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A general entry to linear, dendritic and branched thiourea-linked glycooligomers as new motifs for phosphate ester recognition in water†

José L. Jiménez Blanco,[a] Purificación Bootello,[a] Carmen Ortiz Mellet,*[a] Ricardo Gutiérrez Gallego[b] and José M. García Fernández*[c]

[a] *Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Apartado 553, E-41071 Sevilla, Spain. E-mail: mellet@us.es; Fax: +34 954624960; Tel: +34 954557150*

[b] *Pharmacology Research Unit, Municipal Institute of Medicinal Research (IMIM), Department of Experimental and Health Sciences, University Pompeu Fabra, Doctor Aiguader 80, E-08003 Barcelona, Spain*

[c] *Instituto de Investigaciones Químicas, CSIC, Américo Vespucio s/n, Isla de la Cartuja, E-41092 Sevilla, Spain. E-mail: jogarcia@cica.es; Fax: +34 954460565; Tel: +34 954489559*

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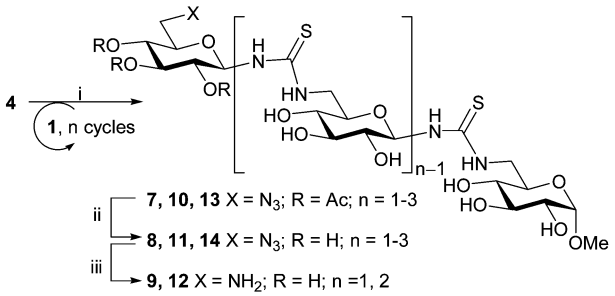
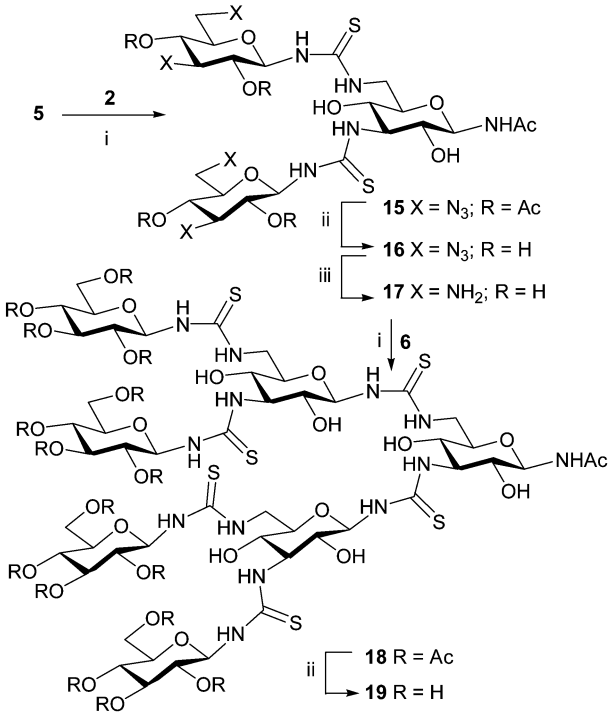
A blockwise iterative synthetic strategy for the preparation of linear, dendritic and branched full-carbohydrate architectures has been developed by using sugar azido(carbamate) isothiocyanates as key templates; the presence of intersaccharide thiourea bridges provides anchoring points for hydrogen bond-directed molecular recognition of phosphate esters in water.

The growing awareness of the role of oligosaccharides as carriers of biological information and the emergence of chemical libraries as potential tools for ligand lead and drug discovery have spurred an aggressive effort towards the development of blockwise synthetic methodologies suitable for the construction of glycodrugs in a combinatorial manner.[1] Inspired by the well-established oligonucleotide and peptide synthetic methods, several groups have worked in the last few years to develop linear and cyclic pseudooligosaccharides incorporating phosphodiester[2] (carbonucleotides) or amide[3] (carbopeptoids, saccharopeptides) intersaccharide functional groups. However, although these non-natural oligomers capture some of the defining characteristics of the natural counterparts, they do not account for the incorporation of branching points into the structure, a unique feature of carbohydrates that is responsible, to a great extent, for their impressive encoding capacity.

