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The Synthesis and Structure of Linear and Dendritic Thiourea-Linked Glycooligomers

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An efficient strategy to produce unnatural glycooligomers containing thiourea intersaccharide bridges has been developed. The presence of the thiourea groups in these oligomers should promote the association with polyphosphates, including nucleic acids, due to the hydrogen-bonding capabilities of the thioureas. Moreover, glycooligomers built from thiourea groups should form complex secondary and tertiary structures due to the interplay of hydrogen bonding and rotational restriction at the bonds adjacent to thiocarbonyl groups. Herein, the preparation and conformational properties of both linear and dendritic architectures are described. This synthetic method relies on the coupling of peracetylated azidoglycosyl isothiocyanates and fully unprotected amine-functionalised growing oligomers. Deacetylation of the hemiacetylated thiourea adduct and reduction of the azido groups allow entry into a new coupling cycle. The procedure is quite general, should be easily extended to molecular diversity-oriented and solid-phase approaches and will allow the investigation of intermolecular interactions.

Introduction

Ligands that sequence-selectively bind to nucleic acids have a number of important uses as tools in chemistry and molecular biology and as therapeutics in medicine. Carbohydrates are a recent addition to the arsenal of such compounds. There are a large number of nucleic acid-binding natural products that contain carbohydrate components, including enediyne antitumour compounds such as calicheamicin γ_1, anthracyclines such as daunorubicin or aminoglycoside antibiotics such as neomycin B.[1] Although the role of these sugars, for the most part, is still unclear, it has been shown in a few cases that the saccharide portion itself mediates the sequence-specific recognition. Understanding the underlying mechanisms involved in carbohydrate–nucleic acid recognition is a prerequisite for the development of new strategies in the design of efficient nucleic acid binding agents and their pharmaceutical application. From the current body of data, some key features can be identified. For instance, the presence of ammonium groups in many of the known nucleic acid-binding carbohydrates can provide charge neutralisation, the selectivity is frequently achieved through a network of hydrogen bonding, and in the case of neutral nucleic acid-binding glucooligo- and -polysaccharides with antitumour activity, such as schizophyllan or lentinan, a minimum length and the existence of a regular β-(1→6), β-(1→3) branching pattern is generally found.[2] The presence of suitably located substructures capable of establishing bidentate hydrogen bonds with phosphodiester groups, e.g. 1,2- and 1,3-hydroxyamine arrangements, also appears to be important, as inferred from the study of aminoglycoside antibiotic–phosphodiester interactions.[3]

The development of unnatural glycooligomers for specific and predictable recognition of nucleic acids has been hampered, however, by the lack of suitable strategies to build polyphosphate-complementary architectures from carbohydrates. Inspired by the well-established oligopeptide synthetic methods, several groups have worked in the last few years on the preparation of linear and cyclic pseudooligosaccharides incorporating amide intersaccharide functional groups (carbopeptoids, saccharopeptides).[4] We speculated that incorporating thiourea groups as intersaccharide bridges instead would provide efficient anchoring points for bidentate hydrogen-bonding recognition of phosphodiesters.[5] Further contributions from the glucidic portions might then modulate the binding event.[6] Actually, preliminary results indicated that neutral thiourea-linked pseudodisaccharides form weak 1:1 complexes with phosphate esters in water.[7] A significant increase in binding strength can be expected for the interaction of higher oligomers with polyphosphates, which might have implications for nucleic acid binding. Moreover, the presence of multiple thiourea groups in the glycooligomers may be expected to

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induce secondary structures, possibly reminiscent of peptide secondary structures, which might provide sequence-specificity upon interaction with oligonucleotides. In order to study such supramolecular architectures from unnatural thiourea-linked pseudooligosaccharides, one must first have an efficient synthetic strategy to produce them. Herein, we describe the synthesis of glycooligomers that alternate β-D-glucopyranosyl units and 1,3-thiourea segments. We focus on the success and difficulties of the method to access both linear and dendritic architectures with (1→6), (1→3) or (1→6) and (1→3) linking patterns, as well as the study of their conformational properties, and leave our supramolecular studies for a future paper.

Results and Discussion

Retrosynthetic Analysis

A thiourea can be formed by the facile coupling of an isothiocyanate with an amine[8] in a reaction that proceeds with total chemoselectivity in the presence of unprotected hydroxy groups.[9] In order to allow the incorporation of distinct monomers in specific positions in an oligomer, we selected monosaccharide building blocks with an isothiocyanate group at the anomeric position and one (for linear construction) or two (for dendritic) azido groups as latent amine functionalities. The retrosynthesis is shown in Figure 1 and involves the addition of O-acetylated glycosyl isothiocyanate derivatives to the growing amine-functionalised oligothiourea. After coupling, deprotection of the hydroxy groups in the thiourea adduct and reduction of the azide group to the corresponding amine, a new monomer could be introduced, thereby incrementally constructing an alternating monosaccharide–thiourea oligomer.

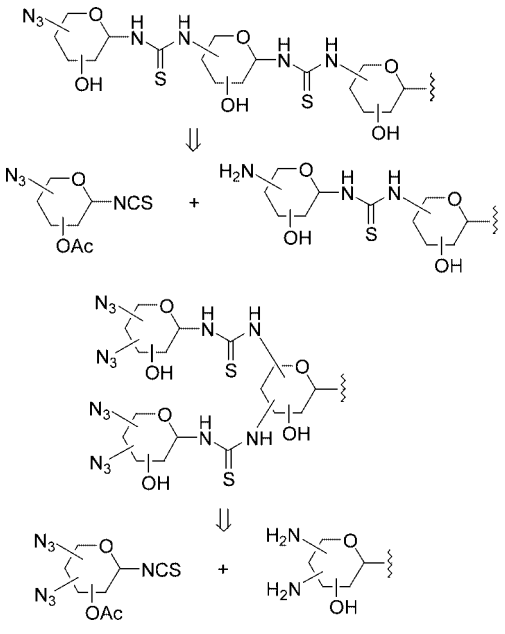


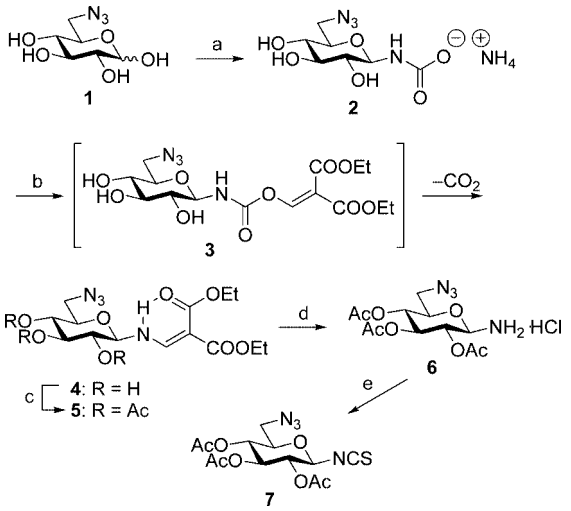
Figure 1. Retrosynthetic analysis for linear and dendritic thiourea-linked glycooligomers.

Monomer Synthesis

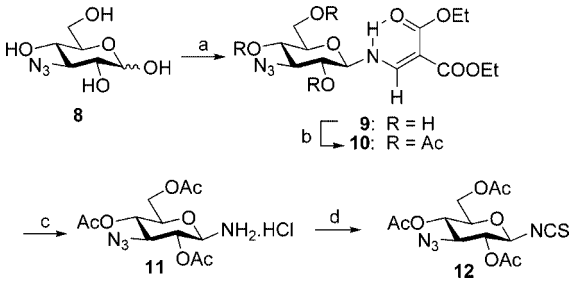
To test the feasibility of such an approach, β-D-glucopyranosyl monomers that could be produced from the inexpensive commercial sugar in a limited number of steps were the initial targets. Even after fixing the monosaccharide hydroxylation profile (D-*gluco*) and the anomeric configuration (β), up to four different pseudodisaccharide positional isomers, 16 linear pseudotri- and 64 pseudotetrasaccharide structures are possible, and many more if we consider the possibility of branching. As a proof of concept of the synthetic strategy, we focused on glucooligomers having a regular structure with a single type of monosaccharidic repeating unit, namely (1→6), (1→3) or [(1→6), (1→3)] oligomeric glucosylthioureas. The synthesis of sugar azidoisothiocyanates having those substitution patterns as building blocks was therefore pursued.

Classically, glycosyl isothiocyanates are prepared by either an S_N2-type reaction of per-O-protected glycosyl halides with thiocyanate salts or the isothiocyanation reaction of glycosylamines.[8] Notwithstanding, attempts to prepare the corresponding tri-O-acetylated glycosyl bromide from 6-azido-6-deoxy-D-glucose (**1**),[10] available in two steps from D-glucose via the 6-*O*-tosyl derivative,[11] by peracetylation and treatment with hydrogen bromide in glacial acetic acid were unsuccessful.[12] Transformation of **1** into the corresponding glycosylamine by treatment with ammonium hydroxide/sodium hydrogencarbonate and decomposition of the resulting ammonium glycosylcarbamate salt **2** by successive lyophilisations[13] also failed, affording extensive formation of bis(glycosylamine) as well as hydrolysis back to **1** (1:1, 85%), as seen by NMR spectroscopy and mass spectrometry. Since compound **2** already possesses a latent amine functionality installed at the anomeric position, we considered the possibility of using the carbamate salt directly for further elaboration without liberating the unstable O-unprotected glycosylamine. We were delighted to confirm that crude **2** readily reacts with diethyl ethoxymethylenemalonate to afford the glycosylenamine **4** in good yield. The reaction probably proceeds by nucleophilic displacement of the ethoxy group by the carbamate anion[14] and subsequent elimination of carbon dioxide from the vinyl carbamate adduct **3** via a four-membered cyclic intermediate, by analogy with the mechanism generally accepted for the known decarboxylation of mixed glycosyl(thio)carbamic anhydrides to give glycosylamides.[15] Conventional acetylation of **4** (→**5**) and chlorolysis of the enamino group yielded the tri-O-acetylated glycosylammonium salt **6**, which was transformed into the target 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl isothiocyanate **7** upon reaction with thiophosgene (Scheme 1).

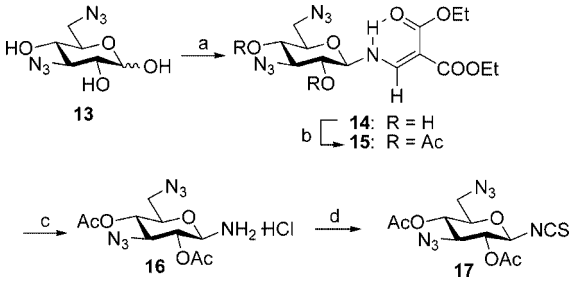
The above approach proved to be of general application to access azidoglycosyl isothiocyanates. Thus, similar reaction sequences starting from 3-azido-3-deoxy- (**8**)[16] (Scheme 2) or 3,6-diazido-3,6-dideoxy-D-glucose (**13**)[17] (Scheme 3) led to the corresponding azidoisothiocyanate **12**, which is a positional isomer of **7**, or the diazidoisothiocyanate **17**, respectively.



Scheme 1. Reagents and conditions: a) NH_4HCO_3, 16 M aq. ammonia, 40 °C, 36 h; b) diethyl ethoxymethylenemalonate, MeOH (62% for two steps); c) 1:1 Ac_2O/pyridine (96%); d) Cl_2, wet CH_2Cl_2, 5 min (97%); e) $CSCl_2$, $CaCO_3$, CH_2Cl_2/water, 30 min (64%).



Scheme 2. Reagents and conditions: a) 1. NH_4HCO_3, 16 M aq. ammonia, 40 °C, 48 h, 2. diethyl ethoxymethylenemalonate, MeOH, 40 °C, 12 h (47%); b) Ac_2O, pyridine (88%); c) Cl_2, wet CH_2Cl_2, 5 °C, 30 min (100%); d) $CSCl_2$, $CaCO_3$, CH_2Cl_2/water, 4 h (50%).

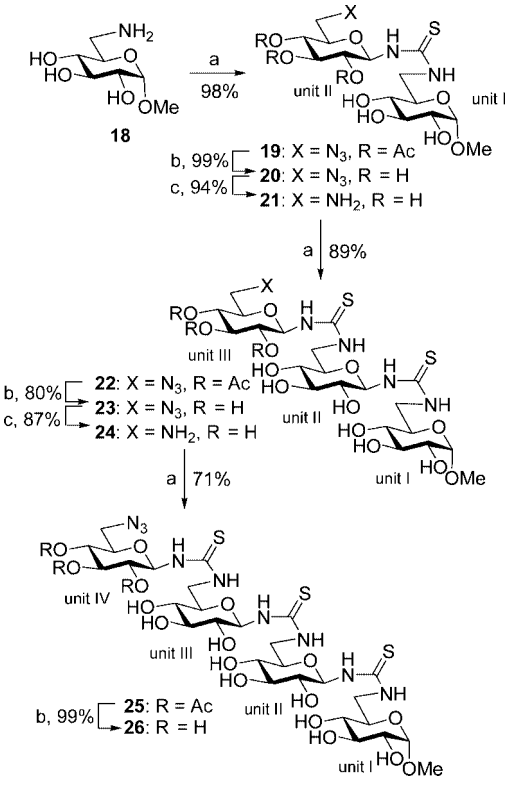


Scheme 3. Reagents and conditions: a) 1. NH_4HCO_3, 16 M aq. ammonia, 40 °C, 36 h, 2. diethyl ethoxymethylenemalonate, MeOH, 40 °C, 12 h (52%); b) Ac_2O, pyridine (89%); c) Cl_2, wet CH_2Cl_2, 5 °C, 30 min (97%); d) $CSCl_2$, $CaCO_3$, CH_2Cl_2/water, 30 min (61%).

Synthesis of Thiourea-Linked Glycooligomers

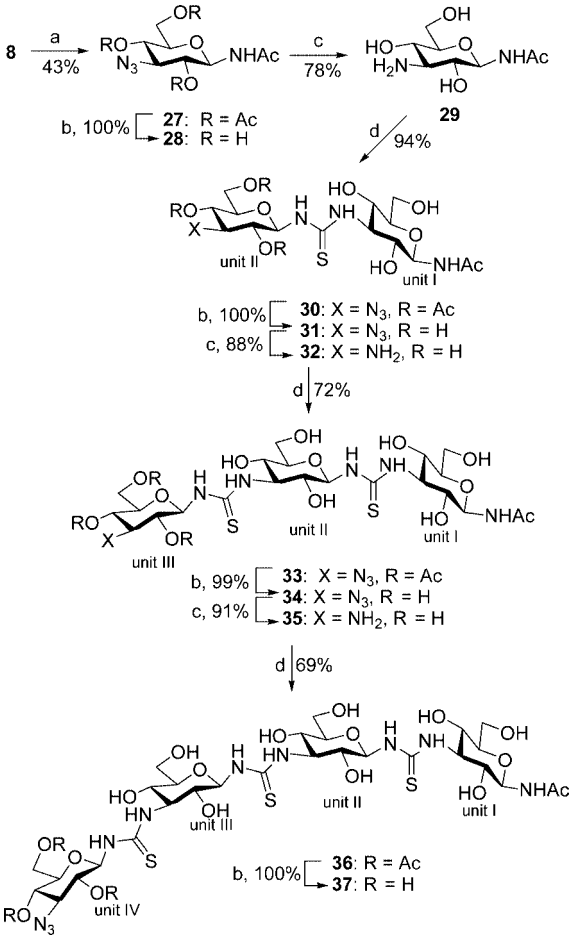
In order to synthesise thiourea-linked glycooligomers, two important considerations had to be taken into account. The first was the possibility of intra- or intermolecular O→N acyl migration during the reduction of azido groups or during the coupling reaction of O-acetylated isothiocyanate and amine precursors. Intramolecular acyl migration was avoided by inserting a deprotection step prior to azide reduction in the thiourea adducts, keeping in mind that the nucleophilic addition of amines to isothiocyanates proceeds with total chemoselectivity in the presence of hydroxy groups. Eventually, water/acetone was used as the coupling reaction solvent in order to avoid the formation of acyl migration by-products during the preparation of sugar-derived polythioureas from polyamines, which generally requires longer reaction times.[18] The second consideration was the choice of an azide reduction method compatible with the presence of the thiourea functionality. Catalytic hydrogenation and Staudinger reduction via the phosphazene[19] were discarded in view of interference with the thiocarbonyl groups. The azide to amine transformation in the oligothioureas was satisfactorily achieved in high yield by treatment with the propanedithiol/triethylamine system.[20]

Our first objective was the preparation of linear β-(1→6) and β-(1→3) thiourea-linked glucooligomers that incorporate a methyl 6-amino-6-deoxy-α-D-glucopyranoside[21] (**18**) or an *N*-acetyl-3-amino-3-deoxy-α-D-glucopyranosylamine unit (**29**), respectively, as the "head" terminus. The latter was prepared from the corresponding reducing azidosugar **8** via the glycosyl carbamate by direct peracetylation (→**27**), followed by selective de-O-acetylation (→**28**) and reduction. Although the pseudotetrasaccharides **26** (Scheme 4) and **37** (Scheme 5) were the initial targets of these syntheses, each intermediate hemiacetylated adduct (**19**, **22**, **25** and **30**, **33**, **36**) and each fully unprotected oligomer (**20**, **23** and **31**, **34**, respectively), bearing an azide-functionalised "tail" ter-



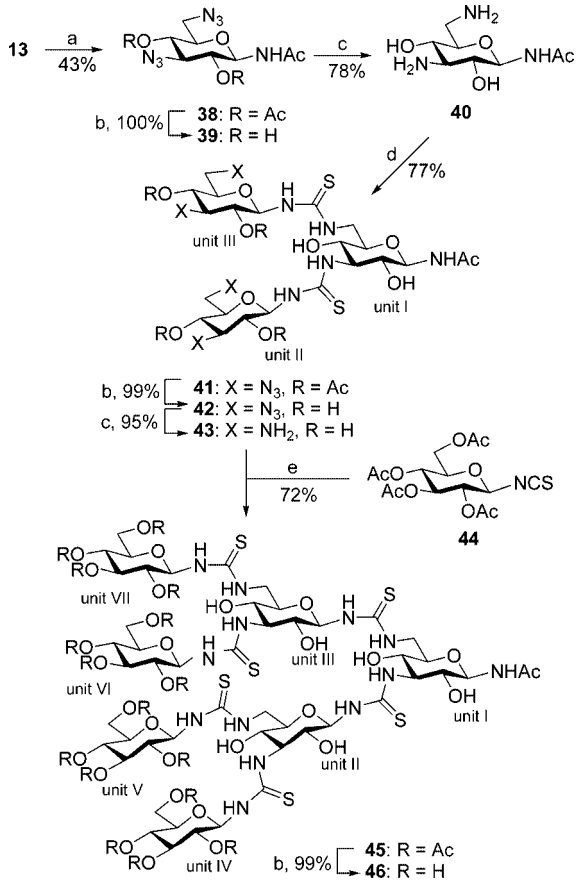
Scheme 4. Reagents and conditions: a) **7**, pyridine, 5–24 h; b) NaOMe, MeOH; Amberlite IR-120 (H^+); c) $HS(CH_2)_3SH$, MeOH, Et_3N, room temp., 16 h.

minus, were also purified and fully characterised. After reduction of the azido group (→**21**, **24** and **33**, **35**, respectively), the isothiocyanate monomer **7** or **12** was added to a solution of the growing amine-armed oligomer in pyridine. As the oligomer length increased, the reaction times for the coupling became longer and the coupling yields were slightly poorer. Nevertheless, the conversions remained satisfactory even after three cycles, and no other by-products were observed in the reaction mixtures. Typically, the first coupling proceeded in 90–95% yield, the second coupling around 88–80% and the third near 70%. No significant differences were observed between the series, in spite of the location of the nucleophilic amine at primary and secondary carbon atoms, respectively.



