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# Urea-, Thiourea-, and Guanidine-Linked Glycooligomers as Phosphate Binders in Water

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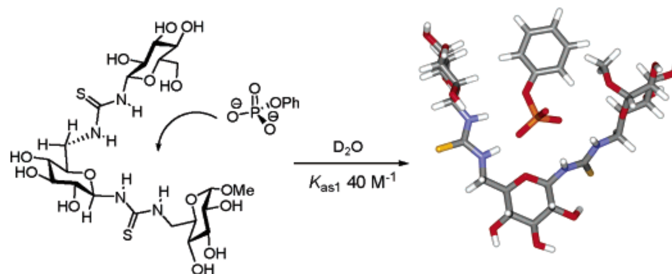


$\beta$-(1→6)-Linked pseudodi- and pseudotrisaccharides incorporating alternating pseudoamide-type (urea, thiourea, guanidine) intersaccharide bridges have been prepared and evaluated as phosphate binders in water. The monosaccharide subunits induce the *Z,Z* rotameric form at the pseudoamide segments, thus favoring their participation in bidentate hydrogen-bond interactions with oxoanions. Moreover, the conformational properties about the anomeric C-1—N bonds and the sugar C-5—C-6 bonds privilege orientations that facilitate both the desolvation of the incoming anionic guest and the stabilization of the complex by cooperative interactions. Measurable association constants ($K_{as}$) toward dimethyl and, especially, phenyl phosphate were obtained from NMR titration experiments for both series of glucooligomers, the binding affinity being strongly dependent on the nature of the pseudoamide functionality. Guanidinium derivatives, for which charge neutralization was expected to contribute to phosphate binding, were superior to the neutral thiourea and urea derivatives ($K_{as} = 48-60\ M^{-1}$ for 1:1 complexes with phenyl phosphate dianion). Interestingly, the thiourea oligomers exhibited association constants of the same order of magnitude ($K_{as} = 25-40\ M^{-1}$), much higher than those observed for the urea analogues ($K_{as} = 2-3\ M^{-1}$), which is ascribed to a less efficient solvation of the thiocarbonyl derivatives.

## 1. Introduction

Phosphate esters exist ubiquitously in nature in the form of nucleotides as components of DNA or RNA, as glycosyl phosphates and glycosyl phosphate esters of nucleotides as glycosylation donors, as glycosylation aceptors, and as chemical mediators. The specific interactions with their biological hosts are known to play a central role in intracellular signaling. Consequently, there is a sustained interest in the identification of natural as well as artificial phosphate receptors capable of differential binding according to the nature of the phosphate esterified groups.[1] Carbohydrates are a recent addition to the arsenal of such compounds. There exist a large number of nucleic-acid-binding natural products that contain carbohydrate components, including enediyne antitumor compounds such as calicheamicin $\gamma_1$, anthracyclines such as daunorubicin, and aminoglycoside antibiotics such as neomycin B,[2] and in a few cases, it has been shown that the saccharide portion itself mediates the sequence-specific recognition. Considering the

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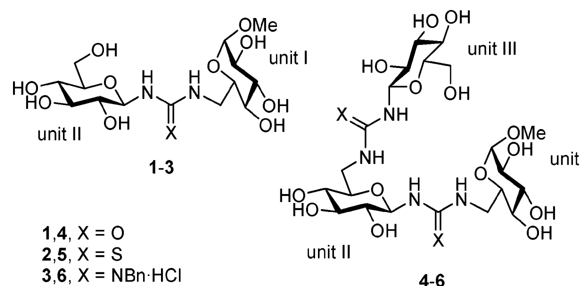
tremendous potential of carbohydrates to generate molecular diversity, the development of unnatural glycooligomers[3] for specific and predictable recognition of nucleic acids seems particularly appealing.

