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Chemoselectively template-assembled glycopeptide presenting clustered cancer related T-antigen

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Abstract—The first synthesis of the tumor-associated α-aminooxy T-antigen **1**, a relevant recognition motif for the direct construction of multitopic carbohydrate architecture of biological interest is described. The usefulness of this building block is emphasized with the efficient preparation through oxime ligation of a neoglycopeptide cluster, which is readily suitable for evaluating the role of multivalency in antigen presentation to the immune system from an anticancer vaccine perspective.

Mucins comprise a large family of heavily glycosylated proteins expressed on the cell surface of epithelial tissues.[1] They are commonly composed of the α-D-GalNAc residue as the core fragment attached in a clustered mode to the hydroxyl side chain of serine or threonine of glycoproteins. These typical structural units are involved in various biological processes and also represent one of the most important class of antigenic markers related to autoimmune or infectious diseases and cancers, making them attractive targets for carbohydrate-based tumor therapy.[2] In particular, molecular constructions presenting clustered cancer-related carbohydrate antigens mimicking the cell surface such as glycopeptide-based vaccines[3] have promising potential for clinical trials and their synthetic access remains a crucial challenge for the future.

Among all the identified mucin-associated antigens, the disaccharide β-D-Gal(1 → 3)-α-D-GalNAc (or T-antigen) represents one of the most prevalent structures to be abundantly expressed on carcinoma malignancies of the colon and the prostate.[4] Thus, many synthetic methods regarding the preparation and the incorporation of such a moiety into peptidic backbone have been described,[5] including the powerful glycal assembly and 'cassette' approach developed by Danishefsky et al.[2b,6] However, due to the polyfunctionality of the target molecules, the control of the alpha anomeric configuration of the *O*-glycosidic linkage between the peptide and the carbohydrate moiety remains the major problem of this methodology, involving many tedious manipulations of orthogonal protecting groups and activation steps.[7] As an attractive alternative, we and others have shown more recently that chemoselective ligation following an oxime-based strategy is of major interest for the convergent assembly of complex macromolecules.[8] In this context, we developed an efficient route providing aminooxy carbohydrate recognition motifs at both the alpha and beta anomeric carbon positions[9] (comprising the mucin-related T_N antigen), which we assembled chemoselectively into oligonucleotides[10] and the cyclodecapeptide RAFT template.[11] Surprisingly, whereas complex aminooxylated cancer-related antigens (sialyl Le^X or sialyl T_N) have been obtained by chemical or chemoenzymatic methods,[8c,12] the preparation of T-antigen is still not described so far (Fig. 1).

To this end, we herein report the first synthesis of the α-aminooxy mucin-related T-antigen and subsequent

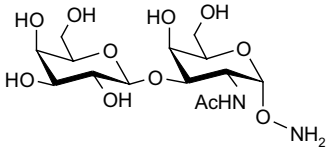

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Figure 1. Alpha aminooxy T-antigen.

Keywords: T-antigen; glycopeptide; multivalency; oxime ligation.
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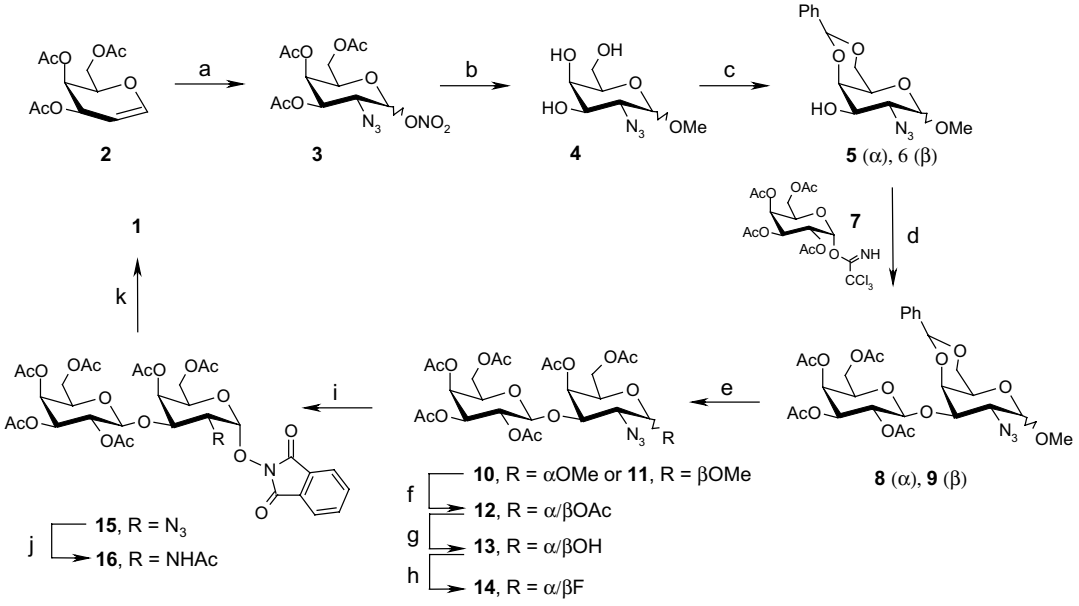