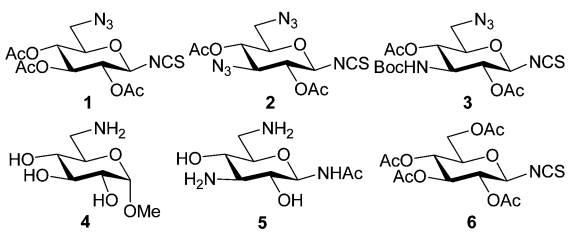
We present here novel linear as well as dendritic and branched glycooligomers consisting of thiourea-linked monomers.[4] Several advantages were envisioned for these molecules, including: (a) chemoselective formation in the presence of hydroxyl groups;[5] (b) possibility of library generation;[6] (c) choice of solution or solid phase synthesis;[6,7] (d) propensity to adopt defined conformations;[8] (e) specific and predictable recognition of complementary functional groups (*e.g.* phosphate, carboxylate, *etc.*) through hydrogen-bonding recognition[8,9] and (f) the thiourea group can be transformed into other functionalities, such as urea, isothiourea or guanidine, through standard transformations.[5–7,10] To demonstrate the above concepts, the glucose derived AB, AB_2 and ABC-type building blocks **1–3**, incorporating isothiocyanate (A), azido (B) and carbamate groups (C), were designed. Methyl 6-amino-6-deoxy-α-D-glucopyranoside (**4**) and 1-*N*-acetyl-3,6-diamino-3,6-dideoxy-β-D-glucopyranosylamine (**5**) have been used as starting ("reducing") ends, while 2,3,4,6-tetra-*O*-acetyl-β-D-gluco-



Scheme 1 *Reagents and conditions*: i, pyridine or water–acetone at pH 8 ($NaHCO_3$), room temperature, 5–24 h, 82–97%; ii, NaOMe, MeOH, room temperature, 2 h, quantitative; iii) 1,3-propanedithiol, Et_3N, room temperature, 16 h, > 95% (NMR).



Scheme 2 *Reagents and conditions*: i–iii have the same meaning as in Scheme 1.



† Electronic supplementary information (ESI) available: preparative procedures for the key building blocks **1–3** and full characterization data for the final thiourea-linked linear (**8, 11** and **14**), dendritic (**16** and **19**) and branched (**21, 24, 26** and **30**) oligosaccharide mimetics, as well as details of the binding studies. See http://www.rsc.org/suppdata/cc/b3/b312743p/

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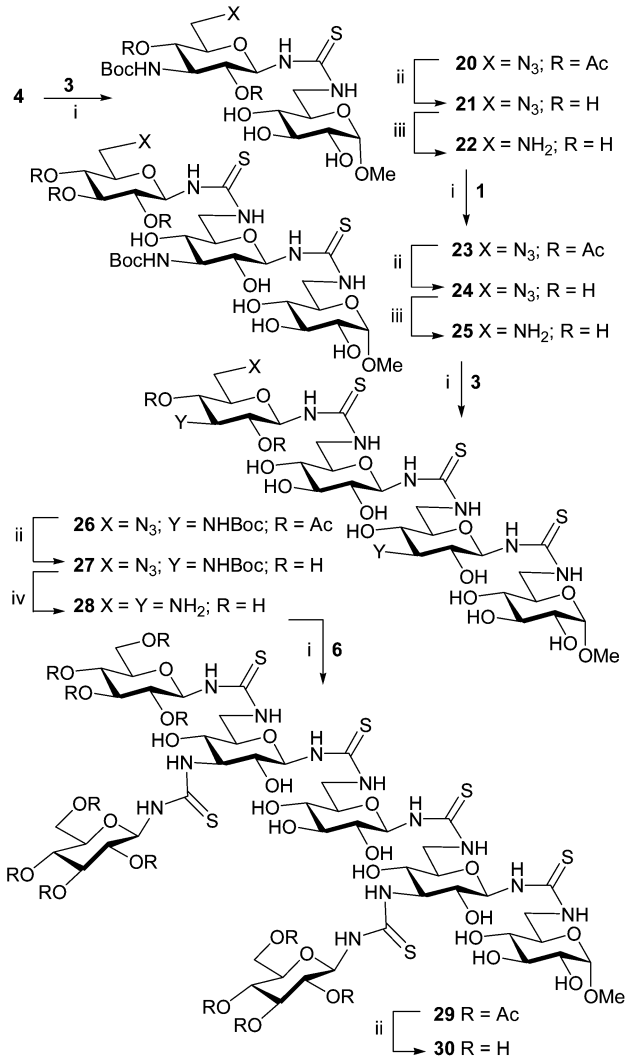
pyranosyl isothiocyanate (**6**) was eventually employed as coating ("non-reducing") unit.‡

