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Oxime-Based Synthesis of New Chromogenic and Fluorogenic Oligosaccharides

Olivier Renaudet*[a] and Pascal Dumy*[a]

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A new simple approach for the synthesis of labelled oligosaccharides is described. Free synthetic carbohydrate recognition building blocks were conjugated with fluorogenic and chromogenic dyes under mild experimental conditions by using oxime ligation. The resulting oligosaccharide probes presenting well-defined anomer configuration might find broad interests for recognition studies with a large panel of carbohydrate-binding proteins.

Introduction

The understanding of the molecular basis of carbohydrate–protein interactions remains a major challenge in glycomics research.[1] For this purpose, diverse analytical tools have been developed either in solution or with immobilized proteins or carbohydrates, not only to assess the binding parameters, but also to discover new active carbohydrate-based therapeutics or diagnostics.[2] For example, inhibition experiments such as hemagglutination (HIA)[3] or enzyme-like lectin assays (ELLA)[4] are commonly used to reflect the relative affinity of a carbohydrate-based ligand for a lectin by calculating an IC_{50} value. Furthermore, accurate association constants can be obtained through experiments such as isothermal titration calorimetry (ITC),[5] surface plasmon resonance (SPR),[6] fluorescence polarization (FP)[7] or frontal affinity chromatography (FAC).[8] By contrast with techniques that involve unlabelled protein or ligand, most of the recognition assays require the labelling of one conterpart to allow for the rapid monitoring of the binding event.

The development of an efficient labelling method for biomolecules, in particular for carbohydrate-based ligands, remains therefore necessary. Indeed, only few reports involving amide coupling, thiol/maleimide ligation or reductive amination have been reported so far.[9] In pursuing our research related to the design and the preparation of bioactive multitopic neoglycopeptides,[10] we focused recently our efforts on exploring a simple and efficient synthetic alternative to prepare diverse oligosaccharides bearing a fluorophore or a chromophore with a well-defined anomer configuration. We report herein a new oxime-based strategy through the synthesis of dabsyl and dansyl-labelled α-D-man, α-L-fuc, β-D-Lac as well as cancer-related Tn antigen (Figure 1).

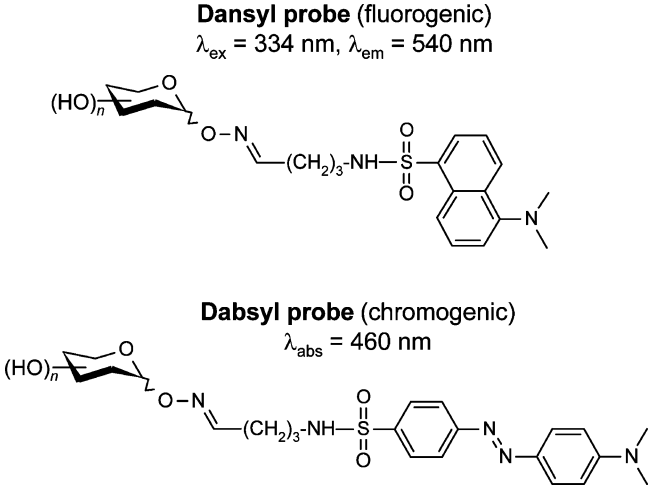

Figure 1. General structure of fluorogenic and chromogenic carbohydrate probes.

