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A Novel Glycosyl Donor for the Synthesis of Cancer Specific Core 5 and Sialyl Core 5 as Glycopeptide Building Blocks

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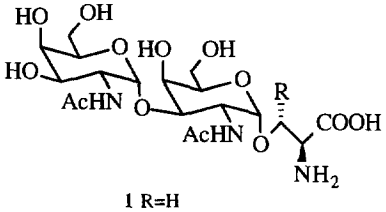
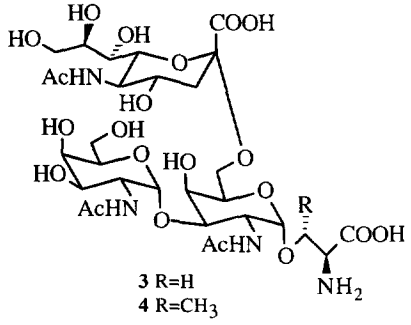
Abstract: Trichloroacetimidate at 1 and 3 position of 4,6-benzylidenyl N-acetylgalactosamine serves as a leaving group for glycosylation and a selective and acid sensitive protecting group respectively. This versatile donor, while forming exclusive α-glycoside with serine/threonine, serves as a fascile precursor to 3-OH which can be generated in acid medium without affecting 4,6-acetal protecting group or the protecting groups of serine/threonine. Synthesis of cancer associated carbohydrate Core 5 and its sialylated analog are accomplished through the use of this donor.

GalNAcα1-3GalNAc, α-linked to serines and threonines (**1**,**2**) of the mucin protein core, is classified as Core 5 structure.[1] This disaccharide has a restricted organ distribution in humans, since it has so far been found only in mucins from fetal intestinal secretions,[2] from meconium [3] and from colon carcinomas[4] where it exists as a mono 6-O-sialyl (**3**,**4**) analog. Predominantly cancer associated carbohydrate antigens like Tn, TF and STn have been successfully tested in experimental immunotherapy of human cancers in spite of their presence, though not in significant quantities, on normal cells.[5] But core 5 and sialyl core 5 structures, mostly restricted to cancer cells, may be effective as cancer specific targets for immunotherapy as well as in the development of diagnostic monoclonal antibodies.



1 R=H
2 R=CH_3

Core 5



3 R=H
4 R=CH_3

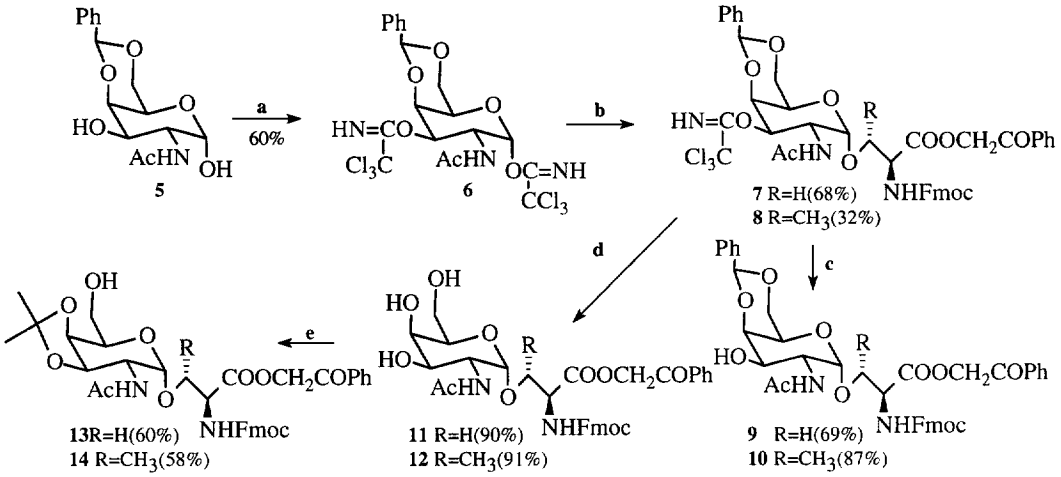
Sialyl Core 5

We have previously reported the use of N-acetylgalactosamine based donors for the synthesis of glycopeptide building blocks Tn /STn[6], TF/STF[7] and Core 2/Core 6/F1-α.[8] We now report a versatile 4,6-benzylidenyl N-acetylglactosamine (**5**) based 1,3-di trichloroacetimidate donor **6**, for the synthesis of core 5 and sialyl core 5 as glycopeptide building blocks.

