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ORIGINAL PAPER

X-Ray Structure of the Key Synthetic Intermediate of a Cancer-Related Sialyl-Tn Antigen Analogue

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Abstract We report herein the synthesis and the X-ray crystal structure of 2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl-*N*-oxyphthalimide **3** which is the key-precursor of a cancer-related STn antigen analogue **5**. Compound **3** crystallizes from a mixture of dichloromethane/pentane as colorless orthorhombic needles in $P2_12_12$ space group with the following cell parameters: $a = 14.039(2)$ Å; $b = 16.574(3)$ Å; $c = 8.038(1)$ Å. The hexapyranose ring of **3** adopts a twisted boat according to the Cremer and Pople puckering parameters (Cremer and Pople, J Am Chem Soc 97:1354, 1975) whereas the structure determination of the corresponding deprotected intermediate **4** (orthorombic colorless prisms, $P2_12_12_1$ space group, $a = 4.927(3)$ Å; $b = 13.423(2)$ Å; $c = 22.744(3)$ Å) has revealed a quasi perfect chair.

Keywords Carbohydrate · Cancer antigen · Pyranose ring

Introduction

The glyco-patterning of the extracellular membrane is the major difference between malignant and normal cells. Tumors are indeed characterized by altered or over-expressed carbohydrate markers, namely Tumor Associated Carbohydrate Antigens (TACAs), which are displayed as repeated and multimeric units by proteins and lipids in the cell membrane [1–3]. Recent reports have clearly shown that these TACAs represent essential building blocks for the construction of therapeutic anti-cancer vaccines [4, 5]. Among other approaches, we have previously reported in our laboratory that synthetic multiepitopic vaccine candidates combining, on a suitable carrier molecule, a cluster of TACAs and immunostimulant peptide [6, 7] or lipopeptide epitopes [8–10] can promote a strong immune response and protection against tumor cells. In the course of our researches in this field, we were interested recently on the sialyl-Tn (STn) antigen. This mucin-type TACA is composed of a sialylated α-GalNAc residue attached to the hydroxyl side-chain of serine or threonine of glycoproteins expressed in many adenocarcinomas cell surface [11].

In order to ensure the controlled molecular assembly of our vaccine prototype, we focused on the synthesis of the aminooxy STn analogue **5** (Scheme 1) as cancer-related carbohydrate antigen to be introduced in a multivalent display into the aldehyde-containing cyclopeptide carrier or conjugated with other molecules by chemoselective oxime ligation [12–26]. This compound **5** was indeed used successfully for the synthesis of mucin mimics following a similar chemoselective procedure [27]. For this purpose, we developed a synthetic route of **5** requiring the preparation the key synthetic intermediate **3** (Scheme 1). Herein, we report the synthesis of this building block **3** which was unexplored to date and compare its X-ray analysis with the corresponding unprotected derivative **4**.

The envisioned chemoselective procedure requires the incorporation of an aminooxy function to the anomer position of the carbohydrate moiety. This can be easily achieved from the fluoride derivative **1** which was glycosylated with *N*-hydroxyphthalimide using boron trifluoride etherate (Scheme 1) following a previously reported protocol [28, 29]. We thus obtained the fully protected glycosylated derivative as an anomer mixture