Results and Discussion

Due to the importance of the spatial display of the sugar ligand in the protein binding site, the reporter molecule has to be incorporated through a linker in a well-defined anomer configuration to both prevent steric hindrance and preserve the recognition efficiency. In order to by-pass the problems related to the control of stereoselectivity encountered during glycosylation, we have selected oxime ligation as a suitable and direct method to incorporate a dye with a pre-defined anomer position. Indeed, oxime bond forma-

[a] Département de Chimie Moléculaire, UMR-CNRS 5250 & ICMG FR 2607, Université Joseph Fourier,
38041 Grenoble Cedex 9, France
Fax: +33-4-76514946
E-mail: olivier.renaudet@ujf-grenoble.fr
pascal.dumy@ujf-grenoble.fr
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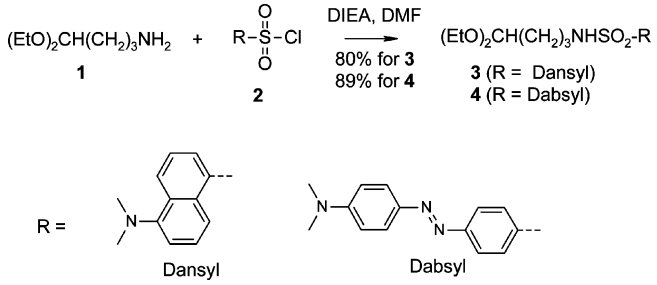
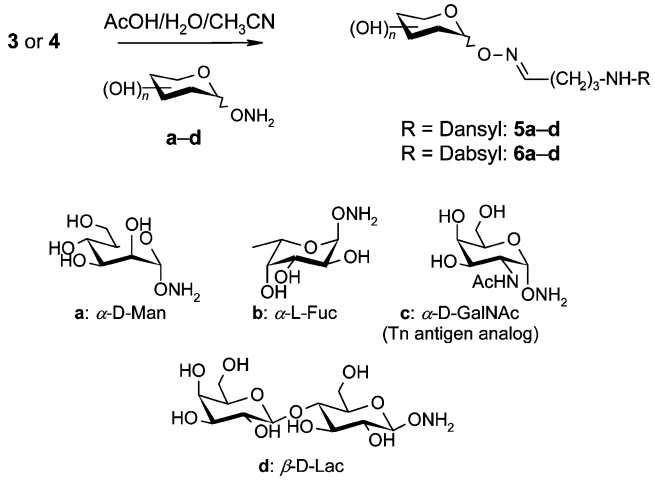
tion relies on the high reactivity between aminooxy and aldehyde (or ketone) functions as illustrated in a number of examples in the last decade.[11] Particularly, aminooxy carbohydrates were shown to provide useful building blocks for the preparation of glycoconjugates presenting well-defined anomer configuration by condensation with other free biomolecules containing aldehydes.[12] In this report, we have investigated for the first time the labelling of aminooxy-containing oligosaccharides with different reporter molecules bearing the complementary aldehyde function. We have first selected the dansyl moiety since its fluorescence properties (λ_{ex} = 334 nm; λ_{em} = 540 nm) make its derivatives useful as a detection agent as well as for binding or competition assays by using FP.[7] Alternatively, we chose dabsyl as a chromophore as it allows a facile and rapid visual detection (λ_{abs} = 466 nm).

The dye functionalization with an aldehyde linker was realized according to the route described in Scheme 1. 4-Aminobutyraldehyde diethylacetal (**1**) was condensed with dansyl chloride (**2**) in dimethylformamide (DMF) in the presence of diisopropylethylamine (DIEA) as a base.[13] After silica gel chromatography, compound **3** was obtained in 80% yield. A similar procedure was applied to prepare **4** from the commercially available dabsyl chloride.



Scheme 1. Synthesis of dansyl and dabsyl probes **3**–**4**.

A few oligosaccharides bearing an α- or β-aminooxy function were prepared next from the corresponding glycosyl fluorides.[14] We focused on α-D-Man (**a**), α-L-Fuc (**b**), α-D-GalNac (**c**) and β-D-Lac (**d**) (Scheme 2) as carbohydrate-based recognition motifs, because they are closely involved in numbers of physiological and/or pathological processes.[15]



Scheme 2. Synthesis of fluorogenic and chromogenic probes **5a**–**d** and **6a**–**b**.