Many of the known nucleic-acid-binding carbohydrates bear ammonium groups, which can provide charge neutralization, whereas selectivity is frequently achieved through a network of hydrogen bonding.[4] The rational design of abiotic hosts for phosphates is generally based on this concept. Thus, most of the known molecular receptors for nucleosides and nucleotides use complementary hydrogen bonding for their recognition. Neutral (urea, thiourea) and charged (isothiouronium, guanidinium) pseudoamide groups[5] have been broadly used as fundamental platforms in such designs,[6] because they can establish a set of two parallel hydrogen bonds in the *Z*,*Z* rotameric form to provide a host−guest complex of well-defined dimensions and orientation. In aqueous medium, such molecular recognition is limited, however, as a result of the competitive hydrogen bonding of the solvent. We speculated that pseudoamide groups inserted into a glycooligomeric backbone could act in a cooperative manner to overcome the solvation energy.[7,8] Further contributions from the glucidic portions might then modulate the binding event.[9] To investigate the potential of this strategy for the design of phosphate binders, a series of pseudodi- (**1**−**3**) and trisaccharides (**4**−**6**) bearing alternating urea, thiourea, and guanidinium segments has now been synthesized (Chart 1). Their association properties toward dimethyl and phenyl phosphate, as models for monoanionic and polyanionic phosphate esters, respectively, have been investigated.

## 2. Results and Discussion

**2.1. Glycooligomer Design and Synthesis.** The $\beta$-(1→6) pseudoamide-linked D-glucopyranosyl substructure was purposely chosen as the repetitive motif in our glycooligomer design on the basis of two important considerations. First, pseudoamide groups $\beta$-linked to the anomeric position of monosaccharides are known to adopt exclusively the anti conformation at the anomeric carbon−N bond and the *Z* configuration at the corresponding N−(C=X) bond,[10] promoting the "active" *Z*,*Z* arrangement at the pseudoamide segment for bidentate recognition of oxoanions. Second, in glucose derivatives, the C-5−C-6 bond tends to adopt a major gauche−trans conformation that orients the substituent at C-6 close to that at C-1, so that these substituents are favorably preorganized to participate in cooperative interactions (Figure 1).[11]

**CHART 1. Pseudoamide-Linked Glucooligomers**




The general synthetic strategy relies on the high-yielding reaction between glycosyl isothiocyanates and amino-functionalized sugars.[10a] Desulfurization of the resulting thiourea adducts provides carbodiimide functionalities that can be further transformed into urea or guanidine derivatives through standard transformations.[12] Bearing in mind that enhancing the hydrophobicity in the vicinity of the hydrogen-bond-donating sites might increase phosphate binding affinity, we chose to prepare N‴-benzylguanidinium-linked glycooligomers for the purpose

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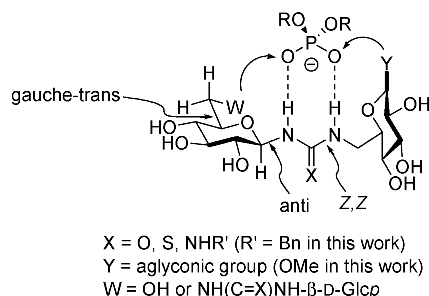



**FIGURE 1.** Conformational properties of pseudoamide-linked glucooligomers and possible cooperative interactions with phosphate anions.

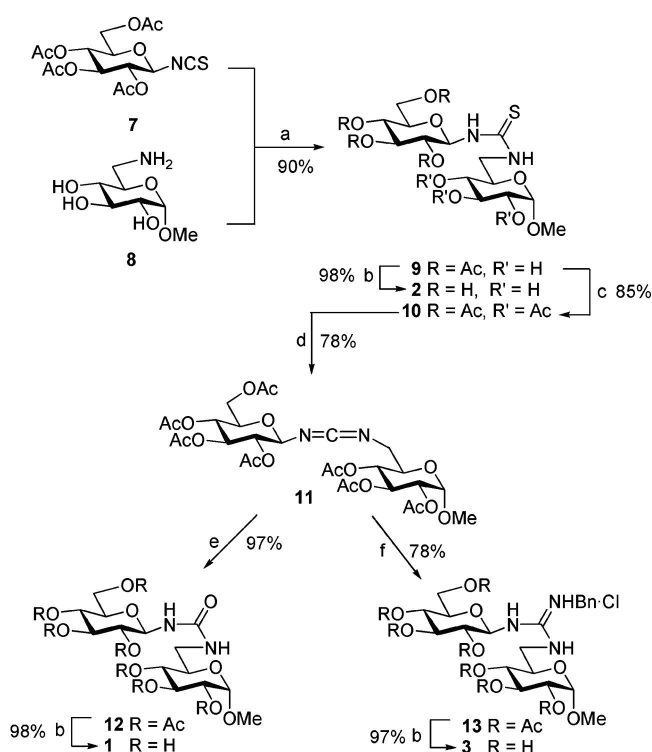
of this study. Collaterally, this choice facilitates chromatographic purification of the adducts as compared with N‴-unsubstituted derivatives.[13] For the preparation of the glucodimers, 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (**7**)[14] and methyl 6-amino-6-deoxy-α-D-glucopyranoside (**8**)[15] were used as the monosaccharide precursors. Their coupling reaction (to give **9**),[16] followed by conventional deacetylation, afforded the thiourea derivative **2**.[17] Alternatively, the hemiprotected adduct **9** was per-*O*-acetylated[18] (to give **10**) and transformed into the pivotal carbodiimide derivative **11** by reaction with mercuric oxide. Nucleophilic addition of water (to give **12**) or benzylamine hydrochloride (to give **13**) to the heteroallene group and final de-*O*-acetylation afforded the corresponding urea- and guanidinium-linked pseudodisaccharides **1**[17] and **3**, respectively (Scheme 1).