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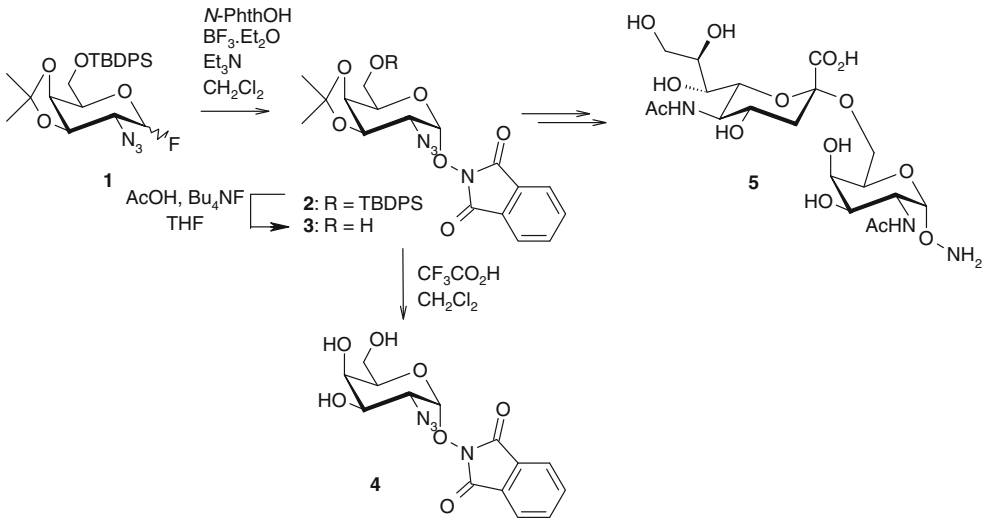

Scheme 1 Synthesis of compounds **3** and **4**

from which the desired alpha anomer **2** was recovered by silica gel chromatography. The subsequently desilylation of **2** afforded the regioselectively protected compound **3**. The latter derivative was next crystallized from a mixture of dichloromethane and pentane. In order to evaluate the influence of isopropylidene protecting group on the sugar ring conformation, compound **3** was further deprotected by acidolysis to afford **4** after crystallisation from methanol.

Experimental Section

Synthesis of 3 and 4

The glycosylation reaction between the D-galactosyl fluoride **1** (450 mg; 0.92 mmol) and *N*-hydroxyphthalimide (150 mg; 1 equiv.) was performed in dichloromethane in the presence of triethylamine (136 µL; 1 equiv.) and $BF_3 \cdot Et_2O$ (470 µL; 4 equiv.) as promoter [28]. The alpha anomer **2** (235 mg; 40% yield) was recovered after classical work-up and silica gel purification (eluant: hexane/ethyl acetate 4:1). The *tert*-butyldiphenylsilyl (TBDPS) protecting group of 2 (875 mg; 1.39 mmol) was finally removed by treatment with acetic acid (150 µL; 2 equiv.) and tetrabutyl ammonium fluoride (2.78 mL; 2 equiv.) to afford **3** which was crystallized as single crystals in a mixture of CH_2Cl_2 and pentane by slow evaporation of the solvent at room temperature (325 mg; 60% yield). NMR spectra were recorded using Bruker AC300 spectrometers. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.89–7.78 (m, 4H), 5.55 (d, 1H, J = 4.2 Hz), 5.01–4.97 (m, 1H), 4.55 (dd, 1H, J = 5.8, 7.3 Hz), 4.42 (dd, 1H, J = 2.4, 5.8 Hz), 4.00–3.87 (m, 3H), 1.54 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 163.3, 134.8, 128.7, 123.8, 110.7, 103.2, 73.6, 73.1, 70.4, 62.6, 59.2, 27.7, 25.9. The electron spray ionization mass spectrometry (ES-MS) was recorded on a VG Platform II (Micromass). ES-MS analysis (positive mode): calcd. 390.3, found 390.9 ($M + H^+$). The compound **4** was prepared from **3** by acidolysis with a solution of trifluoroacetic acid in dichloromethane and was crystallized in methanol. ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ (ppm): 7.88 (s, 4H), 5.51–5.48 (m, 2H), 4.94 (d, 1H, J = 4.2 Hz), 4.59 (bt, 1H, J = 5.4 Hz), 4.42 (bt, 1H, J = 6.4 Hz), 4.01–3.79 (m, 2H), 3.81 (dd, 1H, J = 3.7, 10.7 Hz), 3.58–3.50 (m, 1H), 3.45–3.37 (m, 1H); ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ (ppm): 162.9, 134.9, 128.4, 123.3, 103.4, 72.9, 67.8, 67.4, 59.3, 59.0. ES-MS analysis (positive mode): calcd. 350.3, found 351.0 ($M + H^+$).