The further conjugation of these aminooxy building blocks **a**–**d** with the probes **3**–**4** was realized with a one-pot procedure. The cleavage of the acetal protecting group of **3**–**4** and the final oxime ligation with **a**–**d** were achieved in aqueous acetic acid at room temperature. As illustrated by the RP-HPLC monitoring of the condensation between dabsyl derivative **4** and lactose derivative **d** [see peaks at retention time (t_R) = 4.5 min and 11.2 min, respectively, on the RP-HPLC profiles given in Figure 2], the treatment of **4** under these mild acidic conditions led to the formation

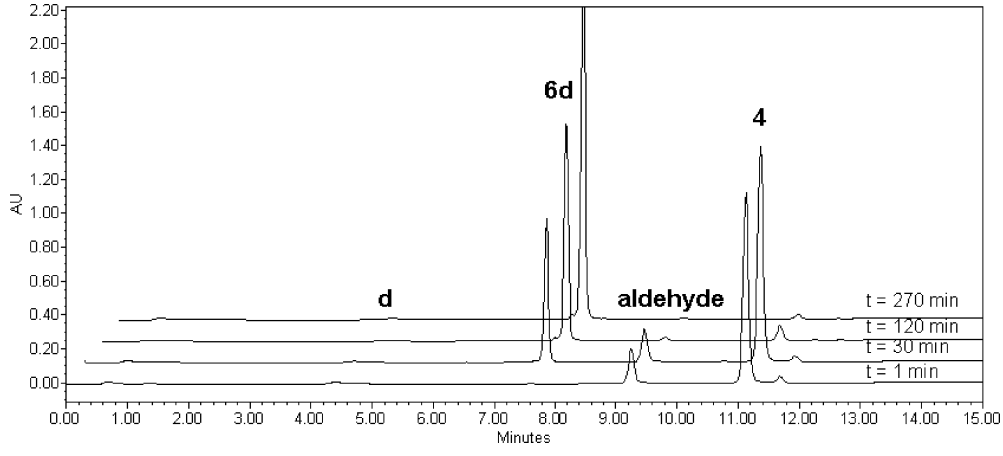


Figure 2. Reverse-phase HPLC of the crude one-pot deprotection/coupling reaction mixture for lactosyl-dabsyl probe **6d** at four different times (from 1 to 270 min). This reaction was performed between a twofold excess of aminooxy lactose **d** in an AcOH/H_2O/CH_3CN mixture and acetal-protected dabsyl derivative **4** (HPLC elution conditions: linear gradient A/B, 95:5 to 0:100 in 15 min, flow: 1.3 mL/min, λ = 214 nm; column: nucleosil 100 Å, 5 μm C_{18} particles, 250 × 4.6 mm; solvent A: 0.09% TFA, solvent B: 0.09% TFA in 90% acetonitrile).

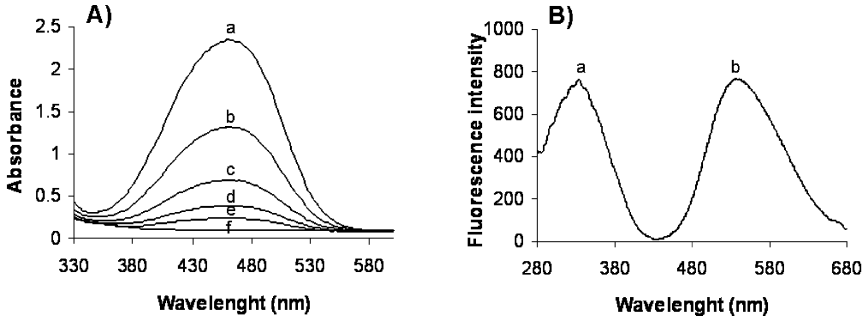


Figure 3. (A) UV spectrum of **6d** at different concentrations in CH_3CN/phosphate buffer (5 mM, pH = 7.4) (1:1) at 23 °C: (a) 1.25 mM, (b) 0.62 mM, (c) 0.31 mM, (d) 0.15 mM, (e) 0.08 mM, (f) 0 mM. (B) Fluorescence spectrum of **5d** (1 mM) in CH_3CN/phosphate buffer (5 mM pH = 7.4) (1:1) at 23 °C: (a) emission spectrum ($\lambda_{em.}$ = 540 nm), (b) excitation spectrum ($\lambda_{ex.}$ = 334 nm).