The synthesis of the homologous thiourea-linked glucotrimer **5** required the insertion of the bifunctional building block **14**[19] in the reaction sequence. Coupling with aminosugar **8** (to give **15**), deacetylation (to give **16**), and reduction of the terminal azido group (to give **17**) provided a new amino nucleophile ready for a final coupling reaction with the capping unit **7**. Deacetylation of the resulting adduct **18** led to the desired bis(thiourea) pseudotrisaccharide **5**, whereas acetylation (to give **19**) and desulfurization yielded the corresponding bis(carbodiimide) intermediate **20**, which was transformed into the target bis(urea) and bis(*N*-benzylguanidine) **4** and **6** via the corresponding per-*O*-acetylated derivatives **21** and **22**, respectively (Scheme 2).

**2.2. Phosphate Binding Evaluation.** NMR titration experiments for the fully unprotected glucooligomers against dimethyl and phenyl phosphate were carried out in pure $D_2O$ at 298 K.[20] Except for the urea-linked pseudodisaccharide **1**, significant changes were observed in the $^{1}H$ and $^{13}C$ spectral profiles with increasing concentration of the guest, which were more evident in the case of the dianionic phosphate ester, indicative of molecular recognition processes. $^{13}C$ NMR spectroscopy was used to determine the stoichiometry and the association constants of the complexes when the proton signals exhibited extensive overlapping. In the pseudodisaccharide series, the continuous variation method (Job's plot)[21] indicated the formation of 1:1 host−guest complexes in all cases. Consistently, the titration curves fitted well to a 1:1 model (Figure 2). The obtained association constants ($K_{as}$) are collected in Table 1.

No complex formation could be detected for the urea derivative **1** with monoanionic dimethyl phosphate, and only a very weak association was observed in the case of phenyl phosphate.[22] The phosphate binding affinity increased considerably for the thiourea and benzylguanidine analogues **2** and **3**, which must be ascribed to the higher acidity,[23] and consequently

**SCHEME 1. Synthesis of Pseudoamide-Linked Glucodimers**[a]




[a] Reagents and conditions: (a) pyridine, room temperature; (b) MeONa, MeOH; (c) 1:1 $Ac_2O$/pyridine, 0 °C; (d) HgO, $CH_2Cl_2/H_2O$; (e) TFA/$H_2O$; (f) $BnNH_2$·HCl, $Et_3N$, DMF, 100 °C.