Schmidt and his colleagues[9] obtained a mixture of 1 and 1,3 di-trichloroacetimidates of 2-azido, 4,6-benzylidenyl galactose using trichloroacetonitrile and sodium hydride in ethyleneglycol dimethylether. Under similar conditions 4,6-benzylidenyl N-acetylgalctosamine **5** failed to yield trichloroacetimidate adducts. However, using methylene chloride as solvent and DBU as a base, trichloroacetonitrile reacted with **5** at -20°C to form 1,3-di-trichloroimidate **6** in 60% yield (scheme 1). The unusual stability of the trichloroacetimidate group at 3-position under glycosylation conditions lead us to its use as a protecting group while the 1-trichloroacetimidate functions as a leaving group. In glycosylation reactions with N-Fmoc and phenacylester protected serine and threonine, mild acid sensitive protecting groups are more desirable than base sensitive groups. Trichloroacetimidate as a non-anomeric hydroxy protecting group fills this need while serving as a

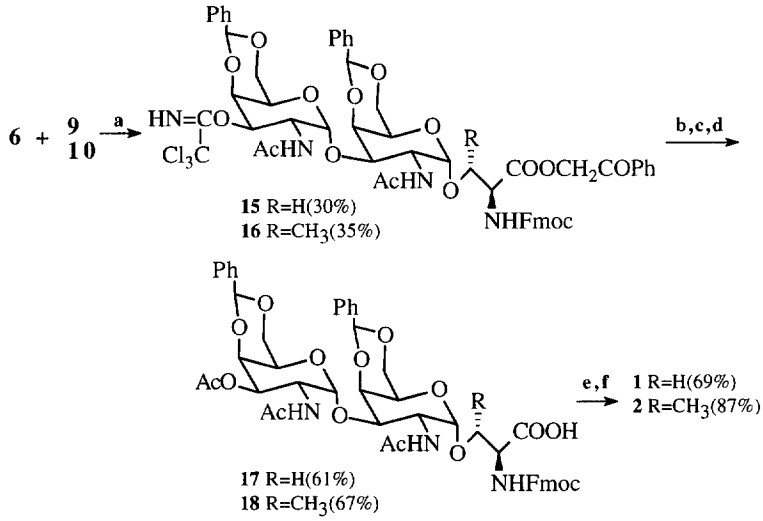
leaving group at the anomeric carbon. Using a donor such as **6**, α-glycosides **7** and **8** are exclusively formed in 68% and 32% yields respectively (scheme 1). The 3-trichloroacetimidate group is selectively removed by stirring **7** and **8** in 80% aqueous acetic acid at room temperature overnight to form **9** and **10** respectively. When the temperature is raised to 80°C for 2 hrs, however, 4,6-benzylidene group is also deblocked forming 3,4,6-trihydroxy intermediates **11** and **12**. Protection of 3,4-hydroxyl group using isopropylidine afforded intermediates **13** and **14**. These intermediates can be used to synthesize core 5 and sialyl core 5 building blocks.

Scheme 1



a. CH_2Cl_2, CCl_3CN, DBU, -20°C; b. N-Fmoc-L-serine or threonine phenacyl ester, BF_3-OEt_2,-20°C, THF; c. 80% CH_3COOH in H_2O, r.t.; d. 80% CH_3COOH in H_2O, 80°C; e. 2,2-Dimethoxypropane, TSOH, DMF.