of the aldehyde function within 4 h (t_R = 9.3 min on the RP-HPLC profiles given in Figure 2). The resulting aldehyde was found to react simultaneously with the free aminooxy carbohydrate **d** to afford **6d** (t_R = 7.5 min on the RP-HPLC profiles given in Figure 2). The completeness of the one-pot deprotection/oxime coupling reactions was ensured after 270 min as confirmed by the clean crude reaction mixture observed in the last RP-HPLC profile (Figure 2).

After removal of excess unreacted carbohydrate by semi-preparative RP-HPLC, the dabsyl-functionalized lactosyl compound **6d** was obtained in a yield (ca. 80%) similar to those usually observed in oxime conjugation between aminooxy and free aldehyde building blocks. The fluorogenic and chromogenic oligosaccharides **5/6a**–**d** were obtained similarly with good yields and purity. Each compound was fully characterized by RP-HPLC, mass spectrometry and NMR spectroscopy (Table 1 and Supporting Information). Furthermore, we observed that the labelled sugars were obtained as a (*Z*)/(*E*) isomer mixture. Each isomer was assigned by further NMR experiments with the (*E*) isomer being the major compound (60–70%).[16] However, several attempts to separate both isomers by changing the RP-HPLC elution gradient were unsuccessful.

Table 1. Analytical data for compounds **5/6a**–**d**.

Probe	Yield	t_R[a]	MS[b]	(*Z*)/(*E*)[c]
5a	83% (21 mg)	5.6 min	498.11 (498.19)	0.4:0.6
5b	87% (21 mg)	5.9 min	482.11 (482.19)	0.35:0.65
5c	65% (20 mg)	5.6 min	539.12 (539.21)	0.35:0.65
5d	54% (10 mg)	5.2 min	660.14 (660.24)	0.35:0.65
6a	89% (25 mg)	7.9 min	552.17 (552.21)	0.4:0.6
6b	85% (33 mg)	8.1 min	536.16 (536.24)	0.4:0.6
6c	84% (21 mg)	7.9 min	593.15 (593.21)	0.35:0.65
6d	82% (33 mg)	7.5 min	714.18 (714.26)	0.3:0.7

[a] Reverse-phase HPLC retention time t_R under conditions described in Figure 2. [b] Mass spectrometry was performed by the electrospray ionisation method in the positive mode. Calculated masses are given in parentheses $[M + H]^+$. [c] Determined by NMR spectroscopy by using DPFGSE NOE experiments.[16]

Spectral data were finally collected for the lactosyl derivatives **5d** and **6d**. The UV properties of the red compound **6d** was firstly recorded from low to high concentrations, showing a maximum absorbance wavelength at 460 nm (Figure 3 A). We next performed fluorescence studies with **5d**, which gave excitation and emission spectra (Figure 3 B) in agreement with the fluorescent properties of the dansyl probe (maximum wavelength at 334 nm and 540 nm, respectively). More interestingly, we measured the fluorescence anisotropy values of **5a** and **5d** in HEPES aqueous buffer, then after adding increasing amounts of the mannose-binding lectin ConcanavalinA (ConA) for **5a** or the galactose-binding lectin from *Arachis hypogaea* (PNA) for **5d** (see Supporting Information).[17] As expected, whereas low fluorescence anisotropy values were measured for both **5a** and **5d** without lectin, we observed a significant increase of anisotropy in the presence of ConA or PNA, respectively, thus confirming that our dansyl probes might provide a useful tool to analyse carbohydrate–protein interactions.