Scheme 5. Reagents and conditions: a) 1. NH_4HCO_3, 16 M aq. ammonia, 40 °C, 36 h, 2. Ac_2O, pyridine; b) NaOMe, MeOH; Amberlite IR-120 (H^+); c) $HS(CH_2)_3SH$, MeOH, Et_3N, 16 h; d) **12**, pyridine, 2–24 h.

Implementing the above methodology to access full-carbohydrate dendritic architectures[22] was a particularly interesting challenge. No examples of branched amide or pseudoamide-linked glycooligomers have been reported so far. On principle, diazidoisothiocyanate **17** can be regarded as a zero-generation AB_2-type glycodendron that is suitable for the preparation of higher-generation dendrimers by iterative coupling-deprotection-reduction cycles. As head terminus we chose an *N*-acetyl-3,6-diamino-3,6-dideoxy-β-D-glucopyranosylamine unit (**40**), obtained from the reducing diazide **13** following a reaction sequence similar to that previously used for the preparation of the related monoamine **29**. The coupling reaction of **40** with two equivalents of the isothiocyanate monomer **17** in pyridine at room temperature afforded the corresponding [(1→6), (1→3)]-branched pseudotrisaccharide **41** in 77% yield. This first-generation dendrimer was activated for the next cycle by deacetylation (→**42**) and reduction (→**43**) following the protocol already employed in the synthesis of linear oligomers (Scheme 6).



Scheme 6. Reagents and conditions: a) 1. NH_4HCO_3, 16 M aq. ammonia, 40 °C, 36 h, 2. Ac_2O, pyridine; b) NaOMe, MeOH; Amberlite IR-120 (H^+); c) $HS(CH_2)_3SH$, MeOH, Et_3N, 18 h; d) **17**, pyridine, 2 h; e) water/acetone, pH 8 (solid $NaHCO_3$) 24 h; f) 1. NaOMe, MeOH, 2. water, (H^+, OH^-).

Attempts to obtain the capped, second-generation analogue **45** under the same reaction conditions by addition of an excess of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**44**)[8] failed, however. Increasing the temperature (50 °C), adding triethylamine as a catalyst and increasing the excess of isothiocyanate reagent allowed us to isolate the desired product, although in low yields (<12%). Extensive formation of bis(glycosylthiourea) from the dimerisation reaction of **44** was observed under these conditions.[14] The accessibility of the amino groups, especially those located at secondary carbon atoms, is probably greatly hampered in the branched arrangement as compared with linear isomers. Nevertheless, the fourfold thiourea-forming reaction proceeded smoothly at room temperature in 1:1 water/

acetone at pH 8 to afford the hemiacetylated hexathiourea **45** in a satisfactory 72% yield. Since precipitation was observed during conventional catalytic transesterification, deacetylation was accomplished by a mixed procedure involving initial treatment with methanolic sodium methoxide and then saponification by addition of water. After demineralisation with mixed (H^+, OH^-) ion-exchange resin, the target dendritic pseudoheptasaccharide **46** was obtained in 99% yield (Scheme 6). An analytical sample was obtained after purification by gel-permeation chromatography.

The NMR spectra of the thiourea-linked glycooligomers are temperature dependent, indicating significant conformational constraints. Different conformations likely result from the well-documented restriction to rotation of the C–N bonds of thioureas and intramolecular hydrogen bonds, which induce secondary structure, the rotameric exchange being slow on the NMR timescale.[8] As a result, the NMR spectra recorded at 298 K exhibit significant line broadening. A fast chemical exchange was achieved at higher temperatures, allowing unequivocal structural assignment.

Previous studies on the conformational behaviour of sugar thioureas by dynamic NMR spectroscopy have shown that β-glycosylthioureas always adopt a rigid *Z-anti* conformation at the anomeric carbon–NH–(C=S) segment.[23] Both the *Z* and *E* arrangements have been found at the NH–(C=S) bond in the case of 6-deoxy-6-thioureido sugars.[24] Similarly, β-(1→6)-linked pseudodisaccharides exhibit an equilibrium between the (*Z*,*Z*) and (*Z*,*E*) conformers in solution.[24b] In the case of **19**, a 1:0.8 relative proportion of both rotamers was found in deuterated methanol at 213 K. On the other hand, the coupling constant values between the methylene H-6^{I} protons and H-5^{I} in **19** (9.3 and 3.3 Hz) are indicative of a *gauche-trans* conformation about the C-5–C-6 bond, that is, an *anti* disposition between C-4 in the ring and the methylene carbon atom C-6 bearing the thiourea group. The low temperature (213–263 K) ^{1}H and ^{13}C NMR spectra of the hemiacetylated higher oligomers (**22** and **25**) prepared in this work, recorded in deuterated methanol, display a much higher complexity, indicative of the presence of several interconverting rotameric forms. The rotational behaviour of each thiourea group is probably independent of the presence of other thiourea segments in the oligomeric chain. The conformational equilibria in a *n*-mer can then be explained as the addition of the individual (*n* – 1) (*Z*,*Z*) and (*Z*,*E*) rotational probabilities (Figure 2).

In order to investigate the conformational properties of β-(1→3)-linked oligoglucosylthioureas, variable-temperature NMR spectra of the pseudodisaccharide **30** were first recorded in methanol. Two rotamers were detected at temperatures below ambient, which were assigned to the (*Z*,*Z*) and (*Z*,*E*) conformers, respectively (1:0.65 relative proportion), on the basis of diagnostic chemical-shift differences (Figure 3). Thus, protons located at carbons that are directly linked to the thiourea group are known to be low-field shifted for rotamers having a (*Z*)-configuration at the corresponding N–(C=S) bond as compared with the (*E*)-conformer.[23] In our case, the strong low-field shift for H-3 in the major (*Z*,*Z*)-rotamer ($\delta_{H\text{-}3}$ = 4.68 and 3.49 ppm for the (*Z*,*Z*)- and (*Z*,*E*)-rotamers, respectively) suggested a parallel arrangement with the thiocarbonyl sulfur atom, that is, an *anti* disposition with respect to the vicinal NH proton. This situation seems to be general for thiourea groups located at secondary carbons in pyranoses. As in the β-(1→6) series, the low-temperature NMR spectra for higher oligomers reflect the expected complexity for all possible combinations of (*Z*,*E*) and (*Z*,*Z*) configurations at every thiourea group in the chain. An identical situation was encountered for the dendritic derivatives **41** and **46**.

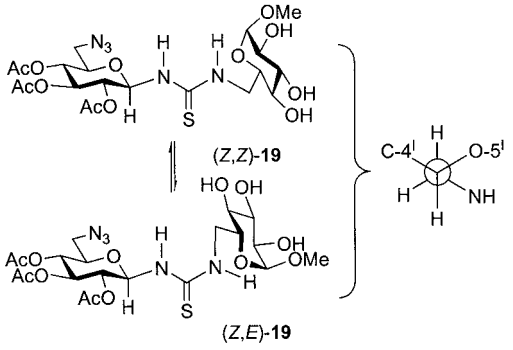


Figure 2. Rotameric equilibria for **19**. The *anti* conformation between the H-1^{II} and NH protons is shown, as well as the *gauche-trans* conformation above the C-5^{I}–C-6^{I} bond (Newman projection).

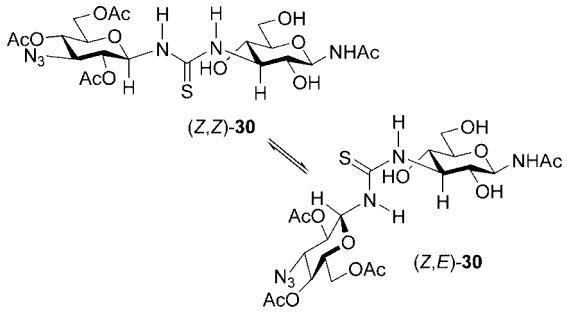


Figure 3. Rotameric equilibria for **30**. The *anti*-conformation between the NH protons and the vicinal H-3 ^{I} and H-1^{II} protons is shown.

Conclusions

An efficient methodology for the modular synthesis of oligomeric sugar thioureas has been developed. Both linear and dendritic architectures can be obtained in high yield from azidoisothiocyanate or diazidoisothiocyanate derivatives, respectively. It is noteworthy that these glycooligomers exhibit a restricted conformational flexibility, probably due to rotational constraints at the thiourea segments and intramolecular hydrogen bonds. Although the given examples are limited to β-D-glucopyranosyl monomers and regular arrangements, the molecular diversity can be easily increased by sequential incorporation of different building blocks. Every subunit in the chain may differ in the sugar configuration, anomeric stereochemistry, linking position

or branching pattern, therefore mimicking the impressive encoding capacity of oligosaccharides. The insertion of non-carbohydrate elements (peptidic, aromatic, aliphatic), by using the appropriate azidoisothiocyanates, could also be used to favour interactions with specific nucleic acid sequences. Moreover, the thiourea group can be transformed into other functionalities, such as urea, isothiourea or guanidine, by standard transformations.[8,25] This method should also be easily adaptable to solid-phase synthetic strategies and combinatorial approaches.[26]

Experimental Section

General Remarks: 6-Azido-6-deoxy-D-glucose[10] (**1**) was obtained from 6-*O*-tosyl-D-glucose[11] by nucleophilic displacement of tosylate by azide anion in *N*,*N*-dimethylformamide. 3-Azido-3-deoxy-D-glucose (**8**) was prepared from 3-azido-3-dideoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose by acid hydrolysis of the acetal groups, as reported previously.[16] 3,6-Diazido-3,6-dideoxy-D-glucose (**13**) was prepared from 3,6-diazido-3,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranose by acid hydrolysis of the acetal group.[17] Methyl 6-amino-6-deoxy-α-D-glucopyranoside (**18**) was obtained from commercial methyl α-D-glucopyranoside in three steps, by selective replacement of the primary hydroxy group by iodo, subsequent treatment with sodium azide and final reduction of the azido group, as described previously.[21a] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate[5] (**47**) was obtained from 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide according to literature procedures.[27] Optical rotations were measured at 22 °C in 1-cm or 1-dm tubes on a Perkin–Elmer 141 MC polarimeter. Infrared (IR) spectra were recorded on a Bomem Michelson MB-120 FTIR spectrophotometer. ^{1}H (and ^{13}C) NMR spectra were recorded at 300 (75.5) or 500 (125.7) MHz with Bruker 300 AMX, 500 AMX and 500 DRX spectrometers. In the case of the thiourea adducts, the spectra recorded at 298 K show broad signals due to slow rotation processes about the NH–C(S) bonds on the NMR timescale. Satisfactory resolutions were achieved after heating above 313 K. 1D TOCSY, 2D COSY, HMQC and HSQC experiments were used to assist with NMR assignments. Thin-layer chromatography (TLC) was carried out on aluminium sheets coated with Kieselgel 60 F254 (E. Merck), with visualisation by UV light or by charring with 10% H_2SO_4. Column chromatography was carried out with silica gel 60 (E. Merck, 230–400 mesh). Analytically pure samples of the fully unprotected thiourea-linked glycooligomers were obtained after gel-permeation chromatography (GPC) using Sephadex G-10 or G-25 and 1:1 MeOH/water as the eluent. FAB mass spectra were obtained with a Kratos MS-80 RFA instrument. The operating conditions were the following: the primary beam consisted of Xe atoms with a maximum energy of 8 keV; the samples were dissolved in thioglycerol, and the positive ions were separated and accelerated over a potential of 7 keV; NaI was added as cationizing agent. MALDI-TOF mass spectra were acquired on a GSG System spectrometer operating in the positive-ion mode with an accelerating voltage of 28 keV. Samples were dissolved in water at millimolar concentration and mixed with a standard solution of 2,5-dihydroxybenzoic acid (DHB; 10 mg mL^{-1} in 10% aq. EtOH, 2 μL) in 1:1 (v/v) relative proportions; 1 μL of the mixture was loaded onto the target plate, then allowed to air-dry at room temperature. Elemental analyses were performed at the Instituto de Investigaciones Químicas (Sevilla, Spain).

6-Azido-6-deoxy-*N*-(2,2-diethoxycarbonylvinyl)-β-D-glucopyranosylamine (4): NH_4HCO_3 (4.20 g, 53.2 mmol) was added to a solution of 6-azido-6-deoxy-D-glucose[10] (**1**; 4.88 g, 24 mmol) in aq. ammonia (16 M, 275 mL), and the mixture was stirred at 40 °C for 36 h. The solvent was concentrated to half volume under reduced pressure. Water (120 mL) was then added and the solution was freeze-dried. The crude carbamate salt **3** thus obtained was dissolved in dry MeOH (50 mL) and diethyl ethoxymethylenemalonate (7.8 mL, 36 mmol) was added. The mixture was stirred at room temperature for 48 h, then concentrated and the residue purified by column chromatography (EtOAc → 45:5:3 EtOAc/EtOH/H_2O) to give **4**. Yield: 5.57 g (62%); R_f = 0.55 (45:5:3 EtOAc/EtOH/H_2O). $[\alpha]_D$ = +9.0 (c = 0.9, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 9.50 (s, 1 H, =CH), 5.83 (d, J = 8.7 Hz, 1 H, H-1), 5.45, 5.73 (2 q, J = 7.2 Hz, 4 H, 2 CH_2), 4.89 (t, J = 8.7 Hz, 1 H, H-4), 4.87 (dd, J = 13.6, 4.8 Hz, 1 H, H-6a), 4.74 (dd, J = 13.6, 6.5 Hz, 1 H, H-6b), 4.71 (ddd, J = 8.7, 6.5, 4.8 Hz, 1 H, H-5), 4.64 (t, J = 8.7 Hz, 1 H, H-3), 4.61 (t, J = 8.7 Hz, 1 H, H-2), 2.60, 2.57 (2 t, J = 7.2 Hz, 6 H, 2 CH_3) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 175.5 (CO chelated), 173.7 (CO free), 165.5 (=CH), 99.1 (=C), 89.4 (C-1), 78.7 (C-4), 78.2 (C-5), 74.6 (C-2), 71.8 (C-3), 67.0 (2 CH_2CH_3), 52.5 (C-6), 20.7 (2 CH_2CH_3) ppm. FAB-MS: m/z = 397 [M + Na]$^+$, 375 [M + H]$^+$. $C_{14}H_{22}N_4O_8$ (374.35): calcd. C 44.92, H 5.92, N 14.97; found C 44.88, H 5.84, N 14.80.