Crystal Mounting and Data Collection

We selected a needle-shape crystal of 0.36 × 0.15 × 0.12 mm and a prismatic crystal of 0.34 × 0.30 × 0.29 mm for **3** and **4**, respectively. Both crystals were stuck on a glass fiber using epoxy resin and mounted at room temperature (293 K) on a Bruker-AXS-Enraf-Nonius CAD-4 automated 4-circle diffractometer, working at the monochromated (graphite) Cu Kα radiation $\lambda =$ 1.54178 Å. We used the CAD-4 software [30] to achieve the data reduction, the cell determination and refinement. For both crystals, the cell determinations were performed using 25 reflections (with $20.27° < \Theta < 23.80°$ for **3** and $20.02° < \Theta < 36.68°$ for **4**) and the data collections were performed up to $\Theta = 75.00°$ (with 2229 reflections for **3** and 1848 for **4**). Since no heavy atom was part of the chemical formula, we did not collect Friedel pairs. The data were corrected for decays and for the Lorentz and

Table 1 Crystal data and structure refinement

Compound	**3**	**4**
Name	2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl-*N*-oxyphthalimide	2-azido-2-deoxy-α-D-galactopyranosyl-*N*-oxyphthalimide
CCDC deposit no.	700851	700852
Color/shape	Colorless/needle	Colorless/prism
Chemical formula	$C_{17}H_{18}N_4O_7$	$C_{14}H_{14}N_4O_7$
Formula weight	390.35	350.29
Temperature (K)	293	293
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12$	$P2_12_12_1$
Unit-cell dimensions (25 reflections)	$a = 14.039(2)$ Å; $b = 16.574(3)$ Å; $c = 8.038(1)$ Å	$a = 4.927(3)$ Å; $b = 13.423(2)$ Å; $c = 22.744(3)$ Å
Unit-cell volume (Å^3)	1870.2(4)	1504.2(7)
Z	4	4
Density (calculated) (g/cm^3)	1.386	1.547
Absorption coefficient (mm^{-1})	0.935	1.090
Diffractometer/scan	Bruker-Enraf-Nonius CAD4/omega	Bruker-Enraf-Nonius CAD4/omega-2theta
θ range for data collection (°)	3.15–74.89	3.29–74.85
Reflections measured	2229	1848
Independent/observed reflections	2229/1644 [$I > 2\sigma(I)$]	1848/1741 [$I > 2\sigma(I)$]
Data/restraints/parameters	1644/0/253	1741/0/227
Goodness-of-fit on F	1.971	1.929
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0615$, $wR_2 = 0.0804$	$R_1 = 0.0310$, $wR_2 = 0.0460$
R indices (all data)	$R_1 = 0.0768$, $wR_2 = 0.0936$	$R_1 = 0.0318$, $wR_2 = 0.0462$

polarization effects. The crystals data are available in Table 1.

Crystal Structures Determinations and Refinement

The structure was solved using direct methods from SIR-92 [31], C, N, O atoms were refined anisotropically by the full matrix least-squares method implemented by TeXsan [32]. H atoms were calculated on idealised positions but H atoms from the hydroxyl groups were located from the Fourier map. Drawings of the molecular structures were performed with the use of ORTEP [33].

Results and Discussion

While the chemical deprotection of the isopropylidene group left most of the molecule unchanged, several variations were observed in the crystallographic parameters of compounds **3** and **4**. By contrast with the crystallographic system which is orthorhombic in both molecules, the space group changes from $P2_12_12$ to $P2_12_12_1$ for **3** and **4**, respectively, and the unit-cell dimensions are quite different (Table 1). These variations can be attributed to the presence of one alcohol functions in **3**, whereas two additional alcohols are released from the acidolysis of the protecting group in **4**. Consequently, the latter structure contains three intermolecular hydrogen bonds (Table 2; Fig. 1), contrarily to the protected molecule **3** which shows only one intramolecular hydrogen bond. As a result, the secondary structure is modified.