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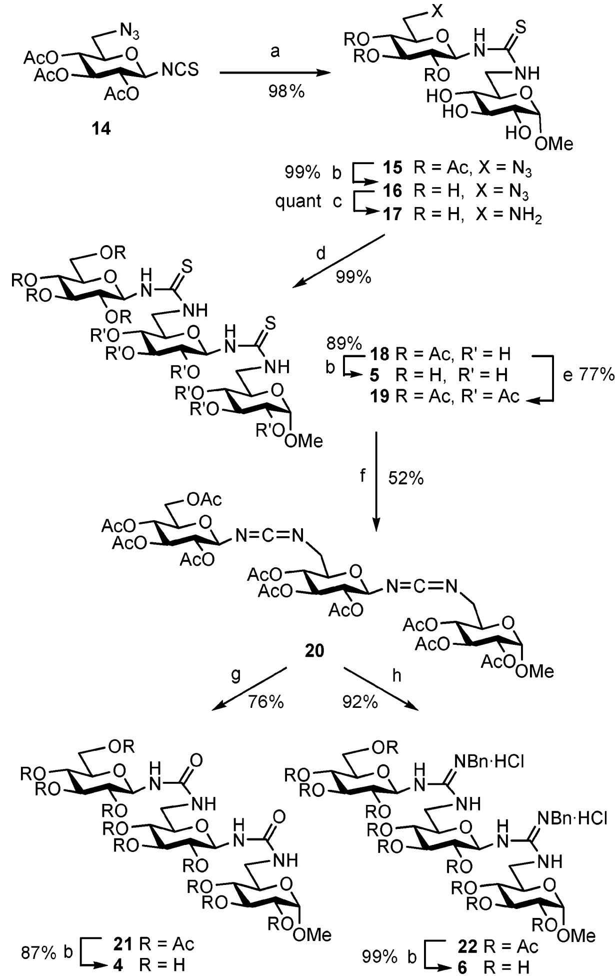
**SCHEME 2. Synthesis of Pseudoamide-Linked Glucotrimers**[a]




[a] Reagents and conditions: (a) **8**, pyridine, room temperature; (b) MeONa, MeOH; (c) 1,3-propanedithiol, $Et_3N$, MeOH; (d) **7**, Pyridine, room temperature; (e) 1:1 $Ac_2O$/pyridine, 0 °C; (f) HgO, $CH_2Cl_2/H_2O$; (g) TFA/$H_2O$; (h) $BnNH_2$·HCl, $Et_3N$, DMF, 100 °C.

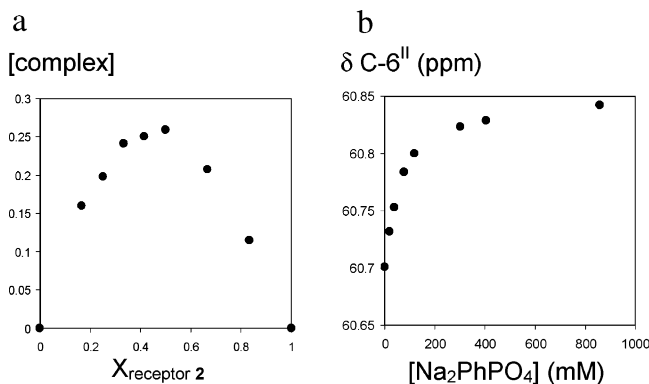



**FIGURE 2.** (a) Job's plot (total concentration = 50 mM) and (b) binding isotherm for compound **2** (78.8 mM) vs $PhPO_4Na_2$ in $D_2O$ at 298 K.

the higher hydrogen-bond-donating ability, of the corresponding NH protons. In the case of the glycosylguanidinium derivative **3**, the interaction is expected to be further reinforced by Coulombic attraction. Nevertheless, a comparison of data for **2** and **3** indicated rather small differences in the corresponding

**TABLE 1. Binding Constants ($K_{as}$)[a] for Receptors 1–6 and Phosphate Anions in $D_2O$ at 298 K**

| receptor | $K_{as(1:1)}$ ($M^{-1}$)/$K_{as(1:2)}$ ($M^{-1}$) | |
|---|---|---|
| | $Me_2PO_4Na$ | $PhPO_4Na_2$ |
| **1** | nd[b] | 2.5 ± 0.5 |
| **2** | 3.0 ± 0.5 | 25 ± 2 |
| **3** | 10 ± 1 | 48 ± 3 |
| **4** | nd | 3.1 ± 0.5 |
| **5** | 3.8 ± 0.6 | 40 ± 3/8.8 ± 2 |
| **6** | 13 ± 3 | 60 ± 3/20 ± 2 |

[a] Average values from at least two separate experiments. [b] Not detected.

$K_{as}$ values. Probably, because the guanidinium cation is much more efficiently solvated in water than the thiourea functionality,[24] its interaction with phosphate anions is mitigated to a greater extent.