2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-*N*-(2,2-diethoxycarbonylvinyl)-β-D-glucopyranosylamine (5): Conventional acetylation of **4** (4.6 g, 12.3 mmol) with 1:1 pyridine/acetic anhydride gave the corresponding triacetate **5**. Yield: 5.90 g (96%); R_f = 0.38 (1:1 EtOAc/petroleum ether). $[\alpha]_D$ = +4.7 (c = 0.42, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ = 3281, 2984, 2106, 1757, 1611, 1223, 1069 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.22 (dd, J = 13.1, 8.8 Hz, 1 H, NH), 7.94 (d, J = 13.1 Hz, 1 H, =CH), 5.28 (t, J = 9.5 Hz, 1 H, H-3), 5.06 (t, J = 9.5 Hz, 1 H, H-2), 5.03 (t, J = 9.5 Hz, 1 H, H-4), 4.57 (t, J = 9.5 Hz, 1 H, H-1), 4.25, 4.19 (2 q, J = 7.2 Hz, 4 H, 2 CH_2), 3.75 (ddd, J = 9.5, 5.0, 3.9 Hz, 1 H, H-5), 3.35 (m, 2 H, H-6a,b), 2.03, 2.02, 2.01 (3 s, 9 H, 3 MeCO), 1.32, 1.29 (2 t, J = 7.2 Hz, 6 H, 2 CH_3) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 169.9, 169.4, 169.2 (3 *C*O ester), 167.5 (CO chelated), 165.3 (CO free), 156.9 (=CH), 95.0 (=C), 86.7 (C-1), 74.8 (C-5), 72.2 (C-3), 70.3 (C-2), 69.0 (C-4), 60.2, 60.0 (2 CH_2), 50.7 (C-6), 20.4, 20.3 (3 *Me*CO), 14.2, 14.1 (2 CH_2CH_3) ppm. FAB-MS: m/z = 523 [M + Na]$^+$, 501 [M + H]$^+$. $C_{20}H_{28}N_4O_{11}$ (500.46): calcd. C 48.00, H 5.64, N 11.20; found C 47.96, H 5.63, N 11.19.

2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosylamine Hydrochloride (6): Enamine **5** (700 mg, 1.4 mmol) was dissolved in a saturated solution of Cl_2 in CH_2Cl_2 (15 mL) at 0 °C. After 5 min, the solution was concentrated, Et_2O (3 × 25 mL) was added and evaporated, and the solid residue was washed with Et_2O and filtered and dried to yield **6**. Yield: 500 mg (97%). $[\alpha]_D$ = +9.5 (c = 1.0, MeOH). IR (KBr): $\tilde{\nu}_{max}$ = 2990, 2108, 1751, 1242, 1215 1036 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 5.36 (t, J = 9.6 Hz, 1 H, H-3), 5.12 (t, J = 9.6 Hz, 1 H, H-2), 5.11 (t, J = 9.6 Hz, 1 H, H-4), 4.95 (d, J = 9.6 Hz, 1 H, H-1), 4.05 (ddd, J = 9.6, 5.5, 2.6 Hz, 1 H, H-5), 3.60 (dd, J = 8.3, 2.6 Hz, 1 H, H-6a), 3.43 (dd, J = 8.3, 5.5 Hz, 1 H, H-6b), 2.09, 2.02, 1.98 (3 s, 9 H, 3 MeCO) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 171.4, 171.3, 171.0 (3 CO), 80.7 (C-1), 76.5 (C-5), 73.7 (C-3), 71.5 (C-2), 69.7 (C-4), 51.8 (C-6), 20.6, 20.5, 20.4 (3 *Me*CO) ppm. FAB-MS: m/z = 389 [M + Na]$^+$, 353 [M – HCl + Na]$^+$ 331 [M – ClH]$^+$. $C_{12}H_{19}ClN_4O_7$ (366.77): calcd. C 39.30, H 5.22, N 30.54; found C 39.29, H 5.05, N 30.19.

2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl Isothiocyanate (7): $CSCl_2$ (239 μL, 1.5 equiv.) was added to a heterogeneous mixture of **6** (769 mg, 2.1 mmol) and $CaCO_3$ (615 mg, 6.15 mmol, 3 equiv.) in H_2O/CH_2Cl_2 (1:1, 20 mL). The mixture was stirred for

30 min in a round-bottomed flask provided with a system for evacuation of gases and diluted with CH_2Cl_2 (10 mL). The organic phase was separated, dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (1:9 → 1:3 EtOAc/petroleum ether) to give **7** (0.5 g, 64%) as an amorphous solid (37% overall yield from **1**); R_f = 0.62 (1:1 EtOAc/petroleum ether). $[\alpha]_D$ = +6.0 (c = 1.0, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ = 2942, 2880, 2106, 2031, 1761, 1236, 909 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.18 (t, J = 9.3 Hz, 1 H, H-3), 5.08 (t, J = 8.8 Hz, 1 H, H-2), 5.04 (t, J = 9.3 Hz, 1 H, H-4), 5.01 (d, J = 8.8 Hz, 1 H, H-1), 3.69 (dt, J = 9.3, 4.5 Hz, 1 H, H-5), 3.35 (m, 2 H, H-6), 2.09, 2.02, 2.00 (3 s, 9 H, 3 MeCO) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 170.1, 169.3, 169.1 (3 CO), 145.0 (NCS), 83.4 (C-1), 75.3 (C-5), 72.3 (C-3), 71.7 (C-2), 68.8 (C-5), 50.8 (C-6), 20.6 (3 *Me*CO) ppm. FAB-MS: m/z = 395 [M + Na]$^+$. $C_{13}H_{16}N_4O_7S$ (372.35): calcd. C 41.93, H 4.33, N 15.05; found C 41.94, H 4.21, N 15.07.

3-Azido-3-deoxy-*N*-(2,2-diethoxycarbonylvinyl)-β-D-glucopyranosylamine (9): NH_4HCO_3 (1.57 g, 19.6 mmol) was added to a solution of 3-azido-3-deoxy-D-glucose (**8**; 1.77 g, 4.90 mmol) in aq. ammonia (16 M, 56 mL), and the mixture was stirred at 40 °C for 36 h. The solvent was concentrated to half volume under reduced pressure. Water (24 mL) was then added and the solution was freeze-dried. The crude product was dissolved in dry MeOH (60 mL) and diethyl ethoxymethylenemalonate (6.06 mL, 29.4 mmol) was added. The mixture was stirred at 40 °C for 12 h, then concentrated and the residue purified by column chromatography (1:1 EtOAc/petroleum ether → EtOAc) to give **9**. Yield: 0.86 g (47%); R_f = 0.56 (EtOAc). $[\alpha]_D$ = –35 (c = 1.1, MeOH). UV (CH_2Cl_2): λ = 216, 275 nm (ε_{mM} = 13.0, 24.7). IR (NaCl): $\tilde{\nu}_{max}$ = 3426, 2982, 2909, 2108, 1665, 1613, 1246, 1098 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 8.14 (s, 1 H, =C*H*), 4.51 (d, J = 8.4 Hz, 1 H, H-1), 4.21, 4.17 (2 q, J = 7.0 Hz, 4 H, 2 CH_2CH$_3$), 3.84 (dd, J = 12.0, 2.4 Hz, 1 H, H-6a), 3.67 (dd, J = 12.0, 5.3 Hz, 1 H, H-6b), 3.44 (ddd, J = 8.5, 5.3, 2.4 Hz, 1 H, H-5), 3.36 (m, 1 H, H-2, H-3, H-4), 1.28, 1.27 (2 t, J = 7.0 Hz, 6 H, 2 CH$_2$CH_3) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 169.3 (CO chelated), 165.6 (CO free), 158.4 (=CH), 93.8 (=C), 91.0 (C-1), 81.4 (C-5), 74.3 (C-2), 72.6 (C-4), 70.7 (C-3), 63.1 (C-6), 61.9 (2 *C*H$_2$CH$_3$), 15.6 (2 CH$_2$*C*H$_3$) ppm. FAB-MS: m/z = 397 [M + Na]$^+$, 375 [M + H]$^+$, 329 [M – OEt]$^+$. $C_{14}H_{22}N_4O_8$ (374.35): calcd. C 44.92, H 5.92, N 14.97; found C 44.78, H 5.77, N 14.81.

2,4,6-Tri-*O*-acetyl-3-azido-3-deoxy-*N*-(2,2-diethoxycarbonylvinyl)-β-D-glucopyranosylamine (10): Conventional acetylation of **9** (0.59 g, 1.58 mmol) with 1:1 pyridine/acetic anhydride gave the corresponding triacetate **10**. Yield: 0.69 g (88%); R_f = 0.35 (1:1 EtOAc/petroleum ether). $[\alpha]_D$ = –31.5 (c = 1.0, CH_2Cl_2). UV (CH_2Cl_2): λ = 274 nm (ε_{mM} = 24.1). IR (NaCl): $\tilde{\nu}_{max}$ = 2982, 2915, 2108, 1753, 1613, 1381, 1227, 1065 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.14 (dd, J = 12.9, 9.0 Hz, 1 H, NH), 7.86 (s, 1 H, =CH), 4.92 (t, J = 9.8 Hz, 1 H, H-4), 4.91 (dd, J = 9.0, 9.8 Hz, 1 H, H-2), 4.46 (t, J = 9.0 Hz, 1 H, H-1), 4.19 (d, J = 12.5 Hz, 1 H, H-6a), 4.19, 4.14 (2 q, J = 7.0 Hz, 4 H, 2 CH_2CH$_3$), 4.02 (dd, J = 12.5, 2.2 Hz, 1 H, H-6b), 3.70 (t, J = 9.8 Hz, 1 H, H-3), 3.70 (m, 1 H, H-5), 2.09, 2.07, 2.05 (3 s, 9 H, 3 MeCO), 1.28, 1.26 (2 t, J = 7.0 Hz, 6 H, 2 CH$_2$CH_3) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 170.6, 169.4, 169.1 (3 CO ester), 167.5 (CO chelated), 165.6 (CO free), 157.4 (=CH), 95.0 (=C), 87.3 (C-1), 74.4 (C-5), 70.7 (C-2), 67.9 (C-4), 64.3 (C-3), 61.6 (C-6), 60.4, 60.2 (2 *C*H$_2$CH$_3$), 20.7, 20.6, 20.5 (3 *Me*CO), 14.3, 14.2 (2 CH$_2$*C*H$_3$) ppm. FAB-MS: m/z = 523 [M + Na]$^+$, 501 [M + H]$^+$, 455 [M – OEt]$^+$. $C_{20}H_{28}N_4O_{11}$ (500.46): calcd. C 48.00, H 5.64, N 11.20; found C 47.95, H 5.25, N 11.09.

2,4,6-Tri-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranosylamine Hydrochloride (11): Enamine **10** (0.69 g, 1.38 mmol) was dissolved in a saturated solution of Cl_2 in CH_2Cl_2 (22 mL) at 0 °C. After 30 min, the solution was concentrated, Et_2O (3 × 20 mL) was added and evaporated, and the solid residue was washed with Et_2O, filtered and dried to yield **11**. Yield: 0.51 g (100%). $[\alpha]_D$ = +12.1 (c = 0.77, MeOH). IR (KBr): $\tilde{\nu}_{max}$ = 3455, 2843, 2114, 1755, 1375, 1209, 1036 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 4.97 (t, J = 9.6 Hz, 1 H, H-4), 4.95 (t, J = 9.6 Hz, 1 H, H-2), 4.87 (m, 1 H, H-1), 4.26 (dd, J = 12.7, 4.9 Hz, 1 H, H-6a), 4.15 (dd, J = 12.7, 4.9 Hz, 1 H, H-6b), 4.09 (t, J = 9.6 Hz, 1 H, H-3), 4.09 (m, 1 H, H-5), 2.17, 2.12, 2.02 (3 s, 9 H, 3 MeCO) ppm. ^{13}C NMR (75.5 MHz, CD_3OD): δ = 172.0, 171.4, 170.9 (3 CO), 80.9 (C-1), 76.3 (C-5), 71.6 (C-2), 68.9 (C-4), 64.9 (C-3), 62.6 (C-6), 20.7, 20.5 (3 *Me*CO) ppm. FAB-MS: m/z = 331 [M – Cl]$^+$. $C_{12}H_{19}ClN_4O_7$ (366.75): calcd. C 39.30; H 5.22, N 15.28; found C 39.46, H 5.24, N 15.52.

2,4,6-Tri-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranosyl Isothiocyanate (12): $CSCl_2$ (0.108 mL, 1.5 equiv.) was added to a heterogeneous mixture of **11** (506 mg, 1.38 mmol) and $CaCO_3$ (0.41 g, 4.14 mmol, 3 equiv.) in H_2O/CH_2Cl_2 (1:1, 40 mL). The mixture was stirred for 4 h in a round-bottomed flask provided with a system for evacuation of gases and diluted with CH_2Cl_2 (15 mL). The organic phase was separated, dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (1:3 → 1:1 EtOAc/petroleum ether) to give **12** (0.26 g, 50%) as an amorphous solid (21% overall yield from **8**); R_f = 0.42 (1:1 EtOAc/petroleum ether). $[\alpha]_D$ = –15.0 (c = 0.8, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ = 2963, 2893, 2108, 2025, 1753, 1377, 1215, 1099, 1051 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.00 (t, J = 9.9 Hz, 1 H, H-2), 4.97 (t, J = 9.9 Hz, 1 H, H-4), 4.92 (d, J = 9.9 Hz, 1 H, H-1), 4.17 (dd, J = 12.5, 5.0 Hz, 1 H, H-6a), 4.10 (dd, J = 12.5, 2.4 Hz, 1 H, H-6b), 3.66 (ddd, J = 9.9, 5.0, 2.4 Hz, 1 H, H-5), 3.61 (t, J = 9.9 Hz, 1 H, H-3), 2.17, 2.11, 2.09 (3 s, 9 H, 3 MeCO) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 170.6, 169.0, 168.9 (3 CO), 144.5 (NCS), 83.7 (C-1), 74.8 (C-5), 71.6 (C-2), 67.7 (C-4), 64.1 (C-3), 61.5 (C-6), 20.7, 20.6 (3 *Me*CO) ppm. FAB-MS: m/z = 503 [M + Na + thioglycerol]$^+$. $C_{13}H_{16}N_4O_7S$ (372.35): calcd. C 41.93, H 4.33, N 15.05; found C 41.86, H 4.31, N 14.1.

3,6-Diazido-3,6-dideoxy-*N*-(2,2-diethoxycarbonylvinyl)-β-D-glucopyranosylamine (14): NH_4HCO_3 (0,76 g, 9.66 mmol) was added to a solution of **13** (1 g, 4.36 mmol) in aq. ammonia (16 M, 50 mL) and the mixture was stirred at 40 °C for 36 h. The solvent was concentrated to half volume under reduced pressure. Water (20 mL) was then added and the solution was freeze-dried. The crude product thus obtained was dissolved in dry MeOH (20 mL) and diethyl ethoxymethylenemalonate (1.00 mL, 4.80 mmol) was added. The mixture was stirred at 40 °C for 12 h, then concentrated and the residue purified by column chromatography (1:2 EtOAc/petroleum ether) to give **14** as a white foam; Yield: 0.90 g (52%); R_f = 0.15 (1:2 EtOAc/petroleum ether). $[\alpha]_D$ = –39 (c = 0.9, CH_2Cl_2). UV (CH_2Cl_2): λ = 274 nm (ε_{mM} = 22.1). IR (KBr): $\tilde{\nu}_{max}$ = 3403, 2982, 2926, 2106, 1670, 1611, 1408, 1384, 1273, 1248, 1098, 1074 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 8.17 (s, 1 H, =C*H*), 4.75 (d, J = 8.5 Hz, 1 H, H-1), 4.22, 4.15 (2 q, J = 6.9 Hz, 4 H, 2 CH_2CH$_3$), 3.63 (ddd, J = 8.5, 6.0, 2.5 Hz, 1 H, H-5), 3.56 (dd, J = 13.5, 2.5 Hz, 1 H, H-6a), 3.43 (dd, J = 13.5, 6.0 Hz, 1 H, H-6b), 3.38 (t, J = 8.5 Hz, 1 H, H-3), 3.36 (t, J = 8.5 Hz, 1 H, H-4), 3.24 (t, J = 8.5 Hz, 1 H, H-2), 1.29, 1.27 (2 t, J = 6.9 Hz,6 H, CH$_2$CH_3) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 169.4 (CO chelated), 167.6 (CO free), 159.5 (=CH), 93.2 (=C), 89.7 (C-1), 79.2 (C-5), 73.4 (C-2), 71.5 (C-4), 70.6 (C-3), 61.1 (2 *C*H$_2$CH$_3$), 52.3 (C-6), 14.7 (2 CH$_2$*C*H$_3$) ppm. FAB-MS: m/z = 422 [M + Na]$^+$, 400 [M + H]$^+$, 354 [M – OEt]$^+$. $C_{14}H_{21}N_7O_7$ (399.36): calcd. C 42.10, H 5.30, N 24.55; found C 42.12, H 5.22, N 24.69.