Scheme 1. Reagents and conditions: (a) CAN, NaN_3, CH_3CN, −15 °C, 77%; (b) MeONa/MeOH, 100% (α/β, 0.3/1); (c) $PhCH(OMe)_2$, *p*-TsOH, CH_3NO_2, 85%; (d) TMSOTf, CH_2Cl_2, −30 °C, 47% for **8** (from **5**) or 50% for **9** (from **6**); (e) (i) aq AcOH 80%, 45 °C; (ii) Ac_2O, pyridine, 85–87% for **10** (from **8**) or for **11** (from **9**); (f) Ac_2O/H_2SO_4 40/1, 0 °C, 95%; (g) ethylenediamine, AcOH, THF, 100% (α/β ratio, 1/1, determined by integration of the anomeric protons in the ^1H spectrum); (h) DAST, THF, 80% (α/β ratio, 1/1, determined by integration of the anomeric protons in the ^1H spectrum); (i) $BF_3{\cdot}Et_2O$, PhtOH, TEA, 40% (+30% of β anomer); (j) H_2, Pd/C, MeOH/Ac_2O, 45%; (k) $MeNHNH_2$, 70%.

glycopeptide cluster formation through oxime ligation as a preliminary study for designing synthetic antitumoral vaccines.

We decided to follow a synthetic route based on the strategy described initially by Paulsen et al. (Scheme 1).[5f] Two protected methyl galactosyl acceptors **5** or **6** were prepared from triacetyl galactal **2** according to the classical azidonitration reaction using cerium ammonium nitrate and sodium azide in acetonitrile.[13] Subsequent treatment of the azido-nitrate mixture **3** with sodium methoxide in methanol[14] followed by the protection of the resulting anomeric mixture of **4** afforded the 4,6-*O*-benzylidene protected methyl galactosides **5** and **6**, which were separated by silica gel chromatography and crystallized from diethyl ether/pentane mixture (Fig. 2).[15]

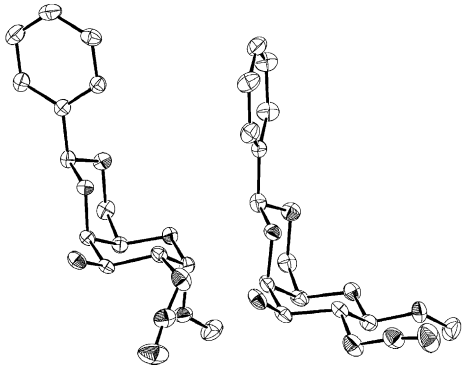


Figure 2. ORTEP drawings of glycosyl acceptors **5** ($C_{14}H_{17}N_3O_5$, FW = 307.31, orthorhombic, it $P2_12_12_1$, $a = 5.085(5)$, $b = 11.820(2)$, $c = 23.990(4)$ Å, $V = 1441(3)$ Å^3, $Z = 4$, $D_{calcd} = 1.416$ g cm^{-3}, $R = 0.045$, $R_w = 0.041$) and **6** ($C_{14}H_{17}N_3O_5$, FW = 307.31, orthorhombic, $P2_12_12_1$, $a = 5.055(4)$, $b = 12.790(3)$, $c = 22.568(5)$ Å, $V = 1456(4)$ Å^3, $Z = 4$, $D_{calcd} = 1.401$ g cm^{-3}, $R = 0.059$, $R_w = 0.027$).[15]

