Synthesis of Novel Pseudodisaccharides and Neoglycoconjugates Containing an *N*-Glycosyl Carbamate Backbone

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Abstract: A new class of pseudodisaccharides is presented in which a *N*-glycosyl carbamate is connected to a monosaccharide unit, using a mild and stereo-controlled isocyanide approach. The methodology was extended to the case of neoglycoconjugates useful as synthons for the construction of molecules with potential therapeutic interest.

Key words: carbohydrates, glycosyl carbamates, oligosaccharides, isocyanate, glycoconjugates

In recent years, increasing attention has been devoted to the synthesis of carbohydrates and carbohydrate analogs, due to their multifaceted biological properties.[1] It is well known that in many saccharidic macromolecules, such as capsular polysaccharides of bacteria, oligosaccharidic antigens or glycoconjugate structures, the glycosidic linkage can give rise to problems of chemical lability.[2] For this reason, many efforts have been directed to the conception and realization of stabilized bridged di- or oligosaccharidic systems.[3]

In the last two decades, a general interest in developing new strategies for the generation of glycosyl isocyanates as building blocks for the synthesis of glycosyl ureas has emerged.[4] By approaching this target, some significant difficulties have been encountered. The main problems involve the stereoselective preparation of the desired anomer and the isolation of the product, which is usually very reactive. Only recently, one example of crystalline peracetylated glucopyranosyl isocyanate was reported.[5] All the methodologies involving carbonylation of the glycosyl amine, included the most widely used employing phosgene or its derivatives, often produce a mixture of α- and β-anomers. Isobe and co-workers described two new strategies for the stereoselective preparation of α- and β-D-glucopyranosyl isocyanates.[6] In one of these methods, they obtain the isocyanate intermediates by mild oxidation of the corresponding isocyanides. In a recent work, we demonstrated the wide feasibility of the isocyanide approach for the synthesis of a series of intersaccharidic urea derivatives, in which the anomeric position of different glycosyl donors was easily connected to primary and secondary positions on the monosaccharidic acceptor.[7]

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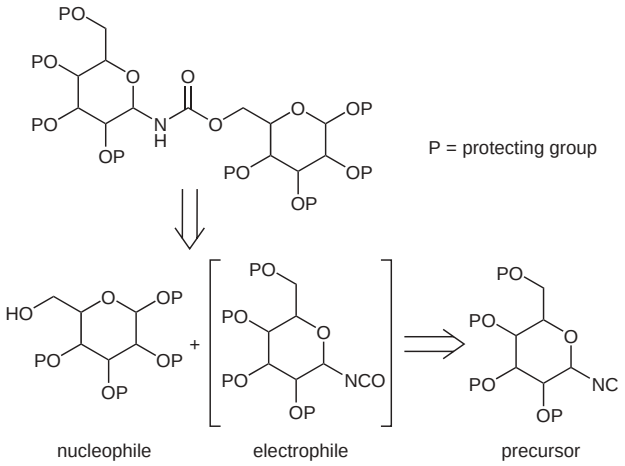
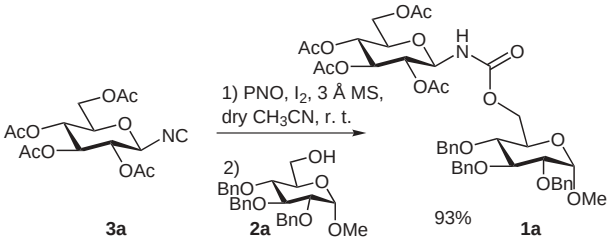
To date, we have found in the literature only two examples of *N*-glycopyranosyl carbamate connecting to a primary position of a sugar acceptor.[5,8] In the present work, we extend the scope of the stereo-controlled isocyanide approach to the synthesis of this new class of pseudooligosaccharidic structures.