2,4-Di-*O*-acetyl-3,6-diazido-3,6-dideoxy-*N*-(2,2-diethoxycarbonylvinyl)-β-D-glucopyranosylamine (15): Conventional acetylation of **14** (0.45 g, 1.13 mmol) with 1:1 pyridine/acetic anhydride followed by column chromatography (1:1 EtOAc/petroleum ether) gave the corresponding diacetate **15**. Yield: 0.48 g (89%); R_f = 0.34 (1:1 EtOAc/petroleum ether). $[\alpha]_D$ = –20 (c = 1.0, CH_2Cl_2). UV (CH_2Cl_2): λ = 274 nm (ε_{mM} = 15.5). IR (KBr): $\tilde{\nu}_{max}$ = 3298, 2984, 2934, 2108, 1755, 1665, 1613, 1408, 1379, 1221, 1098, 1067 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 9.18 (dd, J = 13.2, 8.5 Hz, 1 H, NH) 8.17 (s, 1 H, =C*H*), 4.92 (dd, J = 10.2, 8.5 Hz, 1 H, H-2), 4.87 (t, J = 10.2 Hz, 1 H, H-4), 4.49 (t, J = 8.5 Hz, 1 H, H-1), 4.23, 4.17 (2 q, J = 6.9 Hz, 4 H, 2 CH_2CH$_3$), 3.67 (ddd, J = 10.2, 6.0, 3.3 Hz, 1 H, H-5), 3.34 (dd, J = 13.5, 6.0 Hz, 1 H, H-6a), 3.28 (dd, J = 13.5, 3.3 Hz, 1 H, H-6b), 2.12, 2.09 (s, 6 H, 2 COCH_3), 1.29, 1.26 (2 t, J = 6.9 Hz, 6 H, 2 CH$_2$CH_3) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 169.4, 169.2 (2 CO ester), 167.5 (CO chelated), 165.6 (CO free), 157.1 (=CH), 95.3 (=C), 87.0 (C-1), 75.7 (C-5), 70.7 (C-2), 69.2 (C-4), 64.2 (C-3), 60.3, 60.5 (2 CH_2CH_3), 50.8 (C-6), 20.7, 20.5 (2 *Me*CO), 14.4, 14.2 (2 CH_2CH_3) ppm. FAB-MS: m/z = 523 [M + Na]$^+$, 484 [M + H]$^+$, 438 [M – OEt]$^+$. $C_{18}H_{25}N_7O_9$ (483.43): calcd. C 44.72, H 5.21, N 20.28; found C 44.53, H 5.18, N 20.02.

2,4-Di-*O*-acetyl-3,6-diazido-3,6-dideoxy-β-D-glucopyranosylamine Hydrochloride (16): Enamine **15** (0.45 g, 0.94 mmol) was dissolved in a saturated solution of Cl_2 in CH_2Cl_2 (15 mL) at 0 °C. After 30 min at 5 °C, the solvent was concentrated, Et_2O (3 × 20 mL) was added and evaporated, and the solid residue was washed with Et_2O, filtered and dried to yield **16** as an amorphous solid. Yield: 0.32 g (97%). $[\alpha]_D$ = +37 (c = 1.1, MeOH). IR (KBr): $\tilde{\nu}_{max}$ = 3154, 3048, 2855, 2814, 2106, 1744, 1408, 1223, 1038 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ = 4.95 (t, J = 10.0 Hz, 1 H, H-4), 4.94 (dd, J = 10.0, 8.5 Hz, 1 H, H-2), 4.85 (d, J = 8.5 Hz, 1 H, H-1), 4.64 (br. s, 3 H, NH_3), 4.02 (t, J = 10.0 Hz, 1 H, H-3), 3.97 (ddd, J = 10.0, 6.0, 3.0 Hz, 1 H, H-5), 3.56 (dd, J = 14.0, 3.0 Hz, 1 H, H-6a), 3.41 (dd, J = 14.0, 6.0 Hz, 1 H, H-6b), 2.18, 2.13 (s, 6 H, 2 COCH_3) ppm. ^{13}C NMR (75.5 MHz, CD_3OD): δ = 171.5, 171.0 (2 *C*OCH$_3$), 80.8 (C-1), 76.9 (C-5), 71.4 (C-2), 69.7 (C-4), 64.9 (C-3), 51.7 (C-6), 20.9, 20.6 (2 CO*C*H$_3$) ppm. FAB-MS: m/z = 314 [M – Cl]$^+$. $C_{10}H_{16}ClN_7O_5$ (349.73): calcd. C 34.34, H 4.61, Cl 10.14, N 28.04; found C 34.28, H 4.50, N 27.63.

2,4-Di-*O*-acetyl-3,6-diazido-3,6-dideoxy-β-D-glucopyranosyl Isothiocyanate (17): $CSCl_2$ (107 μL, 1.5 equiv.) was added to a heterogeneous mixture of **17** (330 mg, 0.94 2.1 mmol) and $CaCO_3$ (282 mg, 2.82 mmol, 3 equiv.) in H_2O/CH_2Cl_2 (1:1, 9 mL). The mixture was stirred for 30 min in a round-bottomed flask provided with a system for evacuation of gases and diluted with CH_2Cl_2 (10 mL). The organic phase was separated, dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (1:2 EtOAc/petroleum ether) to give **17** (0.20 g, 61%) as an amorphous solid (46% overall yield from **13**); R_f = 0.69 (1:2 EtOAc/petroleum ether). $[\alpha]_D$ = +9 (c = 1.2, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ = 2926, 2855, 2106, 2023, 1755, 1373, 1213, 1098, 1036 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 4.99 (dd, J = 9.6, 8.7 Hz, 1 H, H-2), 4.93 (d, J = 8.7 Hz, 1 H, H-1), 4.90 (t, J = 9.6 Hz, 1 H, H-4), 3.72 (t, J = 9.6 Hz, 1 H, H-3), 3.63 (ddd, J = 9.6, 6.3, 3.3 Hz, 1 H, H-5), 3.61 (t, J = 9.6 Hz, 1 H, H-3), 3.36 (dd, J = 13.5, 6.3 Hz, 1 H, H-6a), 3.29 (dd, J = 13.5, 3.3 Hz, 1 H, H-6b), 2.19, 2.12 (2 s, 6 H, 2 MeCO) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 169.1, 168.8 (2 CO), 145.1 (NCS), 83.6 (C-1), 76.1 (C-5), 71.6 (C-2), 69.0 (C-4), 64.0 (C-3), 50.8 (C-6), 20.6 (2 *Me*CO) ppm. FAB-MS: m/z = 378 [M + Na]$^+$. $C_{11}H_{13}N_7O_5S$ (355.33): calcd. C 37.18, H 3.69, N 27.59; found C 37.15, H 3.70, N 27.54.

Methyl 6-Deoxy-6-[*N*′-(2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl)thioureido]-α-D-glucopyranoside (19): Compound **19** was obtained by a coupling reaction of amine **18** (83 mg, 0.43 mmol) and isothiocyanate **7** (176 mg, 0.47 mmol, 1.1 equiv.) in pyridine (4 mL) at room temperature for 5 h, followed by column chromatography (EtOAc → 45:5:3 EtOAc/EtOH/H_2O). Yield: 241 mg (98%); R_f = 0.50 (45:5:3 EtOAc/EtOH/H_2O). $[\alpha]_D$ = +6.2 (c = 1.0, CH_2Cl_2). UV (CH_2Cl_2): λ = 253 nm (ε_{mM} = 15.6). IR (KBr): $\tilde{\nu}_{max}$ = 3357, 2920, 2117, 1752, 1565, 1450, 1240, 1093 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 50 °C): δ = 7.11 (br. s, 2 H, NH^I, NH^{II}), 5.77 (t, J = 9.5 Hz, 1 H, H-1^{II}), 5.32 (t, J = 9.5 Hz, 1 H, H-3^{II}), 5.05 (t, J = 9.5 Hz, 1 H, H-2^{II}), 4.99 (t, J = 9.5 Hz, 1 H, H-4^{II}), 4.74 (d, J = 3.3 Hz, 1 H, H-1^I), 3.82 (ddd, J = 9.5, 5.5, 2.9 Hz, 1 H, H-5^{II}), 3.72 (t, J = 9.3 Hz, 1 H, H-3^I), 3.65 (dt, J = 9.3, 3.3 Hz, 1 H, H-5^I), 3.49 (dd, J = 9.3, 3.3 Hz, 1 H, H-2^I), 3.49 (dd, J = 13.5, 2.9 Hz, 1 H, H-6^{II}a), 3.48 (m, 2 H, H-6^I), 3.40 (s, 3 H, OMe), 3.35 (m, 1 H, H-6^{II}b), 3.25 (br. t, J = 9.3 Hz, 1 H, H-4^I), 2.07, 2.06, 2.02 (3 s, 9 H, 3 MeCO) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 184.7 (CS), 171.1, 169.7, 169.5 (3 CO), 99.7 (C-1^I), 83.0 (C-1^{II}), 74.2 (C-5^{II}), 73.6 (C-3^I), 72.9 (C-3^{II}), 72.2 (C-2^I), 70.8 (C-4^{II}), 70.7 (C-5^I), 70.4 (C-4^I), 69.6 (C-2^{II}), 55.5 (OMe), 50.9 (C-6^{II}), 45.1 (C-6^I), 20.7, 20.5, 20.4 (3 *Me*CO) ppm. FAB-MS: m/z = 588 [M + Na]$^+$, 566 [M + H]$^+$. $C_{20}H_{31}N_5O_{12}S$ (565.55): calcd. C 42.47, H 5.53, N 12.38; found C 42.43, H 5.44, N 12.27.

Methyl 6-Deoxy-6-[*N*′-(6-azido-6-deoxy-β-D-glucopyranosyl)thioureido]-α-D-glucopyranoside (20): Deacetylation of the hemiacetylated pseudodisaccharide adduct **19** (44 mg, 0.078 mmol) was effected in methanol (5 mL) by treatment with NaOMe (0.1 equiv. per mol of acetate) for 2 h. The reaction mixture was neutralised with Amberlite IRA 120 (H^+) ion-exchange resin, filtered and concentrated to give **20**. Yield: 34.4 mg (99%); R_f 0.13 (45:5:3 EtOAc/EtOH/H_2O); R_f = 0.63 (6:3:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = +3.2 (c = 1.0, CH_2Cl_2). UV (MeOH): λ = 245 nm (ε_{mM} = 16.1). IR (KBr): $\tilde{\nu}_{max}$ = 3364, 2920, 2117, 1561, 1093 cm^{-1}. ^1H NMR (500 MHz, D_2O, 60 °C): δ = 5.60 (d, J = 9.3 Hz, 1 H, H-1^{II}), 5.08 (d, J = 3.3 Hz, 1 H, H-1^I), 4.29 (m, 1 H, H-6^{I}a), 4.06 (m, 1 H, H-5^I), 4.02 (m, 1 H, H-6^{I}b), 3.95 (dd, J = 13.6, 2.7 Hz, 1 H, H-6^{II}a), 3.94 (t, J = 9.8 Hz, 1 H, H-3^I), 3.93 (ddd, J = 9.4, 5.5, 2.7 Hz, 1 H, H-5^{II}), 3.84 (dd, J = 9.8, 3.3 Hz, 1 H, H-2^I), 3.84 (t, J = 9.4 Hz, 1 H, H-3^{II}), 3.78 (dd, J = 13.6, 5.5 Hz, 1 H, H-6^{II}b), 3.74 (t, J = 9.4 Hz, 1 H, H-2^{II}), 3.70 (t, J = 9.4 Hz, 1 H,H-4^{II}), 3.60 (dd, J = 10.0, 9.8 Hz, 1 H, H-4^I), 3.59 (s, 3 H, OMe) ppm. ^{13}C NMR (125.7 MHz, D_2O, 60 °C): δ = 183.9 (CS), 99.8 (C-1^I), 84.0 (C-1^{II}), 76.8 (C-3^{II}), 76.3 (C-5^{II}), 73.4 (C-3^I), 72.5 (C-2^{II}), 71.8 (C-2^I), 71.7 (C-4^I), 70.7 (C-4^{II}), 70.3 (C-5^I), 55.6 (OMe), 51.4 (C-6^{II}), 45.8 (C-6) ppm. FAB-MS: m/z = 462 [M + Na]$^+$, 440 [M + H]$^+$. $C_{14}H_{25}N_5O_9S$ (439.44): calcd. C 38.26, H 5.73, N 15.94; found C 38.15, H 5.59, N 15.70.

Methyl 6-Deoxy-6-[*N*′-(6-amino-6-deoxy-β-D-glucopyranosyl)thioureido]-α-D-glucopyranoside (21): 1,3-Propanedithiol (26 μL, 2 equiv.) and triethylamine (46 μL, 2 equiv.) were added to a solution of **19** (68 mg, 0.156 mmol) in methanol (2 mL) under Ar. The reaction was stirred at room temperature for 16 h, diluted with water (10 mL) and extracted with CH_2Cl_2 (2 × 5 mL). Freeze-drying of the aqueous solution afforded **21** as a white foam. Yield: 60 mg (94%). $[\alpha]_D$ = +21.3 (c = 0.7, MeOH). ^1H NMR (500 MHz, D_2O, 60 °C): δ = 5.59 (br. s, J = 9.7 Hz, 1 H, H-1^{II}), 5.06 (d, J = 4.0 Hz, 1 H, H-1^I), 4.23 (m, 1 H, H-6^{I}a), 4.05 (m, 2 H, H-5^I, H-6^{I}b), 3.93 (dd, J = 10.1, 9.2 Hz, 1 H, H-3^I), 3.83 (dd, J = 10.1, 4.0 Hz, 1 H, H-2^I), 3.83 (t, J = 9.7 Hz, 1 H, H-3^{II}), 3.77 (m, 1 H, H-5^{II}), 3.73 (t, J = 9.7 Hz, 1 H, H-2^{II}), 3.63 (s, 3 H, OMe), 3.60 (t, J = 9.7 Hz, 1 H, H-4^{II}), 3.59 (dd, J = 10.1, 9.2 Hz, 1 H, H-4^I), 3.38 (dd, J = 13.8, 2.8 Hz, 1 H, H-6^{II}a), 3.07 (dd, J = 13.8, 7.5 Hz, 1 H, H-6^{II}b) ppm. ^{13}C NMR (125.7 MHz, D_2O, 60 °C): δ = 183.2 (CS), 99.8 (C-1^I), 84.1 (C-1^{II}), 77.2 (C-2^I), 77.0 (C-5^{II}), 73.4 (C-3^I), 72.7 (C-2^{II}), 71.8 (C-3^{II}), 71.6 (C-4^I, C-4^{II}), 70.3 (C-5^I), 55.6 (OMe), 45.7 (C-6^I),

41.9 (C-6^{II}) ppm. FAB-MS: *m/z* = 436 [M + Na]$^+$. $C_{14}H_{27}N_5O_9S$ (413.5): calcd. C 40.67, H 6.38, N 10.16; found C 40.72, H 6.56, N 10.14.

Methyl 6-Deoxy-6-[*N*′-{6-deoxy-6-[*N*′-(2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl)thioureido]-6-deoxy-β-D-glucopyranosyl}thioureido]-α-D-glucopyranoside (22): Compound **22** was obtained by a coupling reaction of **21** (42.5 mg, 0.10 mmol) and **7** (45 mg, 0.121 mmol) in pyridine (3 mL) for 16 h, followed by column chromatography (EtOAc → 45:5:3 EtOAc/EtOH/H_2O). Yield: 85 mg (89%); R_f = 0.13 (45:5:3 EtOAc/EtOH/H_2O). $[\alpha]_D$ = +9.3 (*c* = 0.46, pyridine). ^{1}H NMR (500 MHz, D_2O, 70 °C): δ = 6.12 (d, *J* = 9.2 Hz, 1 H, H-1^{III}), 5.78 (t, *J* = 9.5 Hz, 1 H, H-3^{III}), 5.69 (d, *J* = 8.1 Hz, 1 H, H-1^{II}), 5.48 (t, *J* = 9.5 Hz, 1 H, H-2^{III}), 5.47 (t, *J* = 9.5 Hz, 1 H, H-4^{III}), 5.18 (d, *J* = 3.8 Hz, 1 H, H-1^{I}), 4.43 (ddd, *J* = 9.5, 5.5, 2.8 Hz, 1 H, H-5^{III}), 4.29 (m, 1 H, H-6^{II}a), 4.22 (m, 1 H, H-6^{I}a), 4.15 (m, 2 H, H-5^{I}, H-6^{I}b), 4.04 (t, *J* = 9.6 Hz, 1 H, H-3^{I}), 4.00 (m, 2 H, H-5^{II},6^{II}b), 3.96 (dd, *J* = 13.9, 2.8 Hz, 1 H, H-6^{III}a), 3.94 (dd, *J* = 9.6, 3.8 Hz, 1 H, H-2^{I}), 3.94 (t, *J* = 9.4 Hz, 1 H, H-3^{II}), 3.84 (t, *J* = 9.4 Hz, 1 H, H-2^{II}), 3.82 (dd, *J* = 13.9, 5.5 Hz, 1 H, H-6^{III}b), 3.77 (s, 3 H, OMe), 3.71 (t, *J* = 9.6 Hz, 1 H, H-4^{I}), 3.69 (t, *J* = 9.4 Hz, 1 H, H-4^{II}), 2.49, 2.48, 2.46 (3 s, 9 H, 3 MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 60 °C): δ = 183.5 (2 CS), 173.3, 173.2, 173.0 (3 CO), 99.8 (C-1^{I}), 84.1 (C-1^{II}), 82.0 (C-1^{III}), 76.7 (C-2^{I}), 74.0 (C-5^{II},C-5^{III}), 73.9 (C-3^{III}), 73.3 (C-3^{I}), 72.6 (C-2^{II}), 71.7 (C-3^{II}), 71.6 (C-4^{I}), 71.1 (C-4^{II}), 70.3 (C-5^{I}, C-4^{III}), 69.7 (C-2^{III}), 55.6 (OMe), 50.7 (C-6^{III}), 45.7 (C-6^{I}, C-6^{II}) 20.4 (3 *Me*CO) ppm. FAB-MS: *m/z* (%) = 808 (100) [M + Na]$^+$, 786 (25) [M + H]$^+$. $C_{27}H_{43}N_7O_{16}S$ (785.83): calcd. C 41.27, H 5.52, N 12.48; found C 40.90, H 5.52, N 8.16.