The observation of phosphate complex formation in water, although weak, even for the neutral pseudoamide-linked glucodimers is remarkable. Examination of the NMR spectral changes during titration experiments indicated particularly high chemical shift differences for the *O*-methyl protons, the methylene protons at both monosaccharide subunits, and the anomeric carbons (Figure 3). These data are consistent with the location of the phosphate guest in the region occupied by the pseudoamide bridge, in the *Z*,*Z* rotameric form, with the host keeping the initial gauche–trans major conformation at the C-$5^{I}$–C-$6^{I}$ bond (Figure 1). This relative orientation brings the guest toward the aglyconic group, which might facilitate the desolvation process. On the other hand, the primary hydroxyl group at C-$6^{II}$ is favorably positioned to participate in phosphate complexation by acting cooperatively as a hydrogen-bond donor. Control experiments demonstrated that the preorganization provided by the pseudodisaccharide structure is critical for phosphate binding. Thus, replacement of any of the sugar units in the thiourea derivative **2** with an *N*-methyl substituent completely eliminated the recognition event.

Pseudoamide-linked trisaccharides have the potential to form bimolecular tetradentate complexes with phosphates. However, no significant differences were observed in the behavior of the urea-linked glucotrisaccharide **4** as compared to that of the homologous disaccharide **1**. Thus, no complex formation could be detected for dimethyl phosphate in deuterated water, and only a very weak complexation (1:1 host–guest) was measured with phenyl phosphate.[22] It seems that a minimum anchoring ability of the individual pseudoamide group is necessary to elicit any further cooperative interaction that might reinforce the supramolecular association. The phosphate binding behavior of the bis-

(22) Even though these small constants can be determined rather precisely by NMR spectroscopy, their significance must be taken with care. A consequence of very weak complexing is that high concentrations of ligand are generally required to drive the equilibrium, which results in possible medium effects, e.g., the chemical shifts of the host and the complex might not be constant throughout the experimental concentration range. For a discussion on the suitability of NMR spectroscopy to determine association constants, see ref 20a. In any case, the observed experimental values clearly indicate much weaker phosphate complexation by the urea-linked pseudooligosaccharides as compared with the thiourea- or gunidinium-linked analogues.

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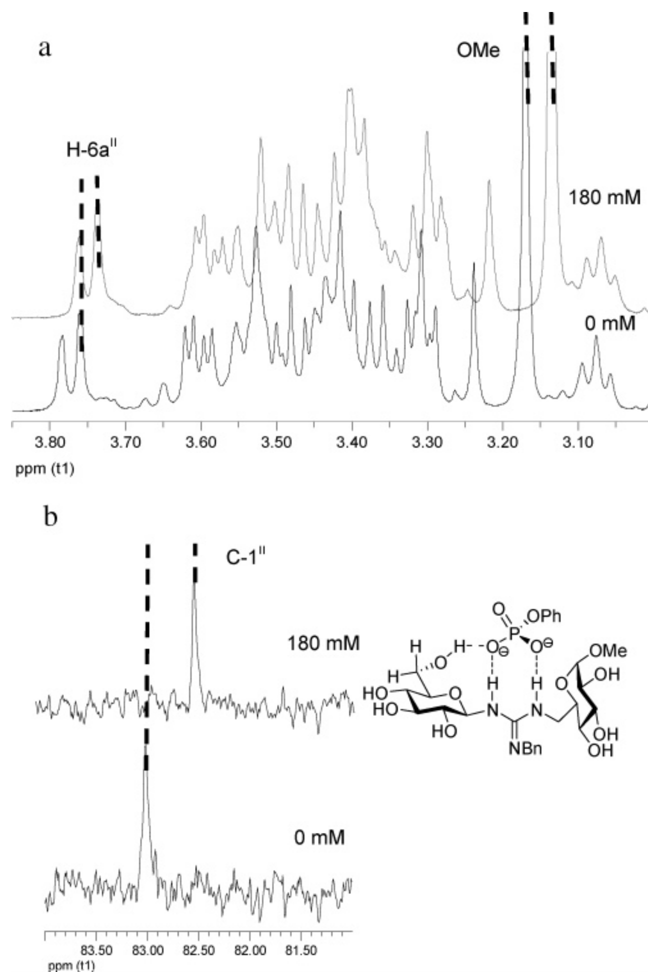



**FIGURE 3.** Stacked selected regions of the (a) $^1$H and (b) $^{13}$C NMR spectra of the guanidinium-linked pseudodisaccharide **3** (58.7 mM) in the absence (lower) and presence (180 mM, upper) of $Na_2PhPO_4$ in $D_2O$ at 298 K. The proposed structure of the 1:1 complex is shown.