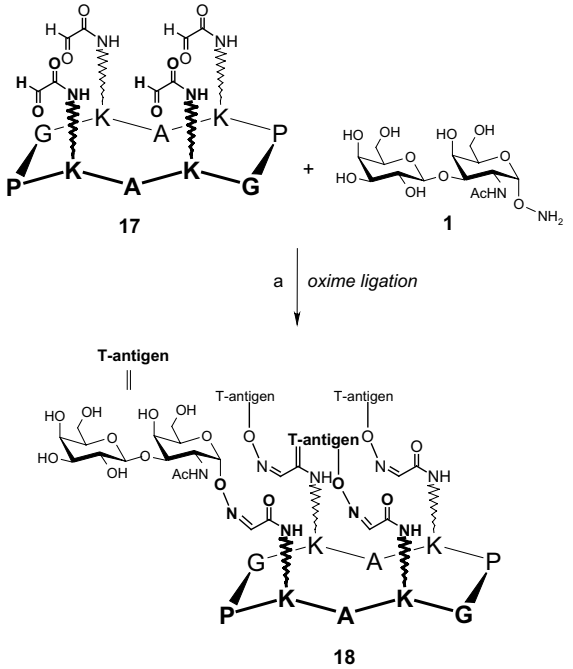
Interestingly, both glycosyl acceptors were suitable for the preparation of the desired compound. Indeed, the Koenigs–Knorr-like coupling reaction between **5** or **6** and the trichloroacetimidate glycosyl donor **7**[16] by treatment with trimethylsilyl triflate as Lewis acid catalyst in dichloromethane gave the corresponding disaccharides **8** or **9** with comparable moderate yields (47–50%). Further mild acidic deprotection with aqueous acetic acid and *O*-acetylation at positions 4 and 6 of **8** and **9** provided the methyl glycosides **10** and **11** in high yields. These two compounds were then converted into the corresponding peracetylated derivatives **12**, using a solution of sulfuric acid and acetic anhydride (1/40),[5f] as an anomeric mixture, which was used without further separation. The regioselective anomeric deacetylation was subsequently achieved with a solution of ethylenediamine and acetic acid (1/1, v/v) in THF at room temperature[17] to obtain the anomerically free derivative **13**.

We previously reported that *N*-hydroxyphthalamide (PhtOH) provides the most suitable precursor for introducing an aminooxy function at the anomeric position of carbohydrates.[9] After activation of **13** with a fluoride atom using DAST, the glycosylation reaction was realized in dichloromethane in the presence of triethylamine and PhtOH by treatment with $BF_3{\cdot}Et_2O$ as promotor. The reaction occurred within 15 min in 70% yield and the corresponding alpha and beta phthalamido derivatives (α/β ratio: 4/3) were separated by silica gel chromatography. The azido function of the desired

alpha compound **15** was then converted into the *N*-acetylated amide group by a controlled catalytic hydrogenation in methanol/acetic anhydride at room temperature to prevent any N–O cleavage under the reductive conditions.[9] The complete deprotection and aminooxy formation of the acetylated derivative **16** was finally achieved in methylhydrazine as solvent to obtain the stable alpha aminooxylated T-antigen **1** in 70% yield after chromatography and lyophilization. Multidimensional NMR and mass spectrometry analysis confirmed the purity and the structure of the desired compound **1**.[18]

To assess the ability of **1** to form a multitopic carbohydrate architecture, we used the RAFT template whose utility as a polyvalent presentation scaffold for sugar recognition motifs has been shown previously with relevant lectins.[11] Thus, the RAFT **17** (Scheme 2) presenting clustered aldehydes was prepared following the classical procedure using serine as precursor of the glyoxo-aldehyde functions, which were liberated by oxidative cleavage with sodium periodate.[19]

The chemoselective oxime ligation between **17** and a slight excess of **1** was finally performed in aqueous sodium acetate buffer at room temperature and was monitored by reverse-phase HPLC. After complete disappearance of **17** (Fig. 3A), semi-preparative HPLC purification afforded the stable tetravalent RAFT/T-antigen conjugate **18** (70% yield), which was characterized by mass spectrometry (Fig. 3B). This neoglycoconjugate **18** is readily suitable for further biological recognition studies.



Scheme 2. Reagents and conditions: (a) 0.1 M sodium acetate buffer pH 4.0, 2 h, 70% after semi-preparative RP-HPLC.