Methyl 6-Deoxy-6-[*N*′-{6-deoxy-6-[*N*′-(6-azido-6-deoxy-β-D-glucopyranosyl)thioureido]-6-deoxy-β-D-glucopyranosyl}thioureido]-α-D-glucopyranoside (23): Conventional Zemplén deacetylation of the hemiacetylated pseudotrisaccharide adduct **22** (50 mg, 0.063 mmol), as described above for the preparation of **20**, afforded **23**. Yield: 34 mg (80%); R_f = 0.64 (6:3:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = –12.0 (*c* = 1.0, H_2O). UV (H_2O): λ = 240 nm (ε_{mM} = 21.5). ^{1}H NMR (500 MHz, D_2O, 343 K): δ = 5.77 (d, *J* = 9.0 Hz, 1 H, H-1^{II}), 5.72 (d, *J* = 9.0 Hz, 1 H, H-1^{III}), 5.27 (d, *J* = 3.5 Hz, 1 H, H-1^{I}), 4.54 (m, 1 H, H-6^{II}a), 4.42 (m, 1 H, H-6^{I}a), 4.24 (m, 2 H, H-5^{I}, H-6^{I}b), 4.17 (ddd, *J* = 9.5, 6.0, 2.0 Hz, 1 H, H-5^{III}), 4.13 (t, *J* = 9.0 Hz, 1 H, H-3^{I}), 4.11 (m, 1 H, H-5^{II}), 4.10 (dd, *J* = 13.5, 2.0 Hz, 1 H, H-6^{III}a), 4.08 (m, 1 H, H-6^{II}b), 4.05 (t, *J* = 9.0 Hz, 1 H, H-3^{III}), 4.04 (dd, *J* = 9.0, 3.5 Hz, 1 H, H-2^{I}), 4.04 (t, *J* = 9.0 Hz, 1 H, H-3^{II}), 3.97 (dd, *J* = 13.5, 6.0 Hz, 1 H, H-6^{III}b), 3.94 (t, *J* = 9.0 Hz, 1 H, H-2^{II}), 3.92 (t, *J* = 9.0 Hz, 1 H, H-2^{III}), 3.88 (t, *J* = 9.0 Hz, 1 H, H-4^{III}), 3.82 (t, *J* = 9.0 Hz, 1 H, H-4^{II}), 3.79 (dd, *J* = 9.5, 9.0 Hz, 1 H, H-4^{I}), 3.54 (s, 3 H, OMe) ppm. ^{13}C NMR (125.7 MHz, D_2O, 80 °C): δ = 183.7 (2 CS), 99.9 (C-1^{I}), 84.3 (C-1^{II}), 83.9 (C-1^{III}), 77.0 (C-3^{II}), 76.9 (C-3^{III}), 76.5 (C-5^{III}), 75.8 (C-5^{II}), 73.5 (C-3^{I}), 72.7 (C-2^{II}), 72.6 (C-2^{III}), 71.9 (C-2^{I}, C-4^{I}), 71.6 (C-4^{II}), 70.9 (C-4^{III}), 70.5 (C-5^{I}), 55.8 (OMe), 51.6 (C-6^{III}), 46.4 (C-6^{II}), 45.8 (C-6^{I}) ppm. FAB-MS: *m/z* = 682 [M + Na]$^+$, 660 [M + H]$^+$. $C_{21}H_{37}N_7O_{13}S$ (659.71): calcd. C 38.23, H 5.65, N 14.86; found C 38.14, H 5.60, N 14.58.

Methyl 6-Deoxy-6-[*N*′-{6-deoxy-6-[*N*′-(6-amino-6-deoxy-β-D-glucopyranosyl)thioureido]-6-deoxy-β-D-glucopyranosyl}thioureido]-α-D-glucopyranoside (24): Reduction of azide **23** (100 mg, 0.15 mmol) in methanol (2 mL) with 1,3-propanedithiol (23 μL, 2 equiv.) and triethylamine (45 μL, 2 equiv.), as described above for the preparation of **21**, afforded **24** as a white foam that was used directly in the next coupling reaction without further purification. Yield: 83.5 mg (87%); R_f = 0.22 (6:3:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = +3.0 (*c* = 1.0, H_2O). UV (H_2O): λ = 245 nm (ε_{mM} = 69.0). ^{13}C NMR (125.7 MHz, D_2O, 80 °C): δ = 183.0 (2 CS), 100.0 (C-1^{I}), 84.5 (C-1^{II}, C-1^{III}), 77.1 (C-5^{III}), 77.0 (C-2^{I}, C-3^{III}), 76.1 (C-5^{II}), 73.7 (C-3^{I}), 72.8 (C-2^{II}, C-2^{III}), 71.9, 72.0 (C-3^{II}, C-4^{III},C-4^{I}, C-4^{II}), 70.5 (C-5^{I}), 55.9 (OMe), 46.5 (C-6^{I}, C-6^{II}), 41.7 (C-6^{III}) ppm. $C_{21}H_{39}N_5O_{13}S_2$ (633.71): calcd. C 39.80, H 6,20, N 11.05; found C 39.75, H 5.97, N 10.89.

Methyl 6-Deoxy-6-[*N*′-{6-deoxy-6-[*N*′-{6-deoxy-6-[*N*′-(2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosyl}thioureido]-β-D-glucopyranosyl}thioureido]-α-D-glucopyranoside (25): Compound **25** was obtained by a coupling reaction of **24** (86 mg, 0.137 mmol) and **7** (56 mg, 0.151 mmol) in pyridine (5 mL) for 24 h, followed by column chromatography (10:1 MeCN/H_2O). Yield: 98 mg (71%); R_f = 0.66 (6:3:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = 0 (*c* = 1.0, H_2O). UV (H_2O): λ = 245 nm (ε_{mM} = 33.0). ^{1}H NMR (500 MHz, D_2O, 60 °C): δ = 6.05 (br. d, *J* = 8.5 Hz, 1 H, H-1^{IV}), 5.72 (t, *J* = 9.5 Hz, 1 H, H-3^{IV}), 5.64 (br. d, *J* = 9.5 Hz, 1 H, H-1^{II}), 5.57 (br. d, *J* = 9.4 Hz, 1 H, H-1^{III}), 5.42 (dd, *J* = 9.5, 8.5 Hz, 1 H, H-2^{IV}), 5.41 (t, *J* = 9.5 Hz, 1 H, H-4^{IV}), 5.11 (d, *J* = 4.0 Hz, 1 H, H-1^{I}), 4.36 (m, 1 H, H-6^{III}a), 4.35 (ddd, *J* = 9.5, 5.0, 2.0 Hz, 1 H, H-5^{IV}), 4.34 (m, 1 H, H-6^{II}a), 4.22 (m, 1 H, H-6^{I}a), 4.07 (m, 2 H, H-5^{I}, H-6^{I}b), 3.96 (t, *J* = 9.5 Hz, 1 H, H-3^{I}), 3.95 (m, 2 H, H-5^{II}, H-6^{II}b), 3.93 (m, 2 H, H-5^{III},6^{III}b), 3.89 (dd, *J* = 14.0, 2.0 Hz, 1 H, H-6^{IV}a), 3.88 (t, *J* = 9.5 Hz, 1 H, H-3^{II}), 3.87 (t, *J* = 9.4 Hz, 1 H, H-3^{III}), 3.85 (dd, *J* = 9.5, 4.0 Hz, 1 H, H-2^{I}), 3.77 (t, *J* = 9.5 Hz, 1 H, H-2^{II}), 3.75 (dd, *J* = 14.0, 5.0 Hz, 1 H, H-6^{IV}b), 3.74 (t, *J* = 9.4 Hz, 1 H, H-2^{III}), 3.72 (s, 3 H, OMe), 3.66 (t, *J* = 9.5 Hz, 1 H, H-4^{II}), 3.60 (t, *J* = 9.5 Hz, 1 H, H-4^{I}), 3.60 (t, *J* = 9.4 Hz, 1 H, H-4^{III}), 2.39, 2.40, 2.42, (3 s, 9 H, 3 MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 60 °C): δ = 183.9, 183.7, 183.6 (3 CS), 173.3, 173.1, 172.9 (3 CO), 99.8 (C-1^{I}), 84.3 (C-1^{II}, C-1^{III}), 82.2 (C-1^{IV}), 76.9 (C-3^{III}), 76.7 (C-3^{II}), 75.8 (C-5^{II}, C-5^{III}), 74.2 (C-5^{IV}), 74.1 (C-3^{IV}), 73.4 (C-3^{I}), 72.6 (C-2^{II}, C-2^{III}), 71.8 (C-2^{I}), 71.7 (C-4^{I}), 71.5 (C-4^{III}), 71.3 (C-4^{II}), 71.2 (C-2^{IV}), 70.3 (C-5), 69.8 (C-4^{IV}), 55.7 (OMe), 51.0 (C-6^{IV}), 46.1 (C-6^{I}, C-6^{II}, C-6^{III}), 20.6 (3 *Me*CO) ppm. FAB-MS: *m/z* = 1028 [M + Na]$^+$, 1006 [M + H]$^+$. $C_{34}H_{55}N_9O_{20}S_3$ (1005.1): calcd. C 40.59, H 5.51, N 12.53; found C 40.30, H 5.24, N 12.35.

Methyl 6-Deoxy-6-[*N*′-{6-deoxy-6-[*N*′-{6-deoxy-6-[*N*′-(6-azido-6-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosyl}thioureido]-β-D-glucopyranosyl}thioureido]-α-D-glucopyranoside (26): Conventional Zemplén O-deacetylation of the hemiacetylated pseudotetrasaccharide adduct **25** (36.7 mg, 0.036 mmol), as described above for the preparation of **20**, afforded **26**. Yield: 32 mg (99%); R_f = 0.33 (6:3:1 MeCN/H_2O/NH_4OH); R_f = 0.43 (10:2:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = +35.3 (*c* = 1.0, H_2O). UV (H_2O): λ = 243 nm (ε_{mM} = 26.9). ^{1}H NMR (500 MHz, D_2O, 70 °C): δ = 5.71 (d, *J* = 9.1 Hz, 1 H, H-1^{IV}), 5.64 (br. d, *J* = 9.1 Hz, 2 H, H-1^{II}, H-1^{III}), 5.18 (d, *J* = 3.3 Hz, 1 H, H-1^{I}), 4.48 (m, 2 H, H-6^{II}a, H-6^{III}a), 4.32 (m, 1 H, H-6^{I}a), 4.18 (m, 2 H, H-5^{I}, H-6^{I}b), 4.06 (t, *J* = 9.3 Hz, 1 H, H-3^{I}), 4.06 (m, 4 H, H-5^{II}, H-5^{III}, H-6^{II}b, H-6^{III}b), 4.04 (ddd, *J* = 9.1, 2.9, 6.0 Hz, 1 H, H-5^{IV}), 3.97 (t, *J* = 9.1 Hz, 3 H, H-3^{II}, H-3^{III}, H-3^{IV}), 3.96 (dd, *J* = 13.5, 6.0 Hz, 1 H, H-6^{IV}a), 3.95 (dd, *J* = 9.3, 3.3 Hz, 1 H, H-2^{I}), 3.86 (t, *J* = 9.1 Hz, 1 H, H-2^{IV}), 3.86 (dd, *J* = 13.5, 2.9 Hz, 1 H, H-6^{IV}b), 3.84 (t, *J* = 9.1 Hz, 2 H, H-4^{II}, H-4^{III}), 3.79 (s, 3 H, OMe), 3.74 (t, *J* = 9.1 Hz, 1 H, H-5^{IV}), 3.73 (t, *J* = 9.1 Hz, 2 H, H-2^{II}, H-2^{III}), 3.71 (t, *J* = 8.7 Hz, 1 H, H-4^{I}) ppm. ^{13}C NMR (125.7 MHz, D_2O, 40 °C): δ = 183.6 (3 CS), 99.2 (C-1^{I}), 84.6 (C-1^{IV}), 83.4 (C-1^{II}, C-1^{III}), 76.3 (C-3^{II}, C-3^{III}, C-3^{IV}), 75.8 (C-5^{II}, C-5^{III}, C-5^{IV}), 72.8 (C-2^{II}, C-2^{III}, C-2^{IV}, C-3^{I}), 72.0 (C-2^{I}), 71.2 (C-4^{I}, C-4^{II}, C-4^{III}), 69.7 (C-5^{I}), 68.5 (C-4^{IV}), 45.2 (C-6^{IV}), 41.8 (C-6^{I}, C-6^{II}, C-6^{III}), 55.0 (OMe) ppm. FAB-MS: *m/z* = 880 (10%, [M + H]$^+$. $C_{28}H_{49}N_9O_{17}S_3$ (879.97): calcd. C 38.22, H 5.61, N 14.33; found C 37.96, H 5.58, N 14.15.

***N*-Acetyl-2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranosylamine (27):** Conventional acetylation of the crude carbamate salt arising from the treatment of **8** (0.3 g, 0.84 mmol) with aq. ammonia and NH_4HCO_3, as described above for the preparation of **9**, followed by purification by column chromatography (2:3 EtOAc/petroleum ether) afforded **27**. Yield: 0.13 g (43%); R_f = 0.39 (1:1 EtOAc/petroleum ether). $[\alpha]_D$ = +4.7 (c = 0.85, CH_2Cl_2). IR (NaCl): $\tilde{\nu}_{max}$ = 3318, 2961, 2108, 1753, 1377, 1223, 1098, 1042 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 6.51 (d, J = 9.3 Hz, 1 H, NH), 5.16 (t, J = 9.8 Hz, 1 H, H-1), 4.88 (t, J = 9.8 Hz, 1 H, H-4), 4.72 (t, J = 9.8 Hz, 1 H, H-2), 4.20 (dd, J = 12.5, 2.9 Hz, 1 H, H-6a), 3.99 (dd, J = 12.5, 1.8 Hz, 1 H, H-6b), 3.72 (t, J = 9.8 Hz, 1 H, H-3), 3.72 (m, 1 H, H-5), 2.07, 2.00, 1.93 (3 s, 9 H, 3 MeCO) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 170.6, 169.2, (4 CO), 78.2 (C-1), 74.1 (C-5), 71.0 (C-2), 68.4 (C-4), 64.5 (C-3), 61.6 (C-6), 23.2 (*Me*NCO), 20.6, 20.5 (2 *Me*CO) ppm. FAB-MS: m/z = 395 [M + Na]$^+$, 373 [M + H]$^+$. $C_{14}H_{20}N_4O_8$ (372.33): calcd. C 45.16, H 5.41, N 15.05; found C 45.03, H 5.34, N 14.92.

***N*-Acetyl-3-azido-3-deoxy-β-D-glucopyranosylamine (28):** O-Deacetylation of **27** (0.13 g, 0.35 mmol) was effected by treatment with methanolic MeONa (0.1 mol per mol of acetate) in MeOH (10 mL) and further neutralisation with Amberlite IR 120 (H^+) cation-exchange resin to give **28**. Yield: 87 mg (100%); R_f = 0.31 (45:5:3 EtOAc/EtOH/H_2O). $[\alpha]_D$ = +14.3 (c = 1.0, MeOH). IR (KBr): $\tilde{\nu}_{max}$ = 3331, 3059, 2926, 2110, 1665, 1292, 1042 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ = 4.92 (d, J = 9.1 Hz, 1 H, H-1), 3.79 (dd, J = 12.0, 2.2 Hz, 1 H, H-6a), 3.63 (dd, J = 12.0, 5.0 Hz, 1 H, H-6b), 3.55 (m, 1 H, H-5), 3.31 (m, 2 H, H-3, H-4), 3.21 (t, J = 9.1 Hz, 1 H, H-2), 1.98 (s, 3 H, MeCO) ppm. ^{13}C NMR (75.5 MHz, CD_3OD): δ = 174.3 (CO), 81.2 (C-1), 80.1 (C-5), 72.7 (C-2), 72.2 (C-4), 70.1 (C-3), 62.3 (C-6), 22.8 (*Me*CO) ppm. FAB-MS: m/z = 247 [M + H]$^+$. $C_8H_{14}N_4O_5$ (246.22): calcd. C 39.02, H 5.73, N 22.75; found C 39.00, H 5.95, N 22.52.

***N*-Acetyl-3-amino-3-deoxy-β-D-glucopyranosylamine (29):** Et_3N (105 μL, 2 equiv.) and propane-1,3-dithiol (57 μL, 2 equiv.) were added to a solution of **28** (81 mg, 0.35 mmol) in freshly distilled MeOH (3 mL) in a vessel previously purged with N_2. The reaction mixture was stirred at room temperature under N_2 for 2 h whilst monitoring by TLC (45:5:3 EtOAc/EtOH/H_2O). Water (10 mL) was then added and the aqueous solution was washed with CH_2Cl_2 (2 × 5 mL). Freeze-drying of the aqueous solution afforded **29** as a white lyophilisate. Yield: 60 mg (78%); R_f = 0.29 (4:1:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = –24.0 (c = 0.9, H_2O). ^1H NMR (300 MHz, D_2O): δ = 4.90 (d, J = 9.8 Hz, 1 H, H-1), 3.82 (dd, J = 12.3, 2.3 Hz, 1 H, H-6a), 3.67 (dd, J = 12.3, 5.3 Hz, 1 H, H-6b), 3.50 (ddd, J = 9.8, 5.3, 2.3 Hz, 1 H, H-5), 3.29 (t, J = 9.8 Hz, 1 H, H-4), 3.26 (t, J = 9.8 Hz, 1 H, H-2), 2.83 (t, J = 9.8 Hz, 1 H, H-3), 2.02 (s, 3 H, MeCO) ppm. ^{13}C NMR (75.5 MHz, D_2O): δ = 177.9 (CO), 82.1 (C-1), 80.8 (C-5), 73.8 (C-2), 71.2 (C-4), 62.9 (C-3), 61.0 (C-6), 24.4 (*Me*CO) ppm. FAB-MS: m/z = 243 [M + Na]$^+$, 221 [M + H]$^+$. $C_8H_{16}N_2O_5$ (220.22): calcd. C 43.63, H 7.32, N 12.72; found C 43.37, H 6.99, N 12.49.