Scheme 1



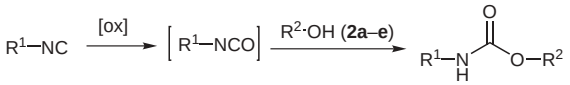
Scheme 2

In Scheme 1, the glycosyl isocyanide is highlighted as the precursor of the reactive isocyanate electrophile. The procedure was first applied to the case of the preparation of the urethane **1a** in which a glucopyranosidic unit is connected to the primary 6-O-position of the methyl glucoside acceptor **2a** through a carbamoyl bond (Scheme 2). Tetraacetyl glucopyranosyl isocyanide **3a**[7] was easily oxidized in dry acetonitrile to the corresponding isocyanate with pyridine *N*-oxide and a catalytic amount of iodine. In these mild conditions, we observed complete disappearance of the starting material in 30 minutes. The benzylated

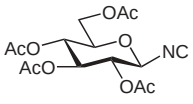
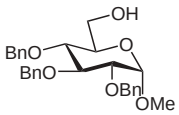
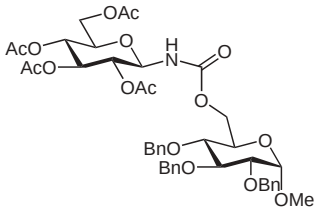
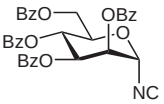
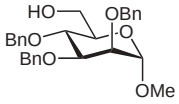
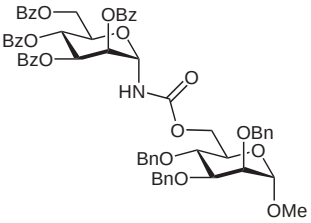
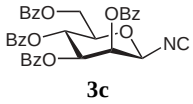
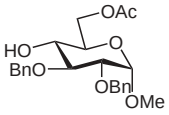
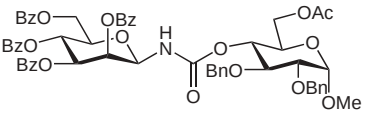
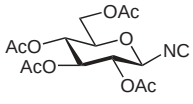
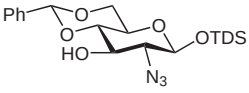
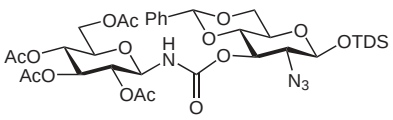
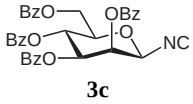
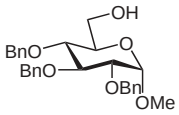
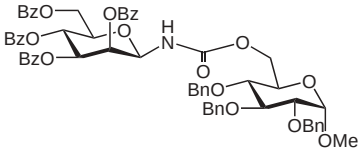
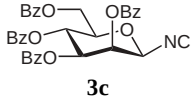
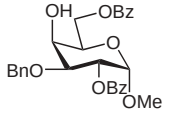
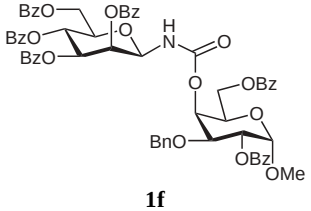
methyl glucoside **2a**, dissolved in dry acetonitrile, was added directly to the reaction mixture, furnishing the desired product **1a** in 93% yield after 30 minutes.[9] We were pleased to observe stereoconservation at the anomeric carbon of the donor during the transformations.

Since the mannopyranosidic unit was identified as one of the most important ligands for C-type animal lectins, which may play a crucial role in providing some protections against infection at the early stage of life, we considered the possibility to employ mannopyranosyl isocyanate as an alternative glycosydic substrate. Tetrabenzoyl

Table 1 Preparation of Carbamoyl Pseudodisaccharides from Glycosyl Isocyanides



3a–c **1a–f**

Entry[a]	Isocyanide[b]	Nucleophile[b]	Product	Time (h)	Yield (%)
1	 **3a**	 **2a**	 **1a**	0.5	93
2	 **3b**	 **2b**	 **1b**	0.5	96
3	 **3c**	 **2c**	 **1c**	1	73
4	 **3a**	 **2d**	 **1d**	2	94
5	 **3c**	 **2a**	 **1e**	0.5	90
6	 **3c**	 **2e**	 **1f**	3	86

