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Olefin self-metathesis as a new entry into xenotransplantation antagonists bearing the Galili antigen

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A hexameric disaccharide cluster bearing the terminal Galα related xenotransplantation antigen was constructed using a sequence of ruthenium carbenoid catalyzed olefin self-metathesis of monoallylated tribenzyl pentaerythritol followed, after interconversion of benzyl ethers into *para*-iodobenzyl ethers, by a single step Sonogashira cross-coupling of six prop-2-ynyl glycosides onto a hexameric aryl iodide scaffold.

Successful pig liver-to-human xenotransplantations are critically hampered by hyperacute organ rejection due to the presence of natural human antibodies (1–2% IgG, 3–8% IgM) recognizing cell surface Galα1-3Galβ epitopes (Galili antigen) covering the epithelial lining of xenografts.[1,2] To overcome this situation and to allow organ 'accommodation', strategies such as immune suppression and serum perfusion through affinity columns that remove circulating anti-Galα antibodies have been accomplished.[3] Analogously, intravenous Galα-OR antagonist therapies have witnessed some successes, at least in baboon allografts.[4] Due to the low affinity of circulating antibodies against monomeric epitopes, few clustered Galili antigens have been prepared[5–7] in order to rely on the well-established increased avidity of multivalent carbohydrate scaffolds.[8,9]

Using a pentaerythritol platform (**1**) functionalised with a unique allyl ether group, olefin self-metathesis provided, as expected,[10] a hexameric cluster (**4**)† according to Scheme 1. Thus, substoichiometric benzylation of tetraol **1** (BnBr, NaH, DMF) provided tribenzyl ether **2** as a thick syrup in 65% yield after silica gel column chromatography. Allylation of the residual hydroxy group (allyl bromide, NaH, DMF, rt) gave fully protected ether **3** in 90% yield. Treatment of olefin **3** with a catalytic amount of ruthenium carbenoid ($Cl_2Ru(P\text{-}Cy_3)_2=CHPh$ (Grubbs′ catalyst), CH_2Cl_2, rt, 12 h) afforded homodimer **4** as an inseparable mixture of *trans*/*cis* stereoisomers (10 : 1) in 85% yield.

Interconversion of the perbenzylated ether **4** into *p*-iodobenzylated ether **5** with concomitant reduction of the alkene functionality was accomplished by complete hydrogenolysis (H_2, Pd/C, 90%) followed by *p*-iodobenzylation in 45% yield (*p*-$IPhCH_2Br$, TBAI, NaH, DMF, rt, 6 h) according to Scheme 2.

The final preparation of the hexameric perglycosylated cluster **8** is illustrated in Scheme 3. Sonogashira[11] cross-coupling of peraryliodide **5** with known prop-2-ynyl disaccharide **6**[5] under palladium-catalyzed conditions in the absence of copper cocatalyst (1.3 eq **6**/iodide, $(Ph_3P)_2PdCl_2$, TEA, DMF, 60 °C, 5 h) provided fully protected hexamer **7** in 75% yield. As previously observed,[5,12] it was remarkable that the efficiency of the cross-coupling could provide multiple carbohydrate attachments in a single step reaction. Finally, complete deprotection of both acetyl and benzoyl ester protecting groups in **7** to afford **8** was accomplished under catalytic transesterification conditions using NaOMe in MeOH (95%). Interestingly, compound **8** was readily soluble in water, a condition judged essential for biological activity. Therefore, the choice of a conformationally restricted glycluster having several aryl–alkyne functionalities does not seem to impair water-solubility, as long as disaccharides or higher oligosaccharides are attached to it. This is in striking contrast to a previously made pergalactosylated cluster into which the hydrophilicity of the glycan portion was insufficient to counterbalance the lipophilic linkers.[12]

Preliminary modelling of the above cluster showed pairs of pentaerythritol substituents to be perpendicular to one another and facing opposite directions, thus allowing easy access to antibody binding sites. The relative topology of this new family of glycoclusters is anticipated to be critical to fully explore receptor specificities.[13]

In conclusion, an effective combination of olefin self-metathesis coupled with a single step multiple Sonogashira cross-coupling strategy allowed an excellent entry toward novel multivalent carbohydrate clusters.