***N*-Acetyl-3-deoxy-3-[*N*′-(2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosylamine (30):** Compound **30** was obtained by a coupling reaction of **29** (39 mg, 0.18 mmol) and **12** (66 mg, 0.18 mmol) in pyridine (3 mL) at room temperature for 2 h, followed by column chromatography (EtOAc → 45:5:3 EtOAc/EtOH/H_2O). Yield: 100 mg (94%); R_f = 0.28 (45:5:3 EtOAc/EtOH/H_2O). $[\alpha]_D$ = –9.8 (c = 0.9, MeOH). UV (MeOH): λ = 250 nm (ε_{mM} = 12.8). IR (KBr): $\tilde{\nu}_{max}$ = 3352, 3073, 2926, 2863, 2110, 1753, 1659, 1227, 1040 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, 40 °C): δ = 5.79 (br. s, 1 H, H-1^{II}), 4.94 (d, J = 9.6 Hz, 1 H, H-1^{I}), 4.89 (t, J = 9.8 Hz, 1 H, H-4^{II}), 4.88 (t, J = 9.8 Hz, 1 H, H-2^{II}), 4.58 (br. s, 1 H, H-3^{I}), 4.20 (dd, J = 12.4, 4.8 Hz, 1 H, H-6^{II}a), 4.05 (dd, J = 12.4, 2.5 Hz, 1 H, H-6^{II}b), 3.99 (t, J = 9.8 Hz, 1 H, H-3^{II}), 3.86 (ddd, J = 9.8, 4.8, 2.5 Hz, 1 H, H-5^{II}), 3.79 (dd, J = 12.7, 1.5 Hz, 1 H, H-6^{I}a), 3.64 (m, 1 H, H-6^{I}b), 3.40 (m, 2 H, H-4^{I}, H-5^{I}), 3.29 (t, J = 9.6 Hz, 1 H, H-3^{I}), 2.08, 2.07, 1.99, 1.97 (4 s, 12 H, 4 MeCO) ppm. ^{13}C NMR (125.7 MHz, CD_3OD, 40 °C): δ = 187.8 (CS), 174.2 (CO amide), 172.4, 171.8, 171.2 (3 CO ester), 84.2 (C-1^{II}), 81.9 (C-1^{I}), 80.5 (C-5^{I}), 75.2 (C-5^{II}), 73.4 (C-2^{I}), 72.4 (C-2^{II}), 70.6 (C-4^{I}), 70.0 (C-4^{II}), 65.9 (C-3^{II}), 64.4 (C-3^{I}), 63.2 (C-6^{II}), 62.5 (C-6^{I}), 26.4 (*Me*NCO), 22.8, 20.6 (3 *Me*OCO) ppm. FAB-MS: m/z = 615 [M + Na]$^+$, 593 [M + H]$^+$. $C_{21}H_{32}N_6O_{12}S$ (592.58): calcd. C 42.56, H 5.44, N 14.18; found C 42.50, H 5.34, N 14.07.

***N*-Acetyl-3-deoxy-3-[*N*′-(3-azido-3-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosylamine (31):** O-Deacetylation of the hemiacetylated pseudodisaccharide adduct **30** (100 mg, 0.17 mmol) was effected in methanol (10 mL) by treatment with NaOMe (0.1 equiv. per mol of acetate) for 2 h. The reaction mixture was neutralised with Amberlite IRA 120 (H^+) ion-exchange resin, then filtered and concentrated to give **31**. Yield: 79 mg (100%); R_f = 0.47 (4:1:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = +1.6 (c = 0.8, MeOH). UV (MeOH): λ = 250 nm (ε_{mM} = 5.8). IR (KBr): $\tilde{\nu}_{max}$ = 3322, 3071, 2924, 2855, 2110, 1254, 1074, 1038 cm^{-1}. ^1H NMR (500 MHz, D_2O, 70 °C): δ = 5.89 (br. d, 1 H, H-1^{II}), 5.43 (d, J = 9.5 Hz, 1 H, H-1^{I}), 5.00 (br. s, 1 H, H-3^{I}), 4.27 (dd, J = 12.4, 2.4 Hz, 1 H, H-6^{II}a), 4.26 (dd, J = 12.3, 2.1 Hz, 1 H, H-6^{I}a), 4.12 (dd, J = 12.3, 4.8 Hz, 1 H, H-6^{II}b), 4.11 (dd, J = 12.3, 5.3 Hz, 1 H, H-6^{I}b), 4.01 (ddd, J = 9.5, 5.3, 2.1 Hz, 1 H, H-5^{I}), 4.00 (ddd, J = 9.8, 4.8, 2.4 Hz, 1 H, H-5^{II}), 3.97 (t, J = 9.8 Hz, 1 H, H-3^{II}), 3.96 (t, J = 9.5 Hz, 1 H, H-4^{I}), 3.88 (t, J = 9.8 Hz, 1 H, H-4^{II}) 3.93 (t, J = 9.5 Hz, 1 H, H-2^{I}), 3.92 (t, J = 9.8 Hz, 1 H, H-2^{II}), 1.95 (s, 3 H, MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 70 °C): δ = 182.3 (CS), 175.8 (CO), 84.5 (C-1^{II}), 80.6 (C-1^{I}), 79.0 (C-5^{I}), 78.4 (C-5^{II}), 71.6 (C-2^{II}), 71.4 (C-2^{I}), 70.0 (C-3^{II}), 69.0 (C-4^{II}), 68.9 (C-4^{I}), 63.4 (C-3^{I}), 61.3 (C-6^{II}), 61.2 (C-6^{I}), 25.4 (*Me*CO) ppm. FAB-MS: m/z = 489 [M + Na]$^+$, 467 [M + H]$^+$. $C_{15}H_{26}N_6O_9S$ (466.47): calcd. C 38.62, H 5.62, N 18.02; found C 38.58, H 5.64, N 17.94.

***N*-Acetyl-3-deoxy-3-[*N*′-(3-amino-3-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosylamine (32):** 1,3-Propanedithiol (25 μL, 2 equiv.) and triethylamine (46 μL, 2 equiv.) were added to a solution of **31** (70 mg, 0.156 mmol) in methanol (2 mL) under Ar. The reaction was stirred at room temperature for 16 h, diluted with water (10 mL) and extracted with CH_2Cl_2 (2 × 5 mL). Freeze-drying of the aqueous solution afforded **32** as a white foam. Yield: 61 mg (88%); R_f = 0.28 (4:1:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = +3.9 (c = 1.1, H_2O). UV (H_2O): λ = 247 nm (ε_{mM} = 13.6). ^1H NMR (500 MHz, D_2O, 60 °C): δ = 5.73 (br. s, 1 H, H-1^{II}), 5.32 (d, J = 9.5 Hz, 1 H, H-1^{I}), 4.50 (br. s, 1 H, H-3^{I}), 4.17 (dd, J = 12.3, 2.3 Hz, 1 H, H-6^{II}a), 4.15 (dd, J = 12.3, 1.9 Hz, 1 H, H-6^{I}a), 4.01 (dd, J = 12.3, 4.8 Hz, 1 H, H-6^{I}b), 4.00 (dd, J = 12.3, 5.4 Hz, 1 H, H-6^{II}b), 3.89 (ddd, J = 9.8, 4.8, 1.9 Hz, 1 H, H-5^{I}), 3.86 (ddd, J = 9.5, 5.4, 2.3 Hz, 1 H, H-5^{II}), 3.86 (t, J = 9.8 Hz, 1 H, H-4^{I}), 3.82 (t, J = 9.5 Hz, 1 H, H-2^{I}), 3.72 (t, J = 9.5 Hz, 1 H, H-2^{II}), 3.66 (t, J = 9.5 Hz, 1 H, H-4^{II}), 3.21 (t, J = 9.5 Hz, 1 H, H-3^{II}), 2.34 (s, 3 H, MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 60 °C): δ = 182.3 (CS), 175.7 (CO), 84.5 (C-1^{II}), 80.5 (C-1^{I}), 78.9 (C-5^{I}), 78.7 (C-5^{II}), 72.1 (C-2^{II}), 71.3 (C-2^{I}), 69.4 (C-4^{II}), 68.8 (C-4^{I}), 63.3 (C-3^{I}), 59.3 (C-3^{II}), 61.3 (C-6^{II}), 61.1 (C-6^{I}), 27.2 (*Me*CO) ppm. FAB-MS: m/z = 397 [M – Ac]$^+$. $C_{15}H_{28}N_4O_9S$ (440.47): calcd. C 40.90, H 6.41, N 12.72; found C 40.82, H 6.27, N 12.52.

***N*-Acetyl-3-deoxy-3-[*N*′-[3-deoxy-3-[*N*′-(2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosyl]thio-**

ureido]-β-D-glucopyranosylamine (33): Compound **33** was obtained by a coupling reaction of **32** (51 mg, 0.116 mmol) and **12** (43 mg, 0.116 mmol) in pyridine (3 mL) at room temperature for 24 h, followed by column chromatography (45:5:3 EtOAc/EtOH/H_2O). Yield: 68 mg (72%); R_f = 0.11 (4:1:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = –8.3 (*c* = 1.0, MeOH). UV (MeOH): λ = 250 nm (ε_{mM} = 22.7). IR (KBr): $\tilde{\nu}_{max}$ = 3351, 3071, 2926, 2855, 2110, 1753, 1231, 1038 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, 50 °C): δ = 5.79 (d, *J* = 8.1 Hz, 1 H, H-1^{III}), 5.39 (br. s, 1 H, H-1^{II}), 4.97 (d, *J* = 9.0 Hz, 1 H, H-1^{I}), 4.89 (t, *J* = 9.9 Hz, 1 H, H-4^{III}), 4.88 (dd, *J* = 9.9, 8.1 Hz, 1 H, H-2^{III}), 4.62 (br. s, 2 H, H-3, H-3^{II}), 4.21 (dd, *J* = 12.4, 4.8 Hz, 1 H, H-6^{III}a), 4.07 (dd, *J* = 12.4, 2.5 Hz, 1 H, H-6^{III}b), 3.98 (t, *J* = 9.9 Hz, 1 H, H-3^{III}), 3.87 (ddd, *J* = 9.9, 2.5, 1.8 Hz, 1 H, H-5^{III}), 3.83 (dd, *J* = 11.8, 1.8 Hz, 1 H, H-6^{II}a), 3.81 (dd, *J* = 11.8, 2.0 Hz, 1 H, H-6^{I}a), 3.66 (dd, *J* = 11.8, 4.8 Hz, 1 H, H-6^{I}b), 3.65 (dd, *J* = 11.8, 5.0 Hz, 1 H, H-6^{II}b), 3.47 (t, *J* = 9.5 Hz, 1 H, H-4^{I}), 3.45 (m, 1 H, H-5^{II}), 3.42 (m, 1 H, H-5^{I}), 3.41 (t, *J* = 9.5 Hz, 1 H, H-4^{II}), 3.39 (t, *J* = 9.5 Hz, 1 H, H-2^{II}), 3.38 (t, *J* = 9.5 Hz, 1 H, H-2^{I}), 2.12, 2.11, 2.09, 2.08 (4 s, 12 H, 4 MeCO) ppm. ^{13}C NMR (125.7 MHz, CD_3OD, 50 °C): δ = 186.8 (2 CS), 173.8 (CO amide), 172.0, 171.6, 170.8 (3 CO ester), 86.0 (C-1^{II}), 84.0 (C-1^{III}), 81.5 (C-1^{I}), 80.2 (C-5^{I}), 80.0 (C-5^{II}), 74.9 (C-5^{III}), 73.3 (C-2^{II}), 72.9 (C-2^{I}), 72.1 (C-2^{III}), 70.1 (C-4^{I}, C-4^{II}), 69.7 (C-4^{III}), 65.6 (C-3^{III}), 64.4 (C-3^{II}, C-3^{I}), 62.9 (C-6^{IV}), 62.3 (C-6^{II}), 62.2 (C-6^{I}), 25.5 (*Me*NCO), 22.6, 20.2 (3 *Me*OCO) ppm. FAB-MS: *m*/*z* = 835 $[M + Na]^+$, 813 $[M + H]^+$. $C_{28}H_{44}N_8O_{16}S_2$ (812.83): calcd. C 41.37, H 5.48, N 13.79; found C 41.24, H 5.53, N 13.57.

***N*-Acetyl-3-deoxy-3-[*N*′-{3-deoxy-3-[*N*′-(3-azido-3-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosyl}thioureido]-β-D-glucopyranosylamine (34):** Conventional Zemplén O-deacetylation of the hemiacetylated pseudotrisaccharide adduct **33** (53 mg, 0.06 mmol), as described above for the preparation of **31**, afforded **34**. Yield: 40 mg (99%); R_f = 0.25 (4:1:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = +0.8 (*c* = 0.7, H_2O). UV (H_2O): λ = 246 nm (ε_{mM} = 17.2). IR (KBr): $\tilde{\nu}_{max}$ = 3345, 3061, 2924, 2110, 1252, 1076, 1038 cm^{-1}. ^1H NMR (500 MHz, D_2O, 80 °C): δ = 6.03 (d, *J* = 9.8 Hz, 1 H, H-1^{II}), 6.00 (d, *J* = 10.0 Hz, 1 H, H-1^{III}), 5.55 (d, *J* = 9.5 Hz, 1 H, H-1^{I}), 5.05 (br. s, 1 H, H-3^{II}), 5.00 (br. s, 1 H, H-3^{I}), 4.41 (dd, *J* = 12.3, 2.5 Hz, 1 H, H-6^{II}a), 4.39 (dd, *J* = 12.5, 2.5 Hz, 1 H, H-6^{III}a), 4.38 (dd, *J* = 12.5, 2.5 Hz, 1 H, H-6^{I}a), 4.25 (dd, *J* = 12.3, 4.0 Hz, 1 H, H-6^{II}b), 4.24 (dd, *J* = 12.5, 4.5 Hz, 1 H, H-6^{III}b), 4.23 (dd, *J* = 4.0, 2.5 Hz, 1 H, H-6^{I}b), 4.18 (m, 1 H, H-5^{II}), 4.16 (t, *J* = 9.8 Hz, 1 H, H-2^{II}), 4.12 (ddd, *J* = 9.5, 4.5, 2.5 Hz, 2 H, H-5^{I}, H-5^{III}), 4.10 (t, *J* = 9.8 Hz, 1 H, H-4^{II}), 4.09 (t, *J* = 10.0 Hz, 1 H, H-3^{III}), 4.07 (t, *J* = 9.5 Hz, 1 H, H-4^{I}), 4.05 (t, *J* = 9.5 Hz, 2 H, H-2^{I}, H-2^{III}), 4.01 (t, *J* = 10.0 Hz, 1 H, H-4^{III}), 2.58 (s, 3 H, MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 60 °C): δ = 182.3 (2 CS), 175.8 (CO),84.6 (C-1^{II}), 84.2 (C-1^{III}), 80.5 (C-1^{I}), 78.9 (C-5^{I}), 78.7 (C-5^{II}), 78.3 (C-5^{III}), 71.6 (C-2^{II}), 71.5 (C-2^{III}), 71.3 (C-2^{I}), 69.9 (C-3^{III}), 68.9 (C-4^{III}), 68.8 (C-4^{I}, C-4^{II}), 63.3 (C-3^{I}, C-3^{II}), 61.2 (C-6^{II}), 61.1 (C-6^{III}), 61.0 (C-6^{I}), 25.1 (*Me*CO) ppm. FAB-MS: *m*/*z* = 709 $[M + Na]^+$. $C_{22}H_{38}N_8O_{13}S_2$ (686.72): calcd. C 38.48; H 5.58, N 16.32; found C 38.38, H 5.34, N 16.12.