Scheme 2

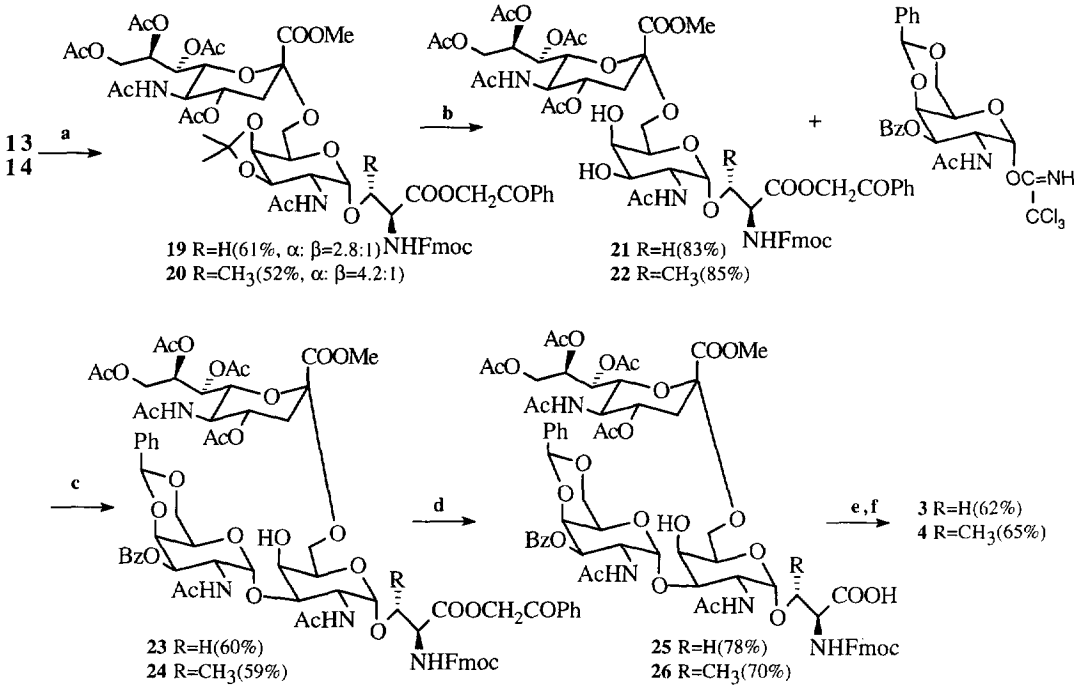


a. BF_3-OEt_2, -20°C, THF; b. CH_3COOH in H_2O, r.t.; c. Ac_2O/Py; d. Zinc/80% CH_3COOH in EtOAc; e. 80% CH_3COOH in H_2O, 80°C; f. 0.1 N NaOH.

Another α-GalNAc can be introduced to the 3-position of **9** and **10** to form protected core 5 structures **15** and **16** using the donor **6**. After selective deblocking and blocking of the 3-position, phenacyl group cleavage gave glycopeptide building blocks **17** and **18** which may be used in solid phase synthesis. Total deblocking afforded free core 5 antigens **1** and **2** (scheme 2).

Sialylation of intermediates **13** and **14** using sialyl phosphite donor[7] in THF gave higher α-selectivty compared to the same procedure using acetonitrile as the solvent. Following the removal of isopropylidine group, α-GalNAc was introduced at 3-position of intermediates **21** and **22** using GalNAc donor[6] to obtain sialyl core 5 analogs **23** and **24** respectively, in about 60% yield. Partial and total deblocking of these analogs gave both sialyl core 5 building blocks **25**, **26** and free antigens **3**, **4** respectively (scheme 3).

Scheme 3



a. Sialic acid donor, TMS-OTf, -20°C, THF; b. 80% CH_3COOH in H_2O, 60°C, 2 hrs; c. BF_3-OEt_2, -20°C, THF; d. Zinc, 80% CH_3COOH in EtOAc; e. 80% CH_3COOH in H_2O, 80°C; f.0.1 N NaOH.