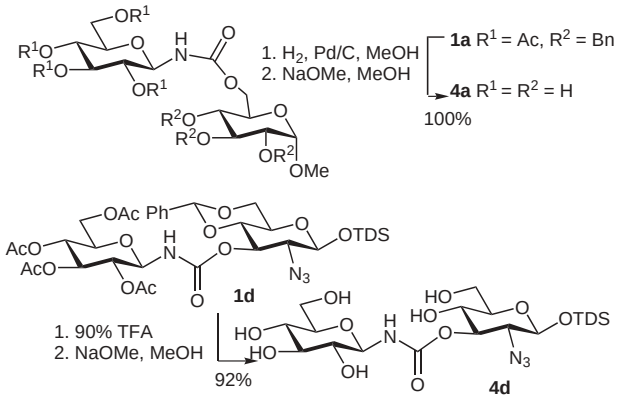
[a] All the reactions were carried out at r.t.

[b] The synthesis of compounds **3a–c**, **2a**, **2b** and **2e** is reported in ref.[7] For the preparation of compound **2c** and **2d** see ref.[10,11]

mannopyranosyl isocyanide, prepared according to our previous work,[7] invariably gave a mixture of α- and β-isomers (compounds **3b** and **3c**) in a 7:3 ratio. These two compounds were easily separated by chromatography and both used to achieve the corresponding carbamoyl linked disaccharides.

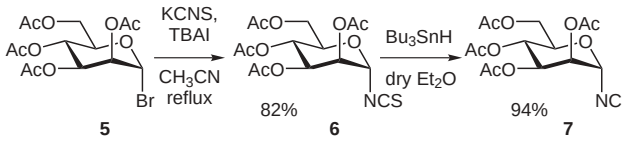
With the compounds **3a**–**c** in hand, we accomplished a small library of glycosyl carbamates, combining the corresponding isocyanates with compounds **2a**–**e**, according to the general procedure as for the synthesis of **1a** (Table 1). The results show the wide applicability of the reaction, employing both primary and secondary hydroxyl groups in the nucleophile, even in the case of axial configuration such as in the methyl galactopyranoside **2e** (entry 6). The equatorial hydroxyl group in position 3 (entry 4) shows a nucleophilic activity comparable to that of primary alcohols (entries 1, 2, 5): the reaction is 4 times slower but the yields are very high.

In order to get a qualitative evaluation of the chemical stability of these products, compounds **1a** and **1d** were deprotected (Scheme 3). Under the required conditions,[12] we did not observe decomposition or anomerization, thus demonstrating high stability of the carbamate bridge both in acidic and in alkaline conditions.



Scheme 3

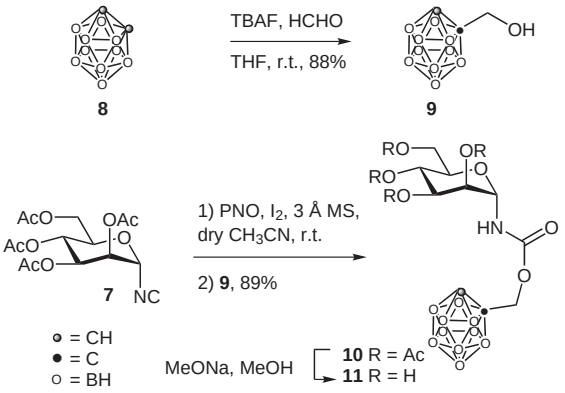
In order to obtain an improved selectivity for the preparation of the α-mannopyranosyl isocyanide for future applications, we also explored a different approach. Tetraacetyl α-mannopyranosyl bromide **5**[13] was converted into the corresponding α-isothiocyanate **6**[14] in 82% yield by reaction with KCNS and tetrabutylammonium iodide in refluxing acetonitrile. Compound **6** was desulfurized with tributyltin hydride, furnishing the isocyanide **7** in 94% yield without epimerization (Scheme 4).



