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Versatile Use of Ytterbium(III) Triflate and Acid Washed Molecular Sieves in the Activation of Glycosyl Trifluoroacetimidate Donors. Assemblage of a Biologically Relevant Tetrasaccharide Sequence of Globo H

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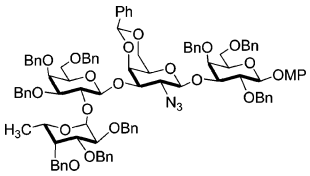
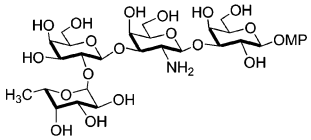


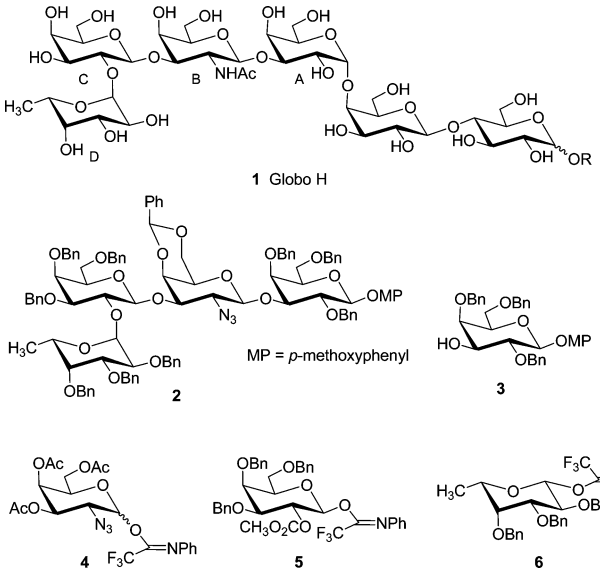


The nonreducing tetrasaccharide terminus of Globo H has been assembled in good yield and excellent stereocontrol exclusively by using mild and moisture stable agents such as $Yb(OTf)_3$ and acid washed molecular sieves for the activation of glycosyltrifluoroacetimidate donors in the glycosylation steps.

In the last years the hexasaccharide Globo H (**1**, Chart 1) has been emerging as an important antigenic oligosaccharide sequence for the development of vaccines against some tumors.[1] Several truncated versions of Globo H have been prepared and biologically evaluated to define synthetically simpler candidates as anticancer vaccines.[2,3] These investigations led to the disclosure of a relevant immunogenic activity associated with the tetrasaccharidic nonreducing end of Globo H.[4]

In this paper an original and efficient approach is proposed for the synthesis of this sequence in a protected

CHART 1. Target Compound and Building Blocks



form (**2**) that is suitable for the construction of novel *N*-derivatized analogues, the 2-azido group functionality representing a useful handle to this purpose. The whole synthetic sequence relies exclusively on glycosidation procedures based on the convenient moisture stable promoters we recently reported. In particular the assemblage of the sequence demonstrates the efficacy of both 4Å acid washed molecular sieves (4Å AW MS)[5] and ytterbium(III) triflate[6] as activators of glycosyl *N*-phenyltrifluoroacetimidate[7] donors and their complementary use in the stereocontrolled construction of three strategically different typologies of glycosidic linkages. In fact, acid washed molecular sieves can both play the usual role of drying agents and promote the stereocontrolled synthesis of 1,2-trans glycosides with donors bearing participating groups at position 2. In the absence of such a group, either 1,2-cis or 1,2-trans selectivity can be attained by the use of $Yb(OTf)_3$ and the suitable choice of the reaction solvent. *N*-Phenyltrifluoroacetimidate donors were chosen for their lower propensity to give undesired side products in the course of glycosidations,[8] and their higher stability in storage than the corresponding trichloroacetimidate analogues.[7] The synthesis was based on monosaccharide building blocks **3**−**6** as the precursors of residues A−D, respectively. Donors **4** and **6** were prepared in high yield (76−99%) by reacting the corresponding known hemiacetals with a slight excess of *N*-phenyltrifluoroacetimidoyl chloride and NaH in anhydrous dichloromethane. The use of a milder base such as DIPEA resulted in sluggish reactions.[6] Donor **5** was prepared as shown in Scheme 1.