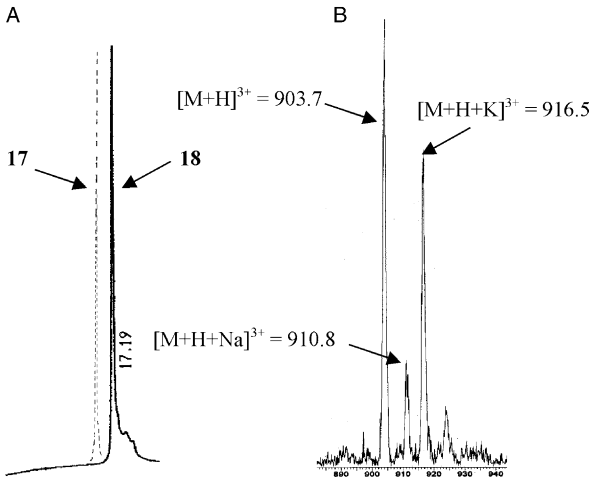


Figure 3. (A) HPLC profile (Nucleosil 100 Å 5 μm C_{18} particles, 250×4.6 mm; solvent B: 0.09% TFA in 90% acetonitrile and solvent A: 0.09% TFA, 1 mL min^{-1}, linear gradient 95:5 A/B to 60:40 A/B in 30 min detection $\lambda = 214$ and 250 nm) of crude reaction mixture of oxime ligation between **1** and **17** after 2 h: RAFT **17** (dotted line): $R_t = 16.9$ min; RAFT/T-antigen conjugate **18**: $R_t = 17.2$ min. (B) ESI-MS (positive mode) of RAFT/T-antigen conjugate **18** (M_{calcd} for $C_{108}H_{174}N_{22}O_{58}$: 2708.7).

In conclusion, we report in this paper the first synthetic route to the alpha aminooxy mucin-related T-antigen **1** starting from two glycosyl acceptors **5** or **6** and the trichloroacetimidate galactosyl donor **7**. Such modified carbohydrate antigens are potentially useful for the rapid and direct assembly of multitopic neoglycoconjugate architectures of biological interest. Indeed, we have shown that the subsequent incorporation of **1** into the cyclodecapeptidic RAFT template **17** presenting aldehydes using an oxime-based strategy efficiently gave a new tetravalent neoglycopeptide T-antigen cluster **18**, which could provide a useful tool for evaluating the role of multivalency in antigen presentation to the immune system. Recognition studies with relevant antibodies and lectins involved in pathogen processes are currently being investigated in our laboratory in the context of synthetic anticancer vaccine research. The results will be reported in due course.

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15. Crystallographic data (excluding structural factors) for the structure **5** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 220302. Copies of the data can be obtained free of charge on application to: The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk. Crystallographic data obtained for the structure **6** are in good agreement with the previous publication of Gururaja, T. L.; Venugopalan, P.; Levine, J. *J. Chem. Cryst.* **1998**, *28*, 747–759.
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18. ^{1}H NMR (300 MHz, D_2O): δ ppm 4.95 (d, 1H, $^3J_{1',2'} = 4.0$ Hz, H-1′), 4.45 (d, 1H, $^3J_{1,2} = 7.7$ Hz, H-1), 4.39 (d, 1H, $^3J_{2',3'} = 11.4$ Hz, H-2′), 4.24 (bd, 1H, $^3J_{3',4'} = 2.8$ Hz, H-4′), 4.02 (bt, 1H, $^3J_{5',6'} = 6.3$ Hz, H-5′), 3.97 (dd, 1H, $^3J_{3',4'} = 2.8$ Hz, $^3J_{2',3'} = 11.4$ Hz, H-3′), 3.91 (bd, 1H, $^3J_{3,4} = 3.2$ Hz, H-4), 3.80–3.72 (m, 4H, H-6, H-6′), 3.66–3.64 (m, 1H, H-5), 3.61 (dd, 1H, $^3J_{3,H} = 3.2$ Hz, $^3J_{2,3} = 9.9$ Hz, H-3), 3.50 (dd, 1H, $^3J_{1,2} = 7.7$ Hz, $^3J_{2,3} = 9.9$ Hz, H-2), 2.03 (s, 3H, CH_3); ^{13}C NMR (75 MHz, D_2O): δ ppm 175.0 (C=O), 105.0 (C-1), 101.1 (C-1′), 77.3 (C-3′) 75.3 (C-5), 72.9 (C-3), 71.1, 70.9 (C-2, C-5′), 69.1, 68.9 (C-4, C-4′), 61.5, 61.3 (C-6, C-6′), 48.2 (C-2′), 22.3 (CH_3); MS (FAB$^+$, glycerol+NaCl): $M_{calcd} = 398$, m/z: $[M+H+K]^+ = 438$.
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