(thiourea) and bis(guanidine) analogues **5** and **6** exposed a completely different scenario. Whereas the Job's plots and binding isotherms for dimethyl phosphate monoanion agreed with 1:1 stoichiometries, the corresponding curves for phenyl phosphate dianion suggested the existence of an equilibrium between 1:1 and 1:2 host−guest complexes (Figure 4a). Nonlinear regression analysis of the titration curves was consistent with this binding model (Figure 4b), affording the corresponding microscopic association constants $K_{as1}$ and $K_{as2}$ (Table 1).

A comparative analysis of the phosphate binding abilities of thiourea- and guanidine-linked pseudodi- and pseudotrisaccharides revealed some common trends. Thus, an increase about of 50% in the association constant for the formation of 1:1 complexes was observed for the bis(pseudoamide) derivatives as compared to the monotopic gluocodimers, which probably accounts for the existence of cooperative hydrogen bonding involving both pseudoamide groups in the higher homologues (Table 1). The observed chemical shift variations, which affect mainly the protons and carbons of units I and II and the anomeric region of unit III, are consistent with this assumption (Figure 5). The increase in $K_{as}$ for the 1:1 complexes ($K_{as1}$), however, is much lower than could be expected from previously reported results for multitopic pseudoamide receptors in aprotic solvents.[10a] Probably, the energetic cost of desolvation and the

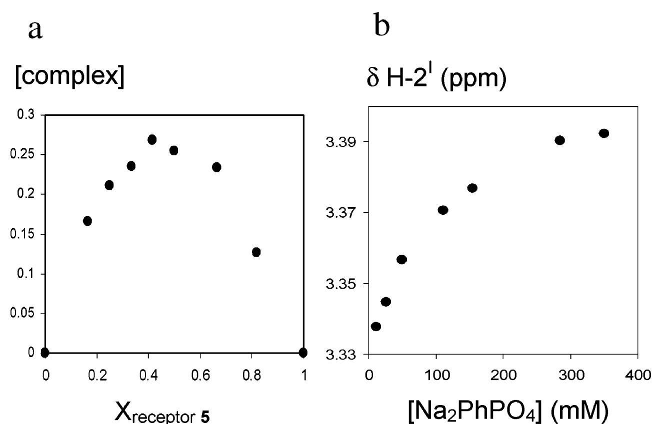
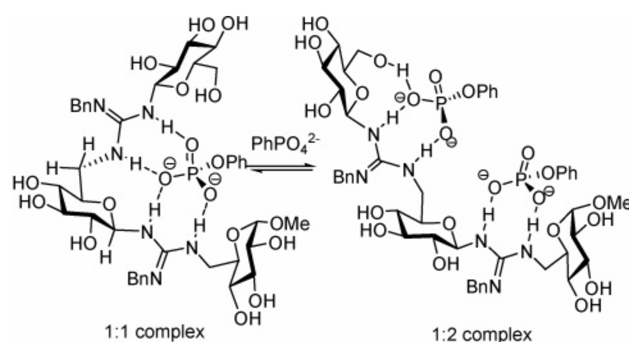



**FIGURE 4.** (a) Job's plot (total concentration = 50 mM) and (b) binding isotherm for compound **5** (29.9 mM) vs $Na_2PhPO_4$ in $D_2O$ at 298 K.



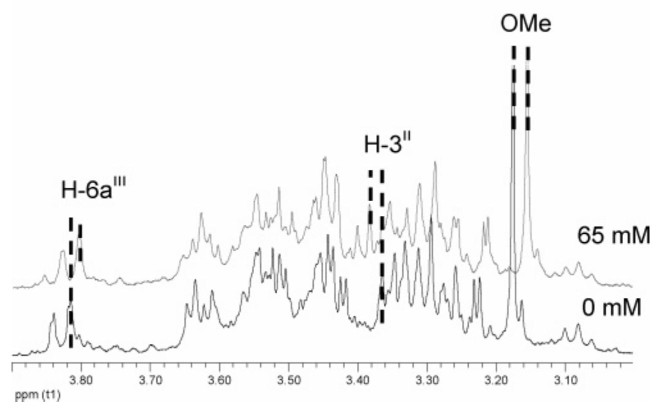




**FIGURE 5.** Stacked selected region of the $^1$H NMR spectra of the bis(guanidinium) pseudotrisaccharide **6** (10 mM) in the absence (lower) and presence (65 mM, upper) of $Na_2PhPO_4$ in $D_2O$ at 298 K. The proposed structures of the 1:1 and 1:2 complexes are shown.