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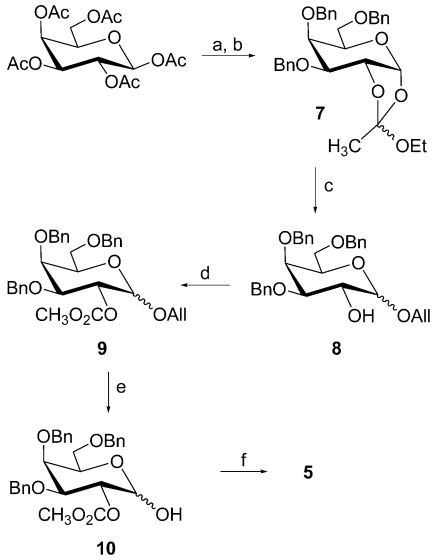
SCHEME 1. Synthesis of Galactosyl Donor 5[a]



[a] Reagents and conditions: (a) I_2, Et_3SiH, DCM reflux, 30 min, then lutidine, ethanol, TBAB, from 0 °C to rt, overnight; (b) KOH, toluene, reflux, then benzyl bromide, 1 h, 50−56% overall yield; (c) allyl alcohol, acetyl chloride, 70 °C, 2 h, 88%; (d) TMEDA, methyl chloroformate, DCM, 0 °C, 30 min, quant; (e) $PdCl_2$, methanol, 5 h, rt; (f) trifluoroacetimidoyl chloride, DIPEA, DCM, 36 h, rt, 73% from **8**.

Ortho ester intermediate **7** (diastereoisomeric mixture) was accessed starting from peracetylated galactopyranose through a one-pot sequence of anomeric iodination, halide promoted ortho esterification, deacetylation, and benzylation followed by a chromatographic purification (50−56% overall yield).[9] **7** was then exposed to allyl alcohol at 70 °C in the presence of in situ generated HCl to achieve simultaneous introduction of the anomeric allyl group and deprotection of the 2-OH. Intermediate **8** (anomeric mixture) was readily purified by chromatography and then protected in quantitative yield with a methoxycarbonyl group. The choice of this unusual protecting group was supported by our previous observations: (a) 2-*O*-methoxycarbonylated donors display less propensity to yield ortho ester-like coupling products than the more canonical acetylated or benzoylated counterparts, especially when glycosidations are conducted under very mild activation conditions;[10] (b) the product of the TMEDA based methoxycarbonylation procedure[11] is recovered pure in quantitative yield after a very short reaction time by a simple extractive workup; and (c) chemical conditions for the removal of this group are comparable to those required by usual *O*-deacylations (see below). Compound **9** was subjected to anomeric deallylation with catalytic $PdCl_2$. Crude compound **10**, isolated by a simple filtration, was directly converted into the corresponding trifluoroacetimidate **5**. In this case DIPEA was a sufficiently strong base to accomplish the reaction with *N*-phenyltrifluoroacetimidoyl chloride in anhydrous DCM. It is worth pointing out that under these conditions the β-anomer was obtained almost exclusively. In contrast, compound **5** was obtained as an approximately equimolar mixture of anomers when **10** was subjected to the procedure reported[7b] by Yu (reaction with an excess of trifluoroacetimidoyl chloride in lab grade non-anhydrous acetone and K_2CO_3 as the base). It also should be noted that the whole synthetic sequence to donor **5** requires eight chemical transformations but only three chromatographic purifications.

With the required building blocks in hand, the linear construction of the tetrasaccharide started with the coupling (Scheme 2) of the known acceptor **3**[12] with donor **4** (anomeric mixture), equipped with a 2-azido functionality. Despite the lack of participating ability of the azide group, the reaction gave excellent results thanks to the activation of catalytic ytterbium(III) triflate (0.1 equiv) and the β-directing effect exerted by the acetonitrile solvent.[6,13] As a matter of fact, the β-linked disaccharide **11** was obtained in high yield (70−77%) and traces of the α-linked disaccharide could be monitored only by a careful inspection of the NMR spectrum of the crude reaction mixture. Interestingly, this result was achieved without resorting to the low temperatures required for the corresponding TMSOTf promoted reactions of 2-azido trichloroacetimidates.[13,14] Moreover, 2-azido-3,4,6-*O*-acetylated trichloroacetimidates were recently reported to provide disappointing results in TMSOTf promoted glycosidatons in nitrile solvents.[14]