Conclusions

We have explored for the first time an oxime-based strategy as a simple method for the preparation of new labelled carbohydrates with well-defined anomer configuration. First, dansyl and dabsyl probes **3** and **4** bearing an acetal-protected linker have been prepared. The final aldehyde release and the subsequent coupling reaction with free aminooxy carbohydrates building blocks **a**–**d** [α-D-Man (**a**), α-L-Fuc (**b**), β-D-Lac (**d**) and cancer-related antigen Tn (**c**)] occurred following a one-pot procedure under mild acidic conditions. The resulting fluorogenic and chromogenic carbohydrates **5/6a**–**d** were thus obtained in good yields and purity without using a coupling reagent or a deprotection step. We thus anticipate that our approach could be further extended to other fluorescent probes largely employed for biomolecules labelling, such as fluorescein and rhodamine derivatives. These new labeled compounds might find promising applications for the measurement of binding interactions with a large panel of proteins by using different techniques. For example, fluorescence polarization assays or HPLC screening procedure with cocktails of carbohydrates might give useful information on lectin glycopatterning.[18] Further investigations in this direction are currently in progress in our laboratory.

Experimental Section

Typical Procedure. Synthesis of 5a: A solution of α-D-Man-ONH_2 (**a**) (10 mg, 0.051 mmol) and protected dansyl derivative **3** (40 mg, 0.10 mmol) in AcOH/H_2O/CH_3CN (3:2:5) (6 mL) was stirred at room temperature for 4 h. The crude mixture was purified by semi-preparative RP-HPLC to obtain **5a** in 83% yield (21 mg, 0.042 mmol) as a lyophilized powder. (*E*)/(*Z*) oxime ratio (determined by NMR spectroscopy):[16] 0.6:0.4. ^{1}H NMR (300 MHz, [D_4]MeOH): δ = 8.59–8.53 (m, 2 H), 8.27 (br. d, J = 7.3 Hz, 1 H), 7.71 (br. t, J = 7.9 Hz, 2 H), 7.58 (br. d, J = 5.2 Hz, 1 H), 7.32 (t, J = 5.7 Hz, 0.6 H), 6.69 (t, J = 5.5 Hz, 0.4 H), 5.32 (d, J = 1.6 Hz, 0.4 H), 5.24 (d, J = 1.6 Hz, 0.6 H), 3.89 (dd, J = 1.6, 3.3 Hz, 0.4 H), 3.85 (dd, J = 1.6, 3.3 Hz, 0.6 H), 3.79–3.59 (m, 4 H), 3.50–3.44 (m, 1 H), 3.12 (s, 3 H), 3.10 (s, 3 H), 2.94–2.88 (m, 2 H), 2.31–2.24 (m, 1 H), 2.13–2.07 (m, 1 H), 1.63–1.54 (m, 2 H) ppm. ^{13}C NMR (75 MHz, [D_4]MeOH): δ = 154.7, 154.3, 137.8, 137.6, 130.9, 130.6, 129.9, 129.8, 129.6, 129.5, 129.0, 128.9, 125.7, 125.6, 123.4, 123.1, 117.9, 117.8, 103.4, 103.2, 75.4, 75.1, 72.8, 72.7, 70.8, 68.3, 62.7, 62.7, 46.5, 46.5, 43.5, 43.1, 27.4, 27.3, 24.1 ppm. Analytical RP-HPLC (5–100% B in 15 min, λ = 250 nm): t_R = 5.6 min. ES-MS (positive mode): calcd. for $C_{22}H_{32}N_3O_8S$ 498.19; found 498.11 [M + H]$^+$.

Supporting Information (see footnote on the first page of this article): Detailed description of synthetic procedure, ^{1}H and ^{13}C NMR spectra, HPLC, MS analysis of each new carbohydrate probe and fluorescence anisotropy assay titration curves.

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