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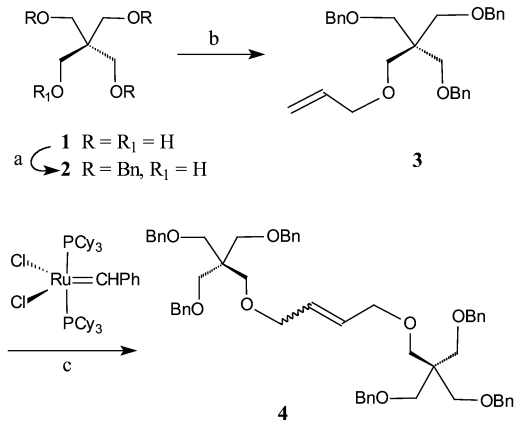
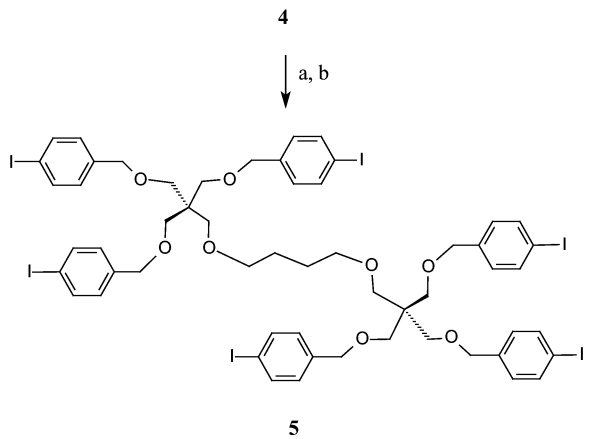


Scheme 1 (a) Benzyl bromide, NaH, DMF, rt, 6 h, 65%; (b) allyl bromide, NaH, DMF, rt, 6 h, 90%; (c) Grubbs′ catalyst, CH_2Cl_2, rt, 12 h, 85%, *E*/*Z* = 10 : 1.



Scheme 2 (a) H_2, Pd/C, solvent, 24 h, 90%; (b) *p*-$IPhCH_2Br$, TBAI, NaH, DMF, rt, 6 h, 45%.

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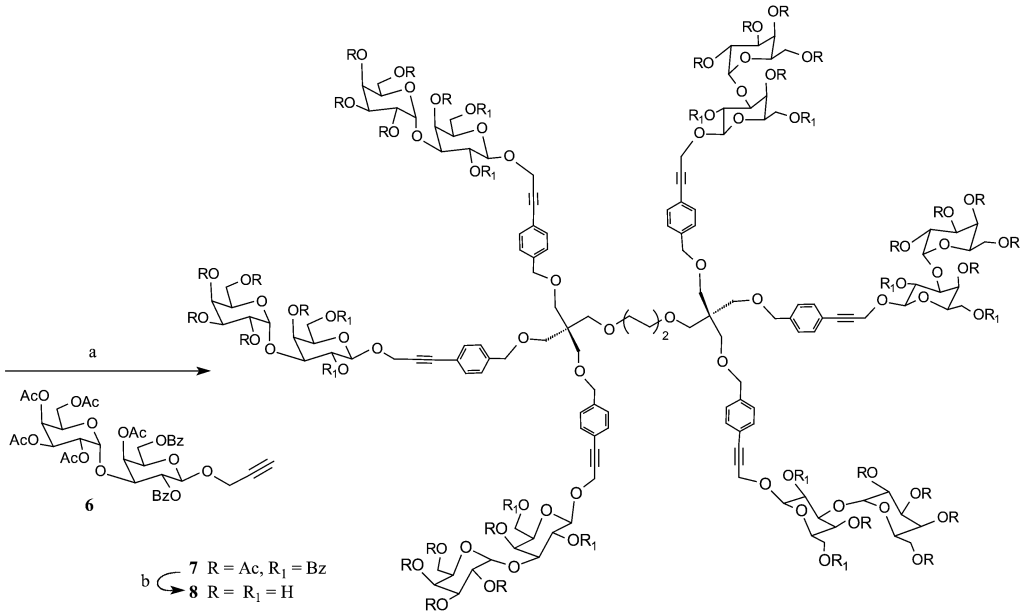