Disaccharide **11** was submitted to a deacetylation−benzylidenation sequence that readily provided the disaccharide acceptor **12** (80% yield over two steps) that was then coupled with the galactose donor **5**. In initial attempts the use of commercially available 4Å acid washed molecular sieves in the double role of activators and drying agents led to satisfying yields (61−64%).[5] Replacement of the 4Å with the 5Å AW MS afforded slightly higher yields (65−70%) within a sensibly shorter reaction time (ca. 24 h vs 48 h). A further improvement (75% yield) was registered with a modified procedure that entails the slow addition of donor **5** to a solution of acceptor **12** in a dichloroethane/cyclohexane mixture containing the 5Å sieves. The resulting trisaccharide **13** was easily deprotected with K_2CO_3 in methanol at 40 °C to yield acceptor **14** (89%). The final sterecontrolled α-L-fucosylation of the sterically encumbered 2-OH was achieved by means of the recently[15] described procedure that combines the efficient activation of catalytic ytterbium(III) triflate with an α-directing solvent (a 4:1:1 dichloromethane/dioxane/diethyl ether mixture). Due to the high reactivity of the perbenzylated fucosyl donor **6**, the reaction was conducted at low temperature (−30 °C) to give the desired α-anomer **2** (66% yield). Hydrogenolysis of **2** led to the removal of benzyl and benzylidene groups and the concomitant reduction of the azide functionality. The resulting compound **15** is expected to be a useful building block for the planned synthesis of

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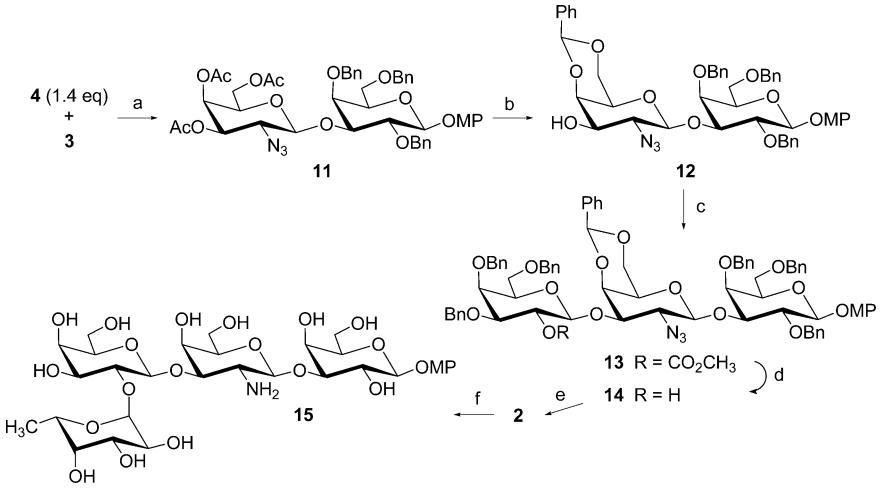
SCHEME 2. Construction of the Tetrasaccharide[a]



[a] Reagents and conditions: (a) $Yb(OTf)_3$, acetonitrile, from 0 °C to rt, overnight, 70−77%; (b) 9:1 MeOH/aq ammonia (32%), 3 h, then acetonitrile, benzaldehyde dimethyl acetal, camphorsulfonic acid (cat.) 70 °C, 3 h, 80% overall yield; (c) **5**, 5Å AW MS, dichloroethane/cyclohexane 5:1, rt, overnight, 75%; (d) K_2CO_3, methanol, 40 °C, 8 h, 89%; (e) **6**, $Yb(OTf)_3$, DCM/diethyl ether/dioxane 4:1:1, from −30 °C to rt, 66%; (f) $Pd(OH)_2$, H_2, 3:3:1 DCM/MeOH/H_2O, rt, 90%.

novel analogues (see above) whose preparation and biological evaluation will be reported in due course.

