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Tetrahedron
Tetrahedron 63 (2007) 2170–2181

Synthesis of biantennary LacNAc-linked *O*-glycan (core 4) and glycopeptide thioester by benzyl protection strategy: rapid zinc reduction of GlcNTCA to GlcNAc by microwave irradiation

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Received 1 December 2006; revised 22 December 2006; accepted 25 December 2006
Available online 22 January 2007

Abstract—A synthetic method for the core 4 *O*-glycan-linked Ser and Thr was developed. Highly stereoselective 3-O- and 6-O-glycosylation was achieved by using two distinctively protected *N*-trichloroacetyllactosaminyl fluorides (**3** and **12**). Microwave-assisted Zn reduction rapidly and efficiently converted *N*-trichloroacetylglucosamine (GlcNTCA) to *N*-acetylglucosamine (GlcNAc). In order to demonstrate the usefulness of the protected core 4 *O*-glycan a segment (Gly^{34}-Gly^{58}) of emmprin (extracellular matrix metalloproteinase inducer), a cancer metastasis-related glycoprotein, was synthesized by the solid-phase method, utilizing the pentasaccharyl Thr (**2**) to introduce an *O*-glycan in place of the native *N*-glycan at Asn^{44}.

1. Introduction

Mucins and their *O*-glycans are of great importance and interest in a number of biological processes. Aberrant features of neoplastic mucins, such as overexpression and altered glycosylation, have attracted particular attention in connection with metastasis.[1] However, only limited knowledge of the biological roles of the alteration in mucins has been obtained so far. By considering the inaccessibility of a homogeneous mucin sample from natural sources, we have studied a synthetic approach to the glycoproteins with *O*-glycan, and recently established an original protocol using the benzyl-protected glycoamino acid building blocks in solid-phase glycopeptide synthesis.[2] In a previous study, we have synthesized the core 3 and core 6 oligosaccharides by glycosylating either the 3- or 6-hydroxyl group of the core *N*-acetylgalactosamine precursor with an *N*-trichloroacetyllactosaminyl glycosyl donor of high reactivity and β-selectivity. Usefulness of the synthetic *O*-glycan building blocks was demonstrated by the synthesis of MUC2 and MUC6 related glycopeptides.[3]

The *N*-acetylglucosaminyl substitution at both 3- and 6-position gives another core class *O*-glycan, known as core 4, which has been identified in the oligosaccharides from human bronchial mucins of cystic fibrosis patients,[4] secreted mucins of a human colonic cancer cell line,[5] human meconium mucins,[6] and sheep gastric mucins.[7] The core 4 oligosaccharides bearing the *N*-acetyllactosamine branches are of particular interest regarding an unanswered question, whether their physical, structural, and biological properties are different from those of the complex-type *N*-glycan as well as those of the core 2 *O*-glycan having an extension of *N*-acetyllactosamine to the core galactose residue.[6,8] To this end our investigations were directed to the synthesis of a glycopeptide with core 4 *O*-glycan. In this paper, we describe preparation of the core 4 glycoserine and glycothreonine building blocks, **1** and **2**, and performance of the solid-phase glycopeptide synthesis with **2** according to the established protocol.[9]

2. Synthesis of the building blocks 1 and 2

We first attempted selective di-O-glycosylation of 3,4,6-*O*-unmasked $GalN_3$–Thr derivative **4**[10] with known *N*-trichloroacetyllactosaminyl fluoride **3**[11] (2.2 equiv) by using $Cp_2ZrCl_2/AgClO_4$ as the promoter[12] in CH_2Cl_2 at −15 °C, since the 4-hydroxyl group of the $GalN_3$ residue was hardly glycosylated in many cases (Fig. 1).[3,10,11] This simple strategy, however, was unsuccessful and gave a complex mixture of a heptasaccharide and three pentasaccharides each in 5–14% yield after consuming the glycosyl fluoride for 3 h. As the second attempt, we reacted **3** and 6-*O*-silylated

Keywords: Glycopeptide; Core 4 *O*-glycan; Solid-phase synthesis; Microwave reaction.
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doi:10.1016/j.tet.2006.12.088