***N*-Acetyl-3-deoxy-3-[*N*′-{3-deoxy-3-[*N*′-(3-amino-3-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosyl}thioureido]-β-D-glucopyranosylamine (35):** Reduction of azide **34** (36 mg, 0.05 mmol) in methanol (1.5 mL) with 1,3-propanedithiol (8 μL, 2 equiv.) and triethylamine (15 μL, 2 equiv.), as described above for the preparation of **32**, afforded **35** as a white foam. Yield: 30 mg (91%); R_f = 0.23 (5:3:5 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = +7.5 (*c* = 1.1, H_2O). UV (H_2O): λ = 246 nm (ε_{mM} = 26.1). ^1H NMR (500 MHz, D_2O, 60 °C): δ = 5.83 (br. d, 1 H, H-1^{II}), 5.80 (br. d, 1 H, H-1^{III}), 5.34 (d, *J* = 9.5 Hz, 1 H, H-1^{I}), 5.02 (m, 2 H, H-3^{I}, H-3^{II}), 4.20 (dd, *J* = 12.3, 1.8 Hz, 2 H, H-6^{II}a, H-6^{III}a), 4.17 (dd, *J* = 12.3, 1.6 Hz, 1 H, H-6^{I}a), 4.04 (dd, *J* = 4.2, 5.2 Hz, 2 H, H-6^{II}b, H-6^{III}b), 4.03 (dd, *J* = 12.3, 4.2 Hz, 1 H, H-6^{I}b), 3.97 (ddd, *J* = 9.8, 5.2, 1.8 Hz, 1 H, H-5^{II}), 3.96 (t, *J* = 9.8 Hz, 1 H, H-2^{II}), 3.91 (ddd, *J* = 9.7, 9.5, 5.2 Hz, 2 H, H-5^{I}, H-5^{III}), 3.90 (t, *J* = 9.7 Hz, 1 H, H-4^{II}), 3.88 (t, *J* = 9.5 Hz, 1 H, H-4^{I}), 3.86 (t, *J* = 9.7 Hz, 2 H, H-2^{III}), 3.85 (t, *J* = 9.5 Hz, 1 H, H-2^{I}), 3.81 (t, *J* = 9.7 Hz, 1 H, H-4^{III}), 3.39 (t, *J* = 9.7 Hz, 1 H, H-3^{III}), 2.36 (s, 3 H, MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 60 °C): δ = 184.3 (2 CS), 175.8 (CO), 84.8 (C-1^{II}, C-1^{III}), 80.5 (C-1^{I}), 78.9 (C-5^{III}), 78.8 (C-5^{I}), 78.7 (C-5^{II}), 71.6 (C-2^{II}), 71.3 (C-2^{III}), 71.0 (C-2^{I}), 68.9 (C-4^{I}, C-4^{II}), 68.3 (C-4^{III}), 63.4 (C-3^{I}, C-3^{II}), 61.2 (C-6^{I}, C-6^{II}), 61.0 (C-6^{III}), 59.4 (C-3^{III}), 22.7 (*Me*CO) ppm. FAB-MS: *m*/*z* = 683 $[M + Na]^+$. $C_{22}H_{40}N_6O_{13}S_2$ (660.72): calcd. C 39.99, H 6.10, N 12.72; found C 39.68, H 6.09, N 12.57.

***N*-Acetyl-3-deoxy-3-[*N*′-{3-deoxy-3-[*N*′-{3-deoxy-3-[*N*′-(2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosyl}thioureido]-β-D-glucopyranosyl}thioureido]-β-D-glucopyranosylamine (36):** Compound **36** was obtained by a coupling reaction of **35** (33 mg, 0.05 mmol) and **12** (20 mg, 0.05 mmol) in pyridine (3 mL) at room temperature for 16 h. After evaporation of the solvent, the residue was dissolved in water (5 mL), washed with EtOAc (2×5 mL) and the aqueous solution was freeze-dried to yield **32**. Yield: 36 mg (69%); R_f = 0.82 (5:3:5 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = 0 (*c* = 1.0, MeOH). UV (MeOH): λ = 249 nm (ε_{mM} = 27.4). IR (KBr): $\tilde{\nu}_{max}$ = 3387, 3059, 2924, 2110, 1740, 1233, 1096, 1040 cm^{-1}. ^1H NMR (500 MHz, D_2O, 60 °C): δ = 6.35 (d, *J* = 9.1 Hz, 1 H, H-1^{IV}), 6.03 (d, *J* = 9.6 Hz, 1 H, H-1^{II}), 6.01 (d, *J* = 9.7 Hz, 1 H, H-1^{III}), 5.53 (d, *J* = 10.1 Hz, 1 H, H-1^{I}), 5.51 (t, *J* = 9.9 Hz, 1 H, H-4^{IV}), 5.47 (dd, *J* = 9.9, 9.1 Hz, 1 H, H-2^{IV}), 5.05 (br. s, 3 H, H-3^{I}, H-3^{II}, H-3^{III}), 4.77 (dd, *J* = 12.6, 4.4 Hz, 1 H, H-6^{IV}a), 4.72 (dd, *J* = 12.6, 3.0 Hz, 1 H, H-6^{IV}b), 4.62 (t, *J* = 9.9 Hz, 1 H, H-3^{IV}), 4.56 (ddd, *J* = 9.9, 4.4, 3.0 Hz, 1 H, H-5^{IV}), 4.40 (br. d, *J* = 12.1 Hz, 1 H, H-6^{III}a), 4.39 (br. d, *J* = 11.6 Hz, 1 H, H-6^{II}a), 4.37 (br. d, *J* = 12.2 Hz, 1 H, H-6^{I}a), 4.24 (br. d, *J* = 12.1 Hz, 1 H, H-6^{III}b), 4.23 (br. d, *J* = 12.1 Hz, 2 H, H-6^{II}b, H-6^{I}b), 4.15 (m, 2 H, H-4^{III}, H-5^{III}), 4.14 (m, 1 H, H-5^{II}), 4.10 (m, 2 H, H-4^{I}, H-5^{I}), 4.10 (t, *J* = 9.7 Hz, 1 H, H-2^{III}), 4.07 (t, *J* = 9.6 Hz, 1 H, H-4^{II}), 4.06 (t, *J* = 10.1 Hz, 1 H, H-2^{I}), 4.05 (t, *J* = 9.6 Hz, 1 H, H-2^{II}), 2.47, 2.44, 2.38, 2.34 (4 s, 12 H, 4 MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 50 °C): δ = 185.3 (3 CS), 175.7 (CO amide), 174.0, 173.3, 173.0 (3 CO ester), 84.5 (C-1^{II}, C-1^{III}), 82.7 (C-1^{IV}), 80.3 (C-1), 78.8 (C-5^{I}, C-5^{II}), 78.6 (C-5^{IV}), 73.8 (C-5^{IV}), 71.2 (C-2^{II}, C-2^{III}), 71.1 (C-2^{I}), 70.8 (C-2^{IV}), 68.8 (C-4^{IV}), 68.6 (C-4^{I}, C-4^{II}, C-4^{III}), 64.3 (C-3^{IV}), 63.1 (C-3^{I}, C-3^{II}, C-3^{III}), 62.4 (C-6^{IV}), 61.0 (C-6^{I}), 60.9 (C-6^{II}, C-6^{III}), 22.5 (*Me*NCO), 20.5 (3 *Me*OCO) ppm. FAB-MS: *m*/*z* = 1055 $[M + Na]^+$. $C_{35}H_{56}N_{10}O_{20}S_3$ (1032.3): calcd. C 40.69, H 5.46, N 13.56; found C 40.43, H 5.29, N 13.38.

***N*-Acetyl-3-deoxy-3-[*N*′-{3-deoxy-3-[*N*′-{3-deoxy-3-[*N*′-(3-azido-3-deoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosyl}thioureido]-β-D-glucopyranosyl}thioureido]-β-D-glucopyranosylamine (37):** Conventional Zemplén O-deacetylation of the hemiacetylated pseudotrisaccharide adduct **36** (36 mg, 0.034 mmol), as described above for the preparation of **31**, afforded **33**. Yield: 31 mg (100%); R_f = 0.84 (5:3:5 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = +5.0 (*c* = 1.1, H_2O). UV (H_2O): λ = 246 nm (ε_{mM} = 26.5). IR (KBr): $\tilde{\nu}_{max}$ = 3331, 3082, 2926, 2112, 1263, 1076, 1038 cm^{-1}. ^1H NMR (500 MHz, D_2O, 80 °C): δ = 6.01 (d, *J* = 9.5 Hz, 2 H, H-1^{II}, H-1^{III}), 5.98 (d, *J* = 9.5 Hz, 1 H, H-1^{IV}), 5.52 (d, *J* = 9.5 Hz, 1 H, H-1^{I}), 5.05 (br. s, 3 H, H-3^{I}, H-3^{II}, H-3^{III}), 4.40 (dd, *J* = 12.0, 1.8 Hz, 1 H, H-6^{II}a), 4.38 (dd, *J* = 12.0, 1.8 Hz, 2 H, H-6^{III}a, H-6^{IV}a), 4.36 (dd, *J* = 12.0, 2.0 Hz, 1 H, H-6^{I}a), 4.24 (dd, *J* = 12.0, 4.5 Hz, 2 H, H-6^{II}b, H-6^{III}b), 4.22 (dd, *J* = 5.0, 4.5 Hz, 2 H, H-6^{I}b, H-6^{IV}b),

4.15 (m, 4 H, H-4^{II}, H-5^{II}, H-4^{III}, H-5^{III}), 4.11 (ddd, J = 9.5, 4.5, 1.8 Hz, 1 H, H-5^{IV}), 4.10 (m, 2 H, H-4^{I}, H-5^{I}), 4.10 (t, J = 9.5 Hz, 2 H, H-2^{II}, H-2^{III}), 4.08 (t, J = 9.5 Hz, 1 H, H-3^{IV}), 4.04 (t, J = 9.5 Hz, 1 H, H-2^{I}), 4.02 (t, J = 9.5 Hz, 1 H, H-2^{IV}), 3.99 (t, J = 9.5 Hz, 1 H, H-4^{IV}), 2.58 (s, 3 H, MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 60 °C): δ = 182.3 (3 CS), 175.8 (CO), 84.6 (C-1^{II}, C-1^{III}, C-1^{IV}), 80.6 (C-1^{I}), 79.0 (C-5^{I}), 78.9 (C-5^{II}, C-5^{III}), 78.5 (C-5^{IV}), 71.8, 71.6 (C-2^{II}, C-2^{III}, C-2^{IV}), 71.4 (C-2^{I}), 70.0 (C-3^{IV}), 69.2 (C-4^{I}), 69.1 (C-4^{II}, C-4^{III}), 69.0 (C-4^{IV}), 63.5 (C-3^{I}, C-3^{II}, C-3^{III}), 61.3, 61.2 (C-6^{I}, C-6^{II}, C-6^{III}, C-6^{IV}), 22.8 (*Me*CO) ppm. MALDI-TOF-MS: m/z = 929 [M + Na]$^+$, 907 [M + H]$^+$. $C_{29}H_{50}N_{10}O_{17}S_3$ (906.96): calcd. C 38.40, H 5.56, N 15.44; found C 38.30, H 5.43, N 15.30.

***N*-Acetyl-2,4-di-*O*-acetyl-3,6-diazido-3,6-dideoxy-β-D-glucopyranosylamine (38):** Conventional acetylation of the crude carbamate salt arising from the treatment of **13** (1 g, 4.36 mmol) with aq. ammonia and NH_4HCO_3, as described above for the preparation of **14**, followed by purification by column chromatography (1:1 EtOAc/petroleum ether) afforded **38**. Yield: 0.66 g (43%); R_f = 0.29 (1:1 EtOAc/petroleum ether). $[\alpha]_D$ = +15.0 (c = 0.9, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ = 3289, 2924, 2853, 2106, 1751, 1686, 1541, 1375, 1219, 1036 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.50 (d, J = 9.3 Hz, 1 H, NH), 5.20 (t, J = 9.3 Hz, 1 H, H-1), 4.89 (t, J = 9.3 Hz, 1 H, H-2), 4.73 (t, J = 9.3 Hz, 1 H, H-4), 3.69 (ddd, J = 9.3, 5.4, 3.3 Hz, 1 H, H-5), 3.38 (t, J = 9.3 Hz, 1 H, H-3), 3.35 (dd, J = 13.5, 3.3 Hz, 1 H, H-6a), 3.23 (dd, J = 13.5, 5.4 Hz, 1 H, H-6b), 2.10, 2.09, 1.96 (s, 9 H, 3 MeCO) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 170.6 (2 CO ester), 169.3 (CO amide), 78.1 (C-1), 75.0 (C-5), 70.9 (C-4), 69.2 (C-2), 64.4 (C-3), 50.6 (C-6), 23.2 (*Me*NCO), 20.6 (2 *Me*CO) ppm. EI-MS: m/z = 356 [M + H]$^+$, 314 [M – Ac$^+$]. $C_{12}H_{17}N_7O_6$ (355.31): calcd. C 40.56, H 4.82, N 27.60; found C 40.74, H 4.60, N 27.78.

***N*-Acetyl-3,6-diazido-3,6-dideoxy-β-D-glucopyranosylamine (39):** Deacetylation of **38** (0.26 g, 0.73 mmol) with methanolic MeONa (0.1 mol per mol of acetate) in MeOH (5 mL) afforded **42**. Yield: 0.20 g (100%); R_f = 0.69 (45:5:3 EtOAc/EtOH/H_2O). $[\alpha]_D$ = +6.0 (c = 0.8, MeOH). IR (KBr): $\tilde{\nu}_{max}$ = 3316, 2926, 2882, 2112, 1663, 1543, 1379, 1246, 1074, 1028 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ = 4.79 (d, J = 8.7 Hz, 1 H, H-1), 3.35 (m, 1 H, H-6a), 3.35 (m, 1 H, H-5), 3.20 (dd, J = 13.5, 5.7 Hz, 1 H, H-6b), 3.16 (t, J = 8.7 Hz, 2 H, H-3, H-4), 3.07 (t, J = 8.7 Hz, 1 H, H-2), 1.83 (s, 3 H, MeCO) ppm. ^{13}C NMR (75.5 MHz, CD_3OD): δ = 174.2 (CO), 81.2 (C-1), 78.7 (C-5), 72.7 (C-2), 72.7 (C-4), 70.8 (C-3), 52.3 (C-6), 22.8 (*Me*CO) ppm. EI-MS: m/z = 272 [M + H]$^+$, 314 [M – H_2O]$^+$. $C_8H_{13}N_7O_4$ (271.10): calcd. C 35.43, H 4.83, N 36.15; found C 35.43, H 4.81, N 36.08.

***N*-Acetyl-3,6-diamino-3,6-dideoxy-β-D-glucopyranosylamine (40):** Et_3N (0.38 mL, 4 equiv.) and propane-1,3-dithiol (0.21 mL, 4 equiv.) were added to a solution of **39** (0.20 g, 0.73 mmol) in freshly distilled MeOH (4 mL) in a vessel previously purged with N_2. The reaction mixture was stirred at room temperature for 2 h under N_2 whilst monitoring by TLC (45:5:3 EtOAc/EtOH/H_2O). Water was then added (50 mL) and the aqueous solution was washed with CH_2Cl_2 (2×25 mL). Freeze-drying of the aqueous solution afforded **40** as a white lyophilisate that was used in the next coupling reaction without further purification. Yield: 0.125 g (78%); R_f = 0.22 (4:1:1 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = –15.0 (c = 1.0, H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 5.12 (d, J = 9.1 Hz, 1 H, H-1), 3.69 (ddd, J = 9.6, 7.6, 2.8 Hz, 1 H, H-5), 3.46 (dd, J = 9.6, 9.1 Hz, 1 H, H-2), 3.39 (t, J = 9.6 Hz, 1 H, H-4), 3.29 (dd, J = 13.9, 2.8 Hz, 1 H, H-6a), 3.00 (t, J = 9.6 Hz, 1 H, H-3), 2.98 (dd, J = 13.9, 7.6 Hz, 1 H, H-6b), 2.02 (s, 3 H, MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O): δ = 175.8 (CO), 80.0 (C-1), 78.1 (C-5), 72.1 (C-2), 70.4 (C-4), 58.9 (C-3), 41.5 (C-6), 22.4 (*Me*CO) ppm. HRFAB-MS: calcd. for $C_8H_{17}N_3O_4$ 220.129731; found 220.128948.

***N*-Acetyl-3,6-dideoxy-3,6-bis[*N*′-(2,4-di-*O*-acetyl-3,6-diazido-3,6-dideoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosylamine (41):** The coupling reaction of diamine **40** (20 mg, 0.091 mmol) and isothiocyanate **17** (64 mg, 0.182 mmol) in pyridine (10 mL) at room temperature for 2 h, followed by column chromatography (1:1 EtOAc/petroleum ether → 45:5:3 EtOAc/EtOH/H_2O), afforded **41**. Yield: 65.8 mg (77%); R_f = 0.64 (45:5:3 EtOAc/EtOH/H_2O). $[\alpha]_D$ = –13.0 (c = 0.8, CH_2Cl_2). UV (CH_2Cl_2): λ = 254 nm (ε_{mM} = 11.8). IR (KBr): $\tilde{\nu}_{max}$ = 3324, 3079, 2932, 2108, 1750, 1541, 1375, 1219, 1099, 1036 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, 50 °C): δ = 5.75 (d, J = 8.5 Hz, 1 H, H-1^{III}), 5.81 (d, J = 8.5 Hz, 1 H, H-1^{II}), 4.93 (d, J = 9.0 Hz, 1 H, H-1^{I}), 4.88 (m, 1 H, H-2^{II}), 4.88 (t, J = 9.8 Hz, 2 H, H-4^{III}, H-4^{II}), 4.87 (m, 1 H, H-2^{III}), 4.03 (m, 1 H, H-6^{I}a), 3.96 (t, J = 9.8 Hz, 1 H, H-3^{II}), 3.95 (t, J = 9.8 Hz, 1 H, H-3^{III}), 3.80 (ddd, J = 9.8, 3.0, 5.9 Hz, 2 H, H-5^{II}, H-5^{III}), 3.56 (m, 1 H, H-6^{I}b), 3.48 (m, 2 H, H-3^{I}, H-5^{I}), 3.45 (dd, J = 13.5, 3.0 Hz, 1 H, H-6^{II}a), 3.40 (dd, J = 13.5, 3.0 Hz, 1 H, H-6^{III}a), 3.33 (t, J = 9.0 Hz, 1 H, H-2^{I}), 3.29 (m, 2 H, H-6^{II}b, H-6^{III}b), 3.24 (t, J = 10.0 Hz, 1 H, H-4^{I}), 2.13 (s, 12 H, 4 MeCO), 2.08 (s, 3 H, MeNHCO) ppm. ^{13}C NMR (125.7 MHz, CD_3OD, 40 °C): δ = 187.3, 185.9 (2 CS), 174.3 (CO amide), 171.9, 171.7, 171.2 (4 CO ester), 84.2 (C-1^{II}), 83.8 (C-1^{III}), 82.0 (C-1^{I}), 78.6 (C-4^{I}), 76.2 (C-5^{II}), 76.1 (C-5^{III}), 72.4 (C-2^{I}, C-2^{II}, C-2^{III}), 70.8 (C-5^{I}, C-4^{II}, C-4^{III}), 66.1 (C-3^{II}), 66.0 (C-3^{III}), 64.1 (C-3^{I}), 52.0 (C-6^{II}, C-6^{III}), 47.3 (C-6^{I}), 22.9 (*Me*NHCO), 20.7 (4 *Me*CO) ppm. FAB-MS: m/z = 952 [M + Na]$^+$. $C_{30}H_{43}N_{17}O_{14}S_2$ (929.26): calcd. C 38.75, H 4.66, N 25.61; found C 38.78, H 4.62, N 25.53.