Scheme 4

The versatility of the scheme of reactions described above was confirmed by two additional examples, where a glycosyl isocyanate was connected to a non-saccharidic alcohol through a carbamate bridge, in order to obtain neoglycoconjugates with potential biological applications.

In the first example, we considered the carborane cage,[15] known for its employment in BNCT (Boron Neutron Capture Therapy) research. BNCT is a binary therapy for the treatment of brain tumor.[16] One of the main questions regarding the carboranyl derivatives administration resides in the exceptional hydrophobic character of the icosahedral dicarba-*closo*-dodecaborane cluster,[17] requiring them to be conjugated with a hydrophilic counterpart. The sugar backbone is certainly an attractive candidate to enhance the water solubility of this interesting class of compounds. In the literature, a number of glycosyl carboranes are reported;[18] however, a possible disadvantage of O-glycosylated carboranes could be encountered in the enzymatic cleavage of such compounds by the action of glycohydrolases. Again, the conjugation of a carborane cage to a glycosyl carbamate could represent a convenient device to avoid this obstacle.



Scheme 5

To reach this goal, we first prepared the *ortho*-carboranylmethanol **9** by reaction of aqueous formaldehyde (37% w/w in H_2O) and *ortho*-carborane **8** with tetrabutylammonium fluoride in THF.[19] This compound was reacted with **7** in the usual conditions, affording rapidly the carborane-bearing α-mannosyl derivative **10** in 89% yield (Scheme 5). Noteworthy, the poor nucleophilic power of the hydroxyl group close to the carborane cage was offset by the high electrophilicity of the isocyanate moiety. Compound **10** was easily deprotected by Zemplén deacylation, giving **11** in quantitative yields.[20]

Finally, the glucosyl isocyanate was reacted with the commercial umbelliferone, also called 7-hydroxy coumarin, furnishing the corresponding carbamate in 98% yield. Unfortunately, any attempt of deacetylating this product was unsuccessfull, since the activated phenolic nature of the coumarin moiety favored transesterification by methanol.

In summary, we have described a simple and versatile route for the synthesis of a new class of pseudodisaccharides, involving the stereoselective isocyanide approach. The carbamate bridge demonstrated to be a useful tool for an easy conjugation with other therapeutically important molecules. Work is in progress to extend this strategy to the preparation of stabilized oligosaccharide analogs of biological interest.

Preparation of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl Isocyanide (7).

A mixture of potassium isothiocyanate (2.0 g, 20 mmol) and tetrabutylammonium iodide (3.7 g, 10 mmol) was dissolved in dry MeCN (250 mL) under nitrogen at r.t. Finely powdered 4 Å molecular sieves (15 g) and **5** (4.1 g, 10 mmol) were added successively and the mixture was refluxed for 3 h under stirring. When the reaction was complete, the mixture was filtered over celite, the crude product was concentrated and purified by flash chromatography (EtOAc–hexane 3:7) furnishing mannosyl isothiocyanate **6** in 82% yield as a pale yellow solid. Compound **6** (1.0 g, 2.57 mmol) was dissolved in dry Et_2O (10 mL) under nitrogen. Tributyltin hydride (680 mL, 2.57 mmol) was added and stirring continued at r.t. for 2 h. The solvent was evaporated and the resulting brown foam was passed through a silica gel column affording 860 mg of pure isocyanide **7** (94%) as a white solid.

General Procedure for the Preparation of the Glycosyl Carbamates 1a–f.