An iterative and very efficient three-step reaction sequence, compatible with the intrinsic polyfunctionality of sugars, was disclosed for the assembly of monosaccharide component units that involves: (i) the thiourea-forming reaction of sugar azido isothiocyanate and aminosugar precursors (→**7,10,13** and **15,18**); (ii) cleavage of the *O*-protecting groups in the adduct (→**8,11** and **16**); and (iii) reduction of the terminal azido functions to generate a new (poly)amine "acceptor" for the next cycle (→**9,12** and **17**). Following this methodology, the linear β-(1→6) tetramer **14** (Scheme 1) and the second generation dendritic β-(1→6), β-(1→3) heptamer **19** (Scheme 2) were prepared.‡

The possibility of introducing branching points at specific locations is exemplified by the synthesis of the thiourea-linked pseudoheptasaccharide **30**, which mimics the branching pattern of a naturally occurring phytoalexin elicitor-active β-glucan.[11] The linear β-(1→6) backbone was built by alternating sequential incorporation of the ABC **3** (first cycle, → **20–22**; third coupling cycle, → **26**) and AB **1** (second cycle, → **23–25**) building blocks into the chain following the above methodology. In the last cycle, deacetylation of **26** (→ **27**) followed by acid-catalysed hydrolysis of the carbamate groups and reduction of the azido group generated



Scheme 3 *Reagents and conditions*: i–iii have the same meaning as in Scheme 1; iv, first iii and then 1 : 1 TFA–water, 2 h, room temperature, quantitative (NMR).

the triamine **28**, which is the requested acceptor for the final coupling step. Reaction of **28** with the coating unit **6** and deacetylation of the resulting adduct **29** yielded the target oligosaccharide mimetic **30** (Scheme 3).‡

To investigate whether this new type of neutral glycooligomer may be involved in phosphate ester binding in aqueous solution, we examined the binding of pseudodisaccharide **7** against dimethyl phosphate and phenyl phosphate (sodium salts) as models for a phosphodiester and a polyanionic phosphate derivative, respectively. Only small changes were observed in the ^{1}H NMR spectrum of **7** upon addition of the anions and their interpretation was complicated by severe overlap of signals. Yet, chemical shifts were clearly observable in the ^{13}C NMR spectrum. Curve-fitting procedures, following the *C*-6′ signal, revealed the formation of 1 : 1 complexes in both cases, with association constants (K_a) of 2.5 ± 0.2 and 39 ± 3 M^{-1}, respectively.

It is remarkable that, although weak, the measured interaction between the saccharide host (neutral) and the phosphate anions in water must be essentially free from any hydrophobic or electrostatic assistance.[12] The present work may thus open a new strategy for the investigation of molecular recognition of highly polar species in water. Moreover, a significant increase in binding strength can be expected for the interaction of the polytopic higher glycooligomers with polyphosphates, which may have implications for nucleic acid binding.[13] Work in that direction is currently in progress in our groups.

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Notes and references

‡ All new compounds gave satisfactory microanalytical, NMR (^{1}H and ^{13}C) and MS data in accord with the proposed structures (see ESI).

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