Scheme 3 (a) $(Ph_3P)_2PdCl_2$, TEA, DMF, 60 °C, 6 h, 75%; (b) NaOMe, MeOH, rt, 2 d, 95%.

Notes and references

† Physical data for representative compounds, NMR assignments are based on COSY and HMQC experiments (500 MHz).

3: ^{1}H NMR ($CDCl_3$) δ 7.25 to 7.32 (m, 15H, ar), 5.83 to 5.90 (m, 1H, vinyl), 5.11 to 5.25 (m, 2H, vinyl), 4.48 (s, 6H, benzylic), 3.93 to 3.95 (m, 2H. allylic), 3.56 (s, 6H, C(CH_2OR)$_4$), 3.53 (s, 2H, C(CH_2OR)$_4$); ^{13}C NMR: δ 138.9 (ar), 135.2 (vinyl), 128.2, 127.3, and 127.2 (ar), 116.1 (vinyl), 73.3 (benzylic), 72.3 (allylic), 69.3 (C(CH_2OR)$_4$), 45.6 (C(CH$_2$OR)$_4$). FAB-MS calcd for $C_{29}H_{34}O_4$ (M + H$^+$) 447.25, found 447.32.

4: ^{1}H NMR ($CDCl_3$) δ 7.23 to 7.32 (m, 30H, aryl), 5.68 (m, 2H, vinyl), 4.48 (s, 12H, benzylic), 3.91 (m, 4H. allylic), 3.56 (s, 12H, C(CH_2OR)$_4$), 3.53 (s, 4H, C(CH_2OR)$_4$); ^{13}C NMR: δ 138.9 (ar), 129.0 (vinyl), 128.2, 127.3 and 127.2 (ar), 73.3 (benzylic), 71.4 (allylic), 69.4 and 65.4 (C(CH_2OR)$_4$), 45.6 (C(CH$_2$OR)$_4$). ESI-MS calcd for $C_{56}H_{64}O_8$(M + H$^+$) 865.4, found 865.2.

5: ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 12H, J = 8 Hz, ar), 6.96 (d, 12H, J = 8 Hz, ar), 4.39 (s, 12H, benzylic), 3.44 (s, 12H, C(CH_2OR)$_4$), 3.40 (s, 4H, C(CH_2OR)$_4$), 3.33 (br s, 4H, −CH_2O-), 1.50 (br s, 4H, −CH_2-); ^{13}C NMR: δ 138.9 (ar), 137.7, 129.6 and 127.3 (ar), 72.9 (benzylic), 71.6 (-CH_2O-), 69.7 (C(CH_2OR)$_4$), 45.8 (C(CH$_2$OR)$_4$), 26.7 (-CH_2-). ESI-MS calcd for $C_{56}H_{60}O_8I_6$ (M + NH_4^+) 1639.4, found 1639.2.