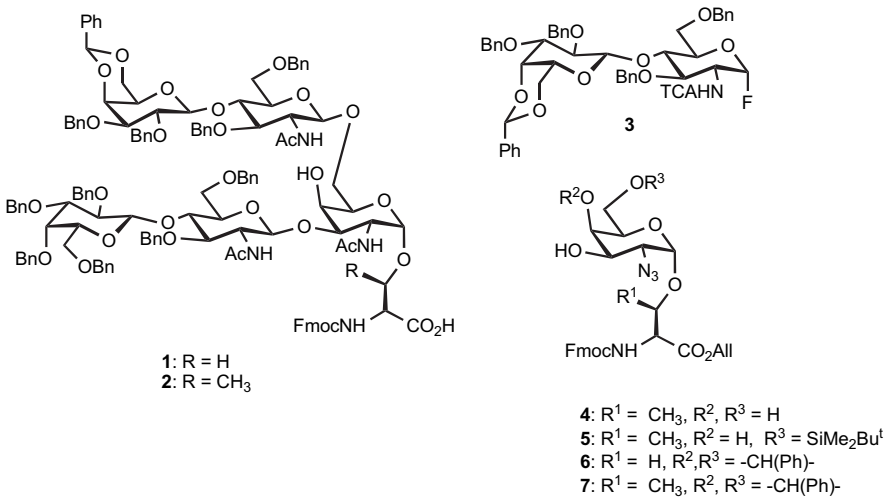
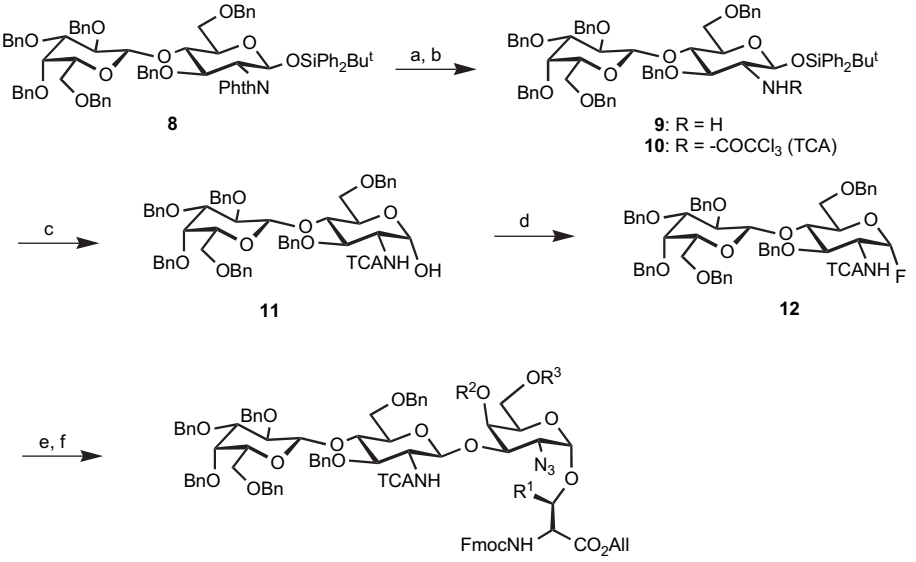


Figure 1. Structures of the protected core 4 pentasaccharyl Ser/Thr (**1** and **2**) and the known intermediates (**3**–**7**).

acceptor **5**[10] in expectation of attaining selective 3-O-glycosylation. But a pentasaccharide (12%) derived by 3,4-di-O-glycosylation was produced along with the desired trisaccharide (13%). In this reaction, an additional complication arose from the departure of the acid-labile silyl group of **5** under the reaction conditions. Thus, we were convinced that the side reaction on the 4-hydroxyl group was unavoidable, when this reactive glycosyl donor was used with the 3,4-unprotected glycosyl acceptors. In order to secure mono 3-O-glycosylation, we decided to use 4,6-*O*-benzylidene $GalN_3$–Ser/Thr derivatives, **6**[13] and **7**,[10] as the glycosyl acceptors, and instead needed a benzylidene group-free glycosyl donor that allowed selective deprotection of the 6-O position of the $GalN_3$ residue at a later stage. Thus, a perbenzylated *N*-trichloroacetyllactosaminyl fluoride was synthesized as an alternative to glycosyl donor **3**. Known lactosamine derivative **8**[14] was heated with ethylenediamine in *n*-BuOH to remove the *N*-phthaloyl group (Scheme 1). The resulting amine **9** was reacted with trichloroacetyl chloride in pyridine to give **10** (81% in two steps). Desilylation of **10** with *n*-Bu_4NF in THF in the presence of excess AcOH afforded hemiacetal **11**, which upon treatment with Et_2NSF_3 gave fluoride **12** (82% in two steps) as a mixture of anomers (α/β=19/1). Fluoride **12** seemed more reactive than **3**, and reacted with glycosyl serine **6** within 0.5 h by activation with $Cp_2Zr(ClO_4)_2$ at −15 °C to afford trisaccharide **13** as



Scheme 1. Synthesis of hexabenzylated glycosyl fluoride **12** and trisaccharyl serine/threonine, **15** and **16**. *Reaction conditions*: (a) 1,2-diaminoethane, *n*-BuOH, 90 °C, 2 days, 96%; (b) trichloroacetyl chloride, pyridine, 0 °C, 1.5 h, 84%; (c) *n*-Bu_4NF, AcOH, THF, room temperature, overnight, 89%; (d) diethylaminosulfur trifluoride, THF, 0 °C, 1 h, 92%; (e) **6** or **7**, Cp_2ZrCl_2, $AgClO_4$, CH_2Cl_2, −15 °C, 1 h, **13** (75%), **14** (80%); (f) 80% aq TFA, CH_2Cl_2, 94% (**15**), 83% (**16**).