In conclusion, the protected form **2** of the tetrasaccharide extremity of Globo H was efficiently accessed resorting exclusively to mild, moisture stable, and easy to handle glycosidation promoters. In the absence of 2-*O*-participating groups on the donor, ytterbium(III) triflate proved efficient in promoting the synthesis of either 1,2-cis or 1,2-trans glycosides, depending on the nature of the adopted solvents. With donors equipped with appropriate participating groups even the sole acid washed molecular sieves can be used to conveniently perform 1,2-trans glycosidations. This work demonstrates that a practical alternative to the harsh and sensitive agents adopted in standard glycosylation protocols is available for the assemblage of nontrivial oligosaccharide sequences.

Experimental Section

Glycosidation Procedures: *p*-Methoxyphenyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-azido-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-D-galactopyranoside (11). Donor 4 (246 mg, 0.49 mmol) and acceptor 3[12] (194 mg, 0.35 mmol) were coevaporated three times with anhydrous toluene and kept for an hour under vacuum. After the addition of freshly activated 4Å AW 300 MS (ca 400 mg in pellets), the mixture was dissolved under argon in anhydrous acetonitrile (1.8 mL) at 0 °C. After 15 min a solution of $Yb(OTf)_3$ (21.7 mg, 0.035 mmol) in acetonitrile (1.1 mL) was added. The mixture was allowed to warm to room temperature and left overnight under stirring to ensure complete glycosidation. The reaction was quenched with a few drops of pyridine and the mixture filtered on a short plug of silica gel eluted with 9:1 dichloromethane/methanol (with a few drops of pyridine). The residue was then chromatographed on a silica gel column eluted with petroleum ether/ethyl acetate (from 8:2 to 7:3) to yield pure disaccharide 11 (211 mg, 70%). $[\alpha]_D$ −32.6 (*c* 0.5 in CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ 7.40−6.80 (aromatic protons), 5.33 (1H, bd, J = 3.4 Hz), 5.11−4.36 (6H, 3 × AB, 3 × benzyl CH_2), 4.86 (2H, 2 × d, J = 7.6 and 8.0 Hz), 4.76 (1H, dd, J = 7.6 and 11.0 Hz), 4.22−4.06 (3H), 3.98−3.90 (2H), 3.78 (3H, s, $-OCH_3$), 3.74−3.56 (5H), 2.16, 2.07, 2.00 (3 × 3H, 3 × s, 3 × $-COCH_3$). ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.2, 170.2, 169.4, 155.3, 151.5, 138.5, 138.5, 137.8, 128.5−127.8, 118.5, 114.5, 103.1, 102.7, 80.4, 79.2, 75.7, 75.3, 74.8, 73.7, 73.6, 70.9, 70.6, 68.8, 66.4, 61.4, 61.1, 55.6, 20.6. $C_{46}H_{51}N_3O_{14}$ calcd C 63.51, H 5.91, found C 63.23, H 5.68.