7: ^{1}H NMR ($CDCl_3$) δ 7.98 to 8.02 (m, 24 H, ar), 7.49 to 7.55 (m, 12 H, ar), 7.39 to 7.47 (m, 12 H, ar), 7.13 to 7.28 (m, 36 H, ar), 5.56 (dd, J = 8.0, 10.0, 6 H, H-2), 5.45 (br d, J = 2.1, 4 H, H-4), 5.20 (d, J < 1, 6 H, H-1′), 5.19 (dd, J = 3.5, 10.4, 6 H, H-2′), 5.03 (dd, J = 3.3, 10.4, 6 H, H-3′), 4.95 (d, J = 8.0, 6 H, H-1), 4.89 (br d, J = 2.1, 6 H, H-4′), 4.58 (s, 12H, H-1″), 4.56 (dd, J = 6.2, 11.0, 6H, H-6a), 4.42 (s, 12H, benzylic), 4.39 (dd, J = 7.0, 11.0, 6 H, H-6b), 4.10 (dd, J = 3.1, 10.0, 6H, H-3), 4.05 (t, J = 6.9, 6H, H-5), 3.97 (t, J = 6.6, 6H, H-5′), 3.73 (dd, J = 6.5, 11.2, 6H, H-6a′), 3.66 (s, 12H, 3.66 (dd, J = 6.5, 11.2, 6H, H-6b′), 3.52 (s, 12H, C(CH_2OR)$_4$), 3.46 (s, 4H, C(CH_2OR)$_4$), 3.36 (s, 4H, −CH_2O-), 2.20, 2.03, 1.99, 1.86 and 1.73 (5s, 90H, CH_3CO), 1.54 (br s, 4H, −CH_2-); ^{13}C NMR: 170.2, 169.8, 169,7 and 169.3 (CH_3CO), 166.0 and 165.0 (PhCO), 139.6, 133.4, 133.3, 132.1, 132.0, 131.7, 129.7, 129.4, 129.3, 128.5, 128.4, 127.1, 126.9 and 121.0 (ar), 98.9 (C-1), 93.7 (C-1′), 86.7 and 83.5 (acetylenic), 73.7 (C-3), 72.8 (benzylic), 71.3 (-CH_2O-), 70.9 (C-5), 70.0 (C-2), 69.7 and 69.6 (C(CH_2OR)$_4$), 67.6 (C-4′), 66.9 (C-3′), 66.5 (C-2′), 66.4 (C-5′), 65.1 (C-4), 61.7 (C-6), 61.2 (C-6′), 56.8 (C-1″), 45.6 (C(CH$_2$O)$_4$), 26.4 (-CH_2-), 20.7, 20.5, 20.4 and 20.3 (CH_3CO). MALDI-TOF-MS calcd for $C_{290}H_{308}O_{116}$ (M + Na$^+$) 5668.8, found 5668.5.

8: ^{1}H NMR (D_2O) δ 7.34 (br s, 12H, ar), 7.06 (br s, 12H, ar), 5.21 (br s, 6H, H-1′), 4.82 (br s, 12H, PhCH_2 or H-1″), 4.63 (br s, 12H, PhCH_2 or H-1″), 4.58 (br s, 6H, H-1), 4.23 (br s, 12H, H-5 and H-5′), 4.06 (br s, 6H, H-4′), 4.02 (br d, J = 10.8, 6H, H-3′), 3.94 (dd, J = 2.9, 10.1, 4H, H-2′), 3.63–3.91 (m, 42H, H-2, H-3, H-4, H-6 and H-6′); 3.42 (m, 20H, C(CH_2OR)$_4$, –CH_2O-), 1.52 (br s, 4H, –CH_2-); ^{13}C NMR: δ 138.6, 131.3, 126.7 and 120.6 (ar), 101.1 (C-1), 95.0 (C-1′), 85.1 and 84.4 (acetylenic), 77.2, 74.1, 71.9, 70.3, 68.7, 67.8 and 64.1 (C-4, C-5, C-2′, C-3′, C-4′, C-5′, PhCH_2, -CH_2O-, C(CH_2OR)$_4$), 60.5 (C-6), 60.1 (C-6′), 56.6 (C-1″), 45.5 (C(CH$_2$O)$_4$), 25.1 (-CH_2-). MALDI-TOF-MS calcd for $C_{146}H_{198}O_{74}$ (M + Na$^+$) 3158.2, found 3158.3.

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