The use of trichloroacetimidate as a protecting group may avoid additional steps of protection/deprotection in repetitive glycosylations and may be useful in generating a succession of 1-3 linkages to produce linear oligosaccharides and a wide variety of O-linked glycoaminoacid building blocks. Trichloroimidate donor of GalNAc with 4,6-benzylidine not only reacts with serine or threonine acceptors but also with carbohydrate acceptors to give exclusive α-glycosides in 30-70% yields. Synthesis of core 3, core 4 and sialyl core 3 using intermediates **9-14** and the applications of a variety of glycosylated amino acids in generating a library of glycoforms of cancer-associated mucin type glycopeptides will be reported in time.

Acknowlegements

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10. NMR data in D_2O for compounds **1-4** and $CDCl_3/CD_3OD$(5:1) for compounds **17** and **18**.

1. δ=5.08 (d, 1 H, J=3.5 Hz, H-1b), 4.95 (d, 1 H, J=3.5 Hz, H-1a), 4.40 (dd, 1 H, J=3.5, 11.0 Hz, H-2b), 4.21 (dd, 1 H, J=3.5, 11.0 Hz, H-2a), 3.70-4.20 (m), 2.07, 2.04 (2 s, 6 H, 2 Ac).

2. δ=5.06 (d, 1 H, J=3.5 Hz, H-1b), 5.00 (d, 1 H, J=3.5 Hz, H-1a), 4.50 (m, 1 H, Thr-α-H), 4.33(dd, 1 H, J=3.5, 11.0 Hz, H-2b), 4.21 (dd, 1 H, J=3.5, 11.0 Hz, H-2a), 3.70-4.20 (m), 2.07, 2.03 (2 s, 6 H, 2 Ac), 1.41 (d, 1 H, J=6.5 Hz, CH_3).

3. δ=5.07 (d, 1 H, J=3.5 Hz, H-1c), 4.94 (d, 1 H, J=3.5 Hz, H-1a), 4.40 (dd, 1 H, J=3.5, 11.0 Hz, H-2), 4.23 (dd, 1 H, J=3.5, 11.0 Hz, H-2), 3.50-4.20 (m), 2.75 (dd, 1 H, J=4.5, 12.5 Hz, H-2b eq), 2.04, 2.01 (2 s, 6 H, 2 Ac), 1.70 (t, 1H, J=12.5 Hz, H-2b ax).

4. δ=5.02 (d, 1 H, J=3.5 Hz, H-1c), 4.96 (d, 1 H, J=3.5 Hz, H-1a), 4.46 (m, 1 H, Thr-α–H), 4.32(dd, 1 H, J=3.5, 11.0 Hz, H-2), 4.22 (dd, 1 H, J=3.5, 11.0 Hz, H-2), 3.40-4.20 (m), 2.73 (dd, 1 H, J=4.5, 12.5 Hz, H-2b eq), 2.05, 2.04 (2 s, 6 H, 2 Ac), 1.69 (t, 1 H, J=12.5 Hz, H-2b ax), 1.45 (bd, 3 H, J=6.5 Hz, CH_3).

17. δ=7.30-7.90 (m, Ar-H), 5.52, 5.51 (2 s, 2 H, 2 CHPh), 5.19 (d, 1 H, J=3.5 Hz, H-1b), 4.97 (dd, 1 H, J=3.0, 11.0 Hz, H-3b), 4.91 (m, 1 H, H-1a). 4.70 (dd, 1 H, J=3.5, 11.5 Hz, H-2b), 4.60 (dd, 1 H, J=3.5, 10.5 Hz, H-2a), 3.70-4.50 (m), 2.01 (s, 6 H, 2Ac), 1.55 (s, 3 H, Ac).

18. δ=7.30-7.90 (m, Ar-H), 5.56, 5.55 (2 s, 2 H, 2 CHPh), 5.19 (d, 1 H, J=3.5 Hz, H-1b), 5.06 (m, 1 H, H-1a), 4.96 (dd, 1 H, J=3.0, 11.0 Hz , H-2b), 4.70 (dd, 1 H, J=3.5, 11.5 Hz, H-2a), 3.70-4.60 (m), 2.02, 2.00 (2 s, 6 H, 2 Ac), 1.52(s, 3 H, Ac), 1.25 (d, 3 H, J=6.5Hz, CH_3).

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