Both compounds display numerous chemical groups around the hexapyranose ring, such as the *N*-hydroxyphthalimido group at C-1, an azido group at C-2 and the alcohol function from the sugar at C-6, which remain unchanged after deprotection. The main structural difference between **3** and **4** is due the isopropylidene removal at C-3 and C-4 to restore the hydroxyl group of the carbohydrate in **4**. While the expected distances and angles are observed [34], a significant modification in the hexapyranose ring conformation is, however, induced. Indeed, the compound **4** stands as a classical chair conformation (Fig. 2) by contrast with the compound **3** which shows a clear distortion between a regular and a twist boat conformation (Fig. 3), presumably due to the constraint of the fused five-membered ring acetal at C-3 and C-4.

This can be revealed by the calculation of the Cremer and Pople parameters [35] which are $Q = 0.703(5)$ Å, $\Phi 2 = 219.3(4)°$, $\Theta = 87.1(4)°$ for compound **3** and $Q =$

Table 2 Hydrogen bonds for compounds **3** and **4**

Compound	A	H	B	B-adc	A···B	A–H	H···B	A–H···B
3	O(6)	H(1)	O(14)	1	2.938(5)	1.13	1.86	156.9
4	O(3)	H(1)	O(6)	75604	2.650(2)	0.93	1.73	173.1
4	O(6)	H(3)	O(3)	64604	2.780(2)	1.01	1.83	155.1
4	O(4)	H(2)	O(14)	75604	3.027(2)	1.01	2.09	152.8

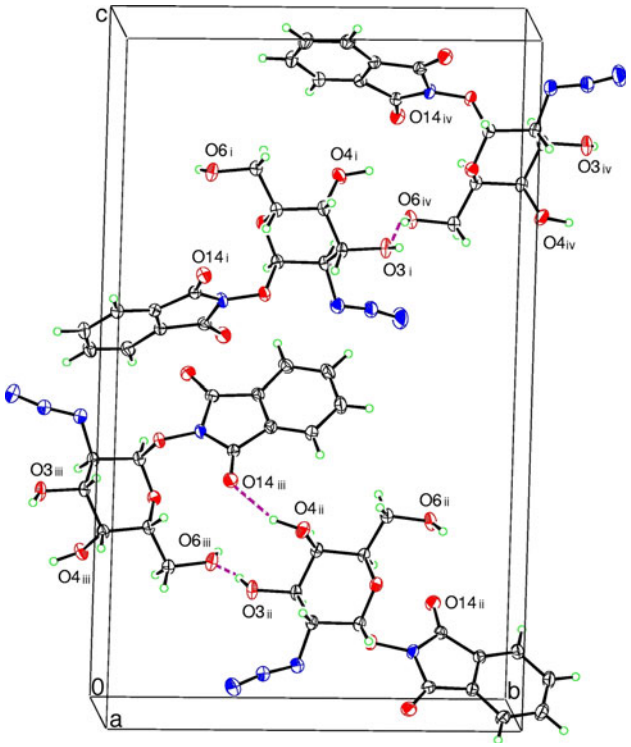

Fig. 1 Ortep view for compound **4** of the cell showing the four molecules generated by the symmetry operations: (i) x, y, z; (ii) $1/2 - x$, $-y$, $1/2 + z$; (iii) $1/2 + x$, $1/2 - y$, $-z$; (iv) $-x$, $1/2 + y$, $1/2 - z$ and the hydrogen bonds between them (*dotted line*)

