) in 87% yield.[4b] The total yield was 40% from the known tri-*O*-acetyl-D-galactal **3**.

The building block **2a** was treated with trifluoroacetic acid at room temperature for 1 h, and the



Scheme 4.

benzylidene was deprotected with the *O*-glycosidic bond staying intact. On this basis, the Fmoc-protected glycopeptide **2a** is a suitable building block to synthesize long chain glycopeptides or glycoproteins using solid-phase synthesis of the Fmoc protocol.[13]

Acknowledgements

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