***N*-Acetyl-3,6-dideoxy-3,6-bis[3-(3,6-diazido-3,6-dideoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosylamine (42):** O-Deacetylation of the hemiacetylated pseudotrisaccharide adduct **41** (38.5 mg, 0.041 mmol) was effected in methanol (5 mL) by treatment with NaOMe (0.1 equiv. per mol of acetate) for 2 h. The reaction mixture was neutralised with Amberlite IRA 120 (H^+) ion-exchange resin, then filtered and concentrated to give **42**. Yield: 31 mg (99%); R_f = 0.54 (45:5:3 EtOAc/EtOH/H_2O. $[\alpha]_D$ = –18 (c = 1.0, MeOH). IR (KBr): $\tilde{\nu}_{max}$ = 3423, 2922, 2882, 2109, 1650, 1540, 1287, 1071, 1028 cm^{-1}. ^{1}H NMR (500 MHz, D_2O, 70 °C): δ = 5.94 (d, J = 8.0 Hz, 1 H, H-1^{III}), 5.79 (br. d, 1 H, J = 9.2 Hz, H-1^{II}), 5.45 (d, J = 9.3 Hz, 1 H, H-1^{I}), 4.83 (m, 1 H, H-6^{I}a), 4.37 (m, 1 H, H-6^{I}b), 4.15 (m, 1 H, H-3^{I}), 4.15 (ddd, J = 9.3, 5.1, 2.1 Hz, 1 H, H-5^{III}), 4.13 (m, 1 H, H-5^{I}), 4.12 (ddd, J = 9.2, 6.6, 3.9 Hz, 1 H, H-5^{II}), 4.12 (dd, J = 13.3, 2.1 Hz, 1 H, H-6^{III}a), 4.07 (dd, J = 14.2, 3.9 Hz, 1 H, H-6^{II}a), 3.98 (t, J = 9.2 Hz, 1 H, H-3^{II}), 3.97 (t, J = 9.3 Hz, 1 H, H-2^{I}), 3.99 (t, J = 9.3 Hz, 1 H, H-3^{III}), 3.96 (t, J = 9.3 Hz, 1 H, H-2^{III}), 3.96 (m, 1 H, H-6^{III}b), 3.92 (t, J = 9.2 Hz, 1 H, H-2^{II}), 3.92 (t, J = 9.3 Hz, 1 H, H-4^{III}), 3.91 (t, J = 9.3 Hz, 1 H, H-4^{I}), 3.91 (m, 1 H, H-6^{II}b), 3.89 (t, J = 9.2 Hz, 1 H, H-4^{II}), 1.97 (s, 3 H, MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 333 K): δ = 184.3, 184.0 (2 CS), 175.7 (CO), 84.5 (C-1^{II}, C-1^{III}), 80.6 (C-1^{I}), 76.8 (C-5^{III}), 76.9 (C-5^{II}), 72.5 (C-2^{I}), 71.4 (C-2^{II}), 71.3 (C-2^{III}), 70.8 (C-5^{I}), 70.3 (C-4^{I}), 69.8, 69.7 (C-3^{II}, C-4^{II}, C-3^{III}, C-4^{III}), 63.0 (C-3^{I}), 51.3 (C-6^{II}, C-6^{III}), 46.0 (C-6^{I}), 22.7 (*Me*CO) ppm. FAB-MS: m/z = 784 [M + Na]$^+$. $C_{22}H_{35}N_{17}O_{10}S_2$ (761.75): calcd. C 34.69, H 4.63, N 31.26; found C 34.50, H 4.43, N 31.33.

***N*-Acetyl-3,6-dideoxy-3,6-bis[*N*′-(3,6-diamino-3,6-trideoxy-β-D-glucopyranosyl)thioureido]-β-D-glucopyranosylamine (43):** 1,3-Propanedithiol (30 μL, 4 equiv.) and triethylamine (54 μL, 8 equiv.) were added to a solution of **42** (40 mg, 0.052 mmol) in methanol (2 mL) under Ar. The reaction was stirred at room temperature for 2 h,

diluted with water (10 mL) and extracted with CH_2Cl_2 (2 × 5 mL). Freeze-drying of the aqueous solution afforded **46** as a white foam that was used directly in the next coupling reaction without further purification. Yield: 32.8 mg (95%); R_f = 0.54 (2:1:1 BuOH/AcOH/H_2O). ^{1}H NMR (500 MHz, D_2O, 70 °C): δ = 5.83 (d, J = 8.5 Hz, 1 H, H-1^{III}), 5.74 (br. d, 1 H, H-1^{II}), 5.43 (d, J = 9.0 Hz, 1 H, H-1^{I}), 4.99 (m, 1 H, H-6^{I}a), 4.37 (m, 1 H, H-6^{I}b), 4.10 (m, 2 H, H-3^{I}, H-5^{I}), 3.96 (m, 2 H, H-2^{I}, H-4^{I}), 3.90, 3.79 (2 m, 2 H, H-5^{II}, H-5^{III}), 3.74 (m, 2 H, H-2^{II}, H-2^{III}), 3.60 (m, 4 H, H-4^{II}, H-4^{III}, H-6^{II}a, H-6^{III}a), 3.28 (m, 4 H, H-3^{II}, H-3^{III}, H-6^{II}b, H-6^{III}b), 1.95 (s, 3 H, MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 60 °C): δ = 184.2 (2 CS), 175.8 (CO), 84.8 (C-1^{II}, C-1^{III}), 80.7 (C-1^{I}), 77.4 (C-5^{I}), 77.2 (C-5^{II}, C-5^{III}), 72.7 (C-2^{I}), 71.6 (C-2^{II}), 71.3 (C-2^{III}), 70.4 (C-4^{I}), 70.2, 69.9 (C-3^{II}, C-3^{III}, C-4^{II}, C-4^{III}), 63.1 (C-3^{I}), 51.3 (C-6^{II}, C-6^{III}), 46.0 (C-6^{I}), 22.8 (*Me*CO) ppm. FAB-MS: m/z = 658 [M + H]$^+$. HRFAB-MS: calcd. for $C_{22}H_{43}N_9O_{10}S_2$ 658.265075; found 658.265258.

***N*-Acetyl-3,6-dideoxy-3,6-bis[3-{3,6-bis[3-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thioureido]-3,6-dideoxy-β-D-glucopyranosyl}-thioureido]-β-D-glucopyranosylamine (45):** Compound **45** was obtained by a coupling reaction of tetraamine **43** (44.7 mg, 0.068 mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**44**; 106 mg, 0.27 mmol) in acetone/water (1:1, 6 mL) at pH 8 (solid $NaHCO_3$) at room temperature for 24 h, followed by column chromatography (EtOAc → 45:5:3 EtOAc/EtOH/H_2O). Yield: 115 mg (77%); R_f = 0.47 (45:5:3 EtOAc/EtOH/H_2O). $[\alpha]_D$ = –7.6 (c = 1.2, MeOH). UV (MeOH): λ = 249 nm (ε_{mM} = 74.4). IR (KBr): $\tilde{\nu}_{max}$ = 3358, 3071, 2930, 1753, 1547, 1377, 1233, 1038 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, 70 °C): δ = 5.81 (d, J = 9.5 Hz, 3 H, H-1^{III}, H-1^{V}, H-1^{VII}), 5.72 (br. s, 3 H, H-1^{II}, H-1^{IV}, H-1^{VI}), 5.31 (t, J = 9.5 Hz, 4 H, H-3^{IV}, H-3^{V}, H-3^{VI}, H-3^{VII}), 5.02 (t, J = 9.5 Hz, 12 H, H-2^{II}, H-2^{III}, H-2^{IV}, H-2^{V}, H-2^{VI}, H-2^{VII}, H-4^{II}, H-4^{III}, H-4^{IV}, H-4^{V}, H-4^{VI}, H-4^{VII}), 4.95 (d, J = 9.0 Hz, 1 H, H-1^{I}), 4.25 (dd, J = 12.5, 5.0 Hz, 6 H, H-6^{II}a, H-6^{III}a, H-6^{IV}a, H-6^{V}a, H-6^{VI}a, H-6^{VII}a), 4.11 (dd, J = 12.5, 2.0 Hz, 6 H, H-6^{II}b, H-6^{III}b, H-6^{IV}b, H-6^{V}b, H-6^{VI}b, H-6^{VII}b), 4.05 (m, 1 H, H-6^{I}a), 3.93 (m, 3 H, H-5^{II}, H-5^{IV}, H-5^{VI}), 3.90 (m, 3 H, H-5^{III}, H-5^{V}, H-5^{VII}), 3.55 (m, 3 H, H-3^{II}, H-3^{III}, H-6^{I}b), 3.42 (m, 2 H, H-3^{I}, H-5^{I}), 3.33 (m, 2 H, H-2^{I}, H-4^{I}), 2.04–1.95 (5 s, 51 H, 17 *Me*CO) ppm. ^{13}C NMR (125.7 MHz, CD_3OD, 40 °C): δ = 187.3, 185.8 (6 CS), 174.4 (CO amide), 173.0–171.4 (16 CO ester), 86.2, 86.1 (C-1^{IV}, C-1^{V} C-1^{VI}, C-1^{VII}), 84.1 83.1 (C-1^{II}, C-1^{III}), 82.1 (C-1^{I}), 78.7, 78.3, (C-5^{I}, C-5^{II}, C-5^{III}), 74.9, 74.8 (C-5^{IV}, C-5^{V} C-5^{VI}, C-5^{VII}), 74.6, 74.5 (C-4^{I}, C-4^{II}, C-4^{III}), 72.2 (C-2^{IV}, C-2^{V} C-2^{VI}, C-2^{VII}), 71.7, 71.5 (C-2^{I}, C-2^{II}, C-2^{III}), 70.0 (C-4^{IV}, C-4^{V} C-4^{VI}, C-4^{VII}), 69.9 (C-3^{IV}, C-3^{V} C-3^{VI}, C-3^{VII}), 64.2 (C-3^{I}, C-3^{II}, C-3^{III}), 63.3 (C-6^{IV}, C-6^{V} C-6^{VI}, C-6^{VII}), 47.3 (C-6^{I}, C-6^{II}, C-6^{III}), 21.5 (*Me*NCO), 19.3 (16 *Me*CO) ppm. FAB-MS: m/z = 2238 [M + Na]$^+$. MALDITOF-MS: m/z = 2237.625 [M + Na]$^+$. $C_{82}H_{119}N_{13}O_{46}S_6$ (2215.3): calcd. C 44.46, H 5.41, N 8.22; found C 44.18, H 5.30, N 8.11.

***N*-Acetyl-3,6-dideoxy-3,6-bis[*N*′-{3,6-bis[*N*′-(β-D-glucopyranosyl)-thioureido]-3,6-dideoxy-β-D-glucopyranosyl}thioureido]-β-D-glucopyranosylamine (46):** Methanolic NaOMe (1 M, 14 μL) was added to a solution of the hemiacetylated pseudoheptasaccharide adduct **45** (20 mg, 0.009 mmol) in methanol (3 mL). After 5 min, a white precipitate was observed. The suspension was stirred for 15 min, then water (1 mL) was added. The clear solution was further stirred for 30 min, neutralised with Amberlite IRA 120 (H^+) ion-exchange resin and demineralized with Duolite MB 6113 (H^+, OH^-) mixed ion-exchange resin. The reaction mixture was filtered, concentrated and the residue was dissolved in water and freeze-dried to give **46** as a white foam. Yield: 15 mg (99%); R_f = 0.19 (5:3:5 MeCN/H_2O/NH_4OH). $[\alpha]_D$ = –9.5 (c = 0.75, H_2O). UV (H_2O): λ = 245 nm (ε_{mM} = 64.8). IR (KBr): $\tilde{\nu}_{max}$ = 3351, 3054, 2922, 2863, 1098, 1038 cm^{-1}. ^{1}H NMR (500 MHz, D_2O, 60 °C): δ = 5.90 (d, J = 10.0 Hz, 1 H, H-1^{II}), 5.81 (d, J = 10.0 Hz, 2 H, H-1^{IV}, H-1^{VI}), 5.80 (br. d, J = 9.0 Hz, 1 H, H-1^{III}), 5.69 (br. d, J = 9.0 Hz, 2 H, H-1^{V}, H-1^{VII}), 5.45 (d, J = 9.0 Hz, 1 H, H-1^{I}), 5.00 (br. t, J = 9.0 Hz, 3 H, H-3^{I}, H-3^{II}, H-3^{III}), 4.42 (m, 1 H, H-6^{II}a), 4.41 (m, 1 H, H-6^{III}a), 4.37 (m, 1 H, H-6^{I}a), 4.28 (dd, J = 12.0, 2.0 Hz, 2 H, H-6^{IV}a, H-6^{VI}a), 4.26 (dd, J = 12.0, 2.0 Hz, 2 H, H-6^{V}a, H-6^{VII}a), 4.19 (m, 1 H, H-5^{II}), 4.17 (m, 2 H, H-6^{I}b, H-6^{III}b), 4.12 (m, 2 H, H-5^{I}, H-6^{II}b), 4.12 (m, 2 H, H-6^{V}b, H-6^{VII}b), 4.11 (m, 2 H, H-6^{IV}b, H-6^{VI}b), 4.10 (m, 1 H, H-5^{III}), 4.06 (t, J = 10.0 Hz, 1 H, H-2^{II}), 3.99 (t, J = 9.0 Hz, 2 H, H-2^{I}, H-2^{III}), 3.98 (t, J = 9.0 Hz, 2 H, H-3^{V}, H-3^{VII}), 3.98 (t, J = 10.0 Hz, 2 H, H-3^{IV}, H-3^{VI}), 3.95 (m, 4 H, H-5^{IV}, H-5^{V}, H-5^{VI}, H-5^{VII}), 3.93 (t, J = 9.0 Hz, 1 H, H-4^{I}), 3.92 (t, J = 10.0 Hz, 1 H, H-4^{II}), 3.90 (t, J = 9.0 Hz, 1 H, H-4^{III}), 3.87 (t, J = 10.0 Hz, 2 H, H-2^{IV}, H-2^{VI}), 3.84 (t, J = 9.0 Hz, 2 H, H-2^{V}, H-2^{VII}), 3.82 (t, J = 9.0 Hz, 4 H, H-4^{IV}, H-4^{V}, H-4^{VI}, H-4^{VII}), 2.49 (s, 3 H, MeCO) ppm. ^{13}C NMR (125.7 MHz, D_2O, 40 °C): δ = 184.0 (6 CS), 175.6 (CO), 84.5 (C-1^{IV}, C-1^{VI}), 83.7 (C-1^{II}, C-1^{V}, C-1^{VII}), 83.5 (C-1^{III}), 80.1 (C-1^{I}), 77.3 (C-3^{IV}, C-3^{VI}), 77.1 (C-3^{V}, C-3^{VII}), 76.7 (C-5^{IV}, C-5^{VI}), 76.6 (C-5^{V}, C-5^{VII}), 76.5 (C-1^{I}, C-1^{II}, C-1^{III}), 72.3 (C-2^{IV}, C-2^{VI}), 72.2 (C-2^{V}, C-2^{VII}), 72.1 (C-2^{I}), 70.9 (C-2^{II}), 70.8 (C-2^{III}), 69.7 (C-4^{I}, C-4^{II}, C-4^{III}), 69.4 (C-4^{IV}, C-4^{VI}), 69.1 (C-4^{V}, C-4^{VII}), 62.7 (C-3^{I}, C-3^{II}, C-3^{III}), 60.9 (C-6^{IV}, C-6^{VI}), 60.7 (C-6^{V}, C-6^{VII}), 45.8 (C-6^{I}, C-6^{II}, C-6^{III}), 22.4 (*Me*CO) ppm. MALDITOF-MS: m/z = 1565.88 [M + Na]$^+$, 1542.05 [M + H]$^+$. $C_{50}H_{87}N_{13}O_{30}S_6$ (1542.7): calcd. C 38.93, H 5.68, N 11.80; found C 39.16, H 5.63, N 11.54.

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