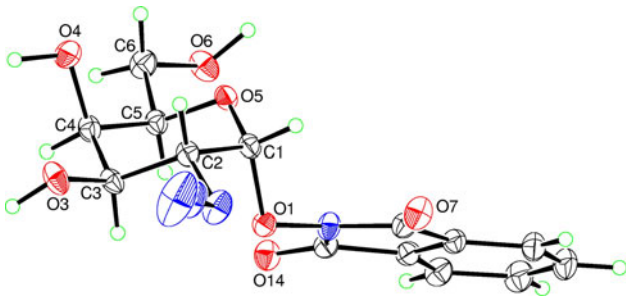

Fig. 2 Ortep drawing for compound **4**

0.549(2) Å, $\Phi 2 = 266.1(3)°$, $\Theta = 0.2(2)°$. Together with this conformational cycle distortion, the intramolecular hydrogen bond between O-6 and O-14 might explain the

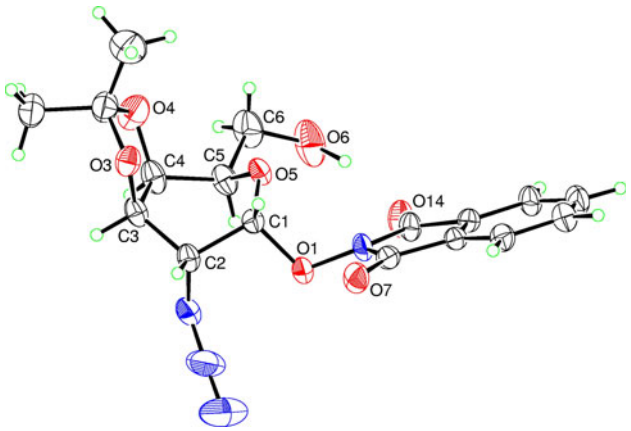

Fig. 3 Ortep drawing for compound **3**

axial orientation of the azido group in compound **3** whereas an equatorial orientation would have been observed as in compound **4**.

As regard to the stereochemistry, the absence of heavy atom in **3** and **4** precludes the direct determination of their absolute configuration. However, we assume that the relative configuration of C-2 to C-6 in compounds **3** and **4** is similar to the starting material, i.e. D-galactose, since both compounds were obtained as enantio pure compounds crystallising in acentric groups. Indeed, no inversion of chirality was induced during the chemical route from this natural carbohydrate. The main uncertainty could concern the stereoselectivity of the glycosylation reaction between **1** and *N*-hydroxyphthalimide. Due to the absence of neighbouring group participation in **1**, i.e. the azido group at C-2 is considered as "non-participating" group in a glycosylation reaction, we expected the formation of the alpha anomer as major stereoisomer by virtue of anomeric effect. This was unambiguously showed by NMR experiments since the coupling constant values (J) measured between the protons H-1 and H-2 in **3** are in perfect agreement with the alpha configuration (J = 4.2 Hz). A similar coupling constant value was observed for **4**, suggesting that the isopropylidene deprotection had no influence on the anomer configuration.

Conclusion

2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl-*N*-oxyphthalimide **3** is a new key building block towards the synthesis of the cancer-related aminooxy STn antigen **5**. This compound was prepared from D-galactosyl fluoride derivative **1** by a glycosylation reaction using *N*-hydroxyphthalimide. The expected alpha anomer was isolated by silica gel chromatography then compound **3** was obtained after desilylation and crystallisation.

Isopropylidene removal of **3** by acidolysis afforded compound **4** in a quantitative yield. NMR, MS and X-ray structures analyses are in perfect agreement with structures **3** and **4**. Interestingly, different hexapyranose ring conformations were observed, depending on the presence of protecting group between alcohols on C-3 and C-4. The fused five-membered isopropylidene ring in compound **3** indeed constrains the sugar cycle, leading to a distortion between a regular and a twist boat, whereas unprotected compound **4** reveals a quasi-perfect chair. This observation was confirmed by the calculation of the Cremer and Pople puckering parameters.

Supplementary Material

Crystallographic data (CIF) for 2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl-*N*-oxyphthalimide and for 2-azido-2-deoxy-α-D-galactopyranosyl-*N*-oxyphthalimide have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 700851 and CCDC 700852, respectively. This material is available free of charge via www.ccdc.can.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: (+44) 1223-336033. E-mail: deposit@ccdc.cam.ac.uk).

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