***p*-Methoxyphenyl 3,4,6-Tri-*O*-benzyl-2-*O*-methoxycarbonyl-*β*-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-azido-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-D-galactopyranoside (13).** A solution of donor **5** (86 mg, 0.12 mmol) in 5:1 dichloroethane/cyclohexane (720 μL) was added in 6 h at room temperature by a syringe pump to a solution of acceptor **12** (53 mg, 0.063 mmol) in 5:1 dichloroethane/cyclohexane (1.2 mL) containing freshly activated 5Å AW molecular sieves in pellets (1.1 g). After completion of the addition the mixture was left under overnight stirring to ensure complete consumption of the donor. The mixture was then filtered on a cotton plug washed repeatedly with 9:1 dichloromethane/methanol (with drops of pyridine). Silica gel chromatography of the residue from the organic phase (eluent: petroleum ether/ethyl acetate from 8:2 to 65:35) afforded pure trisaccharide **13** (63 mg, 75%) as an oil. $[\alpha]_D$ −12.3 (*c* 1.2 in CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$) δ 7.50−6.80 (aromatic protons), 5.51 (1H, s), 5.23 (1H, dd, J = 7.8 and 9.6 Hz), 5.10−4.30 (12H, 6 × AB, 6 × benzyl CH_2), 4.84 (1H, d, J = 7.5 Hz), 4.71 (1H, d, J = 7.8 Hz), 4.68 (1H, d), 4.26−4.20 (2H), 4.12−4.04 (2H), 3.94−3.78 (4H), 3.77 and 3.74 (2 × 3H, 2 × s, 2 × $-OCH_3$), 3.70−3.40 (7H), 3.24 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.1, 155.0, 151.6, 138.6, 138.5, 138.3, 138.0, 137.8, 137.8, 137.4, 128.6−126.3, 118.4, 114.4, 103.1, 103.0, 102.4, 100.6, 81.0, 80.5, 79.1, 78.1, 75.8, 75.6, 75.2, 74.7, 74.5, 73.9, 73.4, 73.0, 72.6, 69.2, 69.0, 66.5, 62.9, 55.6, 55.0. MALDI-TOF MS for $C_{76}H_{79}N_3O_{18}$ (*m/z*): M_r (calcd) 1321.54, M_r (found) 1344.80 $(M + Na)^+$. $C_{76}H_{79}N_3O_{18}$ calcd C 69.02, H 6.02, found C 68.88, H 6.21.

***p*-Methoxyphenyl 2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-*β*-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-azido-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-*β*-D-galactopyranoside (2).** Trisaccharide **14** (69 mg, 0.055 mmol) and the fucosyl donor **6** (99 mg, 0.16 mmol) were coevaporated three times in anhydrous toluene. After adding 4Å AW 300 MS, the mixture was dissolved under argon in 4:1 dichloromethane/diethyl ether (1.5 mL) and immediately cooled to −30 °C. After the mixture was stirred for 15 min, a solution of ytterbium triflate (3.4 mg, 5.5 μmol) in dioxane (300 μL) was added dropwise. After 3 h at −30 °C the mixture was allowed to warm to room temperature to ensure the consumption of residual amounts of the donor and the reaction was then quenched with pyridine. The mixture was filtered on a short plug of silica gel washed with 9:1 dichlo-

romethane/methanol (with drops of pyridine). The residue was then purified on a silica gel column eluted with toluene/ethyl acetate (from 5:1 to 3:1) to yield tetrasaccharide **2** (61 mg, 66%) as the only detectable anomer. $[\alpha]_D$ −38.6 (*c* 0.5 in CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ 7.50−6.80 (aromatic protons), 5.61 (1H, d, J = 3.2 Hz), 5.18−4.40 (18 H, 9 × AB, 9 × benzyl CH_2), 5.54 (1H, s), 4.89 (1H, d, J = 7.6 Hz,), 4.78 (1H, d, J = 8.0 Hz), 4.75 (1H, d, J = 7.6 Hz), 4.34 (1H, bq, J = 6.8 Hz), 4.28−4.15 (5H), 4.10−3.90 (4H), 3.79 (3H, s, $-OCH_3$), 3.80−3.50 (9H), 3.26 (1H, s), 0.69 (3H, d, J = 6.8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.2, 151.6, 139.0, 139.0, 138.9, 138.6, 138.4, 138.3, 138.2, 138.0, 137.9, 137.9, 128.5−126.3, 118.5, 114.4, 103.6, 103.3, 102.9, 101.2, 97.8, 84.0, 81.2, 79.9, 79.1, 78.4, 76.2, 75.5, 75.4, 75.3, 74.9, 74.5, 74.0, 73.5, 73.0, 72.8, 72.6, 72.4, 71.4, 69.1, 68.9, 66.7, 66.4, 55.6, 16.1. MALDI-TOF MS for $C_{101}H_{105}N_3O_{20}$ (*m/z*): M_r (calcd) 1679.72, M_r (found) 1702.40 $(M+Na)^+$. $C_{101}H_{105}N_3O_{20}$ calcd C 72.17, H 6.30, found C 71.90, H 6.45.

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Supporting Information Available: Synthesis procedures and characterization data, including ^1H and ^{13}C NMR spectra, for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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