entropic cost of fixing a defined conformation at the glycooligomer backbone partially cancel the advantages of multipoint hydrogen-bonding interactions. Formation of 1:2 complexes at high phenyl phosphate concentrations is comparatively more favorable for the bis(guanidine) compound as compared to the bis(thiourea). Charge neutralization likely favors the approach of the second anionic guest molecule to form two independent sets of bidentate four-center hydrogen bonds. The observed chemical shift variations for the methylene protons in unit III (Figure 5) at high concentrations of the guest implicates the terminal primary hydroxyl group in phosphate binding, as previously observed in the pseudodisaccharide homologues.

**2.3. Summary and Conclusions.** In the present work, we have shown that alternating monosaccharide−pseudoamide oligomers can bind phosphate anions in water. Our results indicate that the binding strength depends strongly on the nature of the pseudoamide group, especially the acidity of the NH protons and the solvation properties. The less acidic and highly solvated urea groups only form complexes with very weak associations affinities with dianionic phosphate, whereas the less solvated thioxo analogues exhibit significantly higher binding capabilities. In the case of guanidinium groups, the interplay of hydrogen bonds, Coulombic interactions, and a high desolvation energy results in association constants rather similar to those encountered with the neutral thioureas. In any case, a preorganization of the binding site to favor multitopic hydrogen bonding and desolvation of the incoming phosphate anion is an essential prerequisite for complex formation. Cooperative interactions involving several units in the chain per phosphate group as well as polyphosphate binding models are possible for higher pseudoamide-linked glycooligomers. Moreover, the synthetic strategy is compatible with the introduction of molecular diversity by modifying the linking positions and the configuration of the monosaccharide building blocks or by introducing branching units, which might result in specific interactions with phosphate side chains. On the whole, a powerful methodology for carbohydrate-based phosphate binding in water can be established on this new conceptual approach.

## Experimental Section

**Methyl 6-Deoxy-6-[*N*′-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)thioureido]-α-D-glucopyranoside (9).** A solution of methyl 6-amino-6-deoxy-α-D-glucopyranoside (**8**,[15] 150 mg, 0.76 mmol) and 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate (**7**,[14] 0.84 mmol, 324 mg) in pyridine was stirred for 2 h at room temperature. The solvent was evaporated, and the residue was chromatographed on silica gel (45:5:3 EtOAc/EtOH/water) to afford the thiourea adduct **9** in 90% yield (426 mg). The $^{1}$H and $^{13}$C NMR data were in full agreement with those reported in the literature.[16]

**Methyl 6-Deoxy-6-(*N*′-*β*-D-glucopyranosylthioureido)-α-D-glucopyranoside (2).** To a solution of **9** (100 mg, 0.17 mmol) in methanol (5 mL) was aded NaOMe (3.7 mg, 68 $\mu$mol), and the reaction mixture was stirred at room temperature for 1 h. Neutralization with Amberlite IRA 120 ($H^+$) ion-exchange resin afforded **2** in quantitative yield. The $^{1}$H and $^{13}$C NMR data were in full agreement with those reported in the literature.[10b]

**Methyl 2,3,4-Tri-*O*-acetyl-6-deoxy-6-[*N*′-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)thioureido]-α-D-glucopyranoside (10).** A solution of **9** (300 mg, 0.51 mmol) in 1:1 $Ac_2O$/pyridine (3 mL) was stirred at 4 °C for 2 h. Evaporation of the solvents under reduced pressure and purification by column chromatography (1:1 EtOAc/petroleum ether) of the resulting residue afforded the per-*O*-acetylated derivative **10** (310 mg, 85%). The $^{1}$H and $^{13}$C NMR data were in full agreement with those reported in the literature.[17]