To a mixture of glycosyl isocyanide **3a**–**c** (0.40 mmol) and powdered 3 Å molecular sieves (300 mg) in dry MeCN (5 mL), were added pyridine *N*-oxide (115 mg, 1.2 mmol) dissolved in dry MeCN (0.5 mL) and iodine (8 mg, 0.03 mmol) under nitrogen. After 10 min, a solution of alcohol **2a**–**e** (0.40 mmol) dissolved in dry MeCN (1 mL) was added under stirring. The reaction was monitored by TLC (EtOAc–hexane 3:7). The mixture was quenched with a sat. solution of $NaHSO_3$ (5 mL), extracted with CH_2Cl_2 (20 mL) and washed with brine (20 mL). After drying over anhyd Na_2SO_4 and evaporation of the solvent, the crude product was purified by flash chromatography (EtOAc–hexane 3:7), affording glycosyl carbamate **1a**–**f** (yields reported in Table 1).

Acknowledgment

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(9) Selected data for compound **1a**. Mp 63–65 °C; $[\alpha]_D^{23}$ +14.1 (*c* 1.00, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$): δ = 1.94 (3 H, s, OAc), 2.01 (3 H, s, OAc), 2.02 (3 H, s, OAc), 2.07 (3 H, s, OAc), 3.35 (3 H, s, OMe), 3.41 (1 H, dd, *J* = 9.6, 1.5 Hz, H-6a), 3.51 (1 H, dd, *J* = 9.6, 3.4 Hz, H-6b), 3.72–3.86 (2 H, m, H-5, H-5′), 3.99 (1 H, t, *J* = 9.2 Hz, H-3), 4.07 (1 H, dd, *J* = 12.6, 1.1 Hz, H-6a′), 4.19–4.37 (2 H, m, H-4, H-6b′), 4.48–5.13 (9 H, m, H-1, H-2′, H-4′, 3 CH_2Ph), 5.01 (1 H, d, *J* = 8.9 Hz, H-1′), 5.29 (1 H, t, *J* = 9.3 Hz, H-3′), 5.63 (1 H, br d, *J* = 9.5 Hz, NH), 7.20–7.41 (15 H, m, Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.5, 55.2, 61.5, 64.4, 68.0, 68.6, 70.2, 72.7, 73.2, 73.4, 75.0, 75.7, 77.6, 79.8, 80.7, 81.8, 97.9, 127.6, 127.9, 128.1, 128.4, 137.8, 137.9, 138.5, 155.2, 169.5, 169.9, 170.5.

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(12) (a) **Procedure for the Deprotection of 1a:** Compound **1a** (90 mg, 0.11 mmol) was hydrogenated in MeOH (2 mL) in the presence of a catalytic amount of Pd/C; the crude product was deacetylated in methanolic NaOMe (0.02 mmol), giving 44 mg of **4a**, with a 100% overall yield. (b) **Deprotection of 1d:** To a solution of compound **1d** (109 mg, 0.13 mmol) in CH_2Cl_2 (2 mL), 90% aq TFA (620 μL) was added; after 1.5 h, the mixture was diluted with CH_2Cl_2 (15 mL) and washed with a sat. solution of $NaHCO_3$ (10 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The crude product was dissolved in dry MeOH (4 mL) and 70 μL of a 1 M solution of NaOMe (0.07 mmol) were added, furnishing 60 mg of **4d** (92%) as a white solid after flash chromatography.

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(20) Selected data for Compound **11**: $[\alpha]_D^{21}$ +40.1 (*c* 1.00, MeOH). ^1H NMR (300 MHz, CD_3OD): δ = 1.11–3.16 (10 H, m, BH), 3.45 (1 H, ddd, *J* = 9.0, 4.8, 3.1 Hz, H-5), 3.66 (1 H, t, *J* = 9.0 Hz, H-4), 3.70–3.77 (3 H, m, H-3, H-6a, H-6b), 3.80, (1 H, dd, *J* = 2.8, 1.8 Hz, H-2), 4.58 (1 H, br s, carboranyl CH), 4.63 (2 H, s, CH_2), 5.26 (1 H, d, *J* = 1.8 Hz, H-1). ^{13}C NMR (75 MHz, CD_3OD): δ = 63.0, 66.4, 69.0, 72.0, 72.5, 76.1, 82.6, 156.8.