**Methyl 2,3,4-Tri-*O*-acetyl-6-deoxy-6-[*N*′-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)carbodiimido]-α-D-glucopyranoside (11).** To a heterogeneous mixture of **10** (300 mg, 0.41 mmol) in $H_2O/CH_2Cl_2$ (1:1, 15 mL) was added yellow HgO (265 mg, 1.23 mmol). The mixture was stirred at room temperature for 30 min. The organic phase was separated, dried ($MgSO_4$), filtered through Celite, and concentrated. The residue was purified by column chromatography (1:1 EtOAc/petroleum ether) to furnish **11** (220 mg, 78%). The $^{1}$H and $^{13}$C NMR data were in full agreement with those reported in the literature.[17]

**Methyl 2,3,4-Tri-*O*-acetyl-6-deoxy-6-[*N*′-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)ureido]-α-D-glucopyranoside (12).** To a solution of **11** (81 mg, 0.12 mmol) in $H_2O$/acetone (1:2, 3 mL) was added TFA (50 $\mu$L), and the reaction mixture was stirred at room temperature for 15 min. The solvent was removed under vacuum, and the resulting residue was purified by column chromatography (2:1 EtOAc/petroleum ether) to give **12** (80 mg, 97%). The $^{1}$H and $^{13}$CNMR data were in full agreement with those reported in the literature.[17]

**Methyl 6-Deoxy-6-(*N*′-*β*-D-glucopyranosylureido)-α-D-glucopyranoside (1).** Conventional deacetylation of **12** (60 mg, 85 $\mu$mol), as described above for **2**, afforded **1** (35 mg, 98%) having analytical and spectroscopic data identical to those reported previously.[17]

**Methyl 2,3,4-Tri-*O*-acetyl-6-deoxy-6-[*N*′-(2,3,4,6-tetra-O-acetyl-*β*-D-glucopyranosyl)-*N*″-benzylguanidinio]-α-D-glucopyranoside Hydrochloride (13).** To a solution of **11** (110 mg, 0.16 mmol) in DMF (3 mL) was added benzylamine hydrochloride (135 mg, 5.8 equiv, 0.94 mmol), and the reaction mixture was stirred at 60 °C for 2 h. The solvent was removed under vacuum; the residue was dissolved in $CH_2Cl_2$; and the organic solution was washed with water, dried ($MgSO_4$), and concentrated. The resulting residue was purified by column chromatography (gradient from 9:1 to 3:1 EtOAc/MeOH) to yield **13** (78%, 185 mg). $R_f$ 0.14 (9:1 EtOAc/MeOH). $[\alpha]_D$ +61.0 (*c* 1.1, $CH_2Cl_2$). IR (KBr): 3038, 2942, 2861, 1751, 1647, 1616, 1370, 1225, 1044 cm$^{-1}$. $^{1}$H NMR (500 MHz, $CDCl_3$, 313 K): $\delta$ (ppm) 7.39 (bs, 3H, NH), 7.35−7.21 (m, 5H, Ph), 5.41 (t, 1H, $J_{2,3} = J_{3,4} = 10.2$ Hz, H-3$^{I}$), 5.30 (t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3$^{II}$), 5.23 (bd, 1H, $J_{1,2} = 9.5$ Hz, H-1$^{II}$), 5.05 (t, 1H, $J_{4,5} = 9.5$ Hz, H-4$^{II}$), 4.97 (t, 1H, H-2$^{II}$), 4.82 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1$^{I}$), 4.75 (dd, 1H, H-2$^{I}$), 4.70 (t, 1H, $J_{4,5} = 10.2$ Hz, H-4$^{I}$), 4.69 (d, 1H, $^{2}J_{H,H} = 15.3$ Hz, C$H_a$H$_b$Ph), 4.53 (d, 1H, CH$_a$$H_b$-Ph), 4.26 (dd, 1H, $J_{6a,6b} = 12.6$ Hz, H-6$^{II}$a), 4.22 (m, 1H, $J_{5,6a} = 5.0$ Hz, H-5$^{II}$), 4.14 (d, 1H, H-6$^{II}$b), 3.83 (ddd, 1H, $J_{5,6b} = 6.2$ Hz, $J_{5,6a} = 2.0$ Hz, H-5$^{I}$), 3.75 (bs, 1H, H-6$^{I}$a), 3.19 (s, 3H, OMe), 3.10 (bd, 1H, $J_{6a,6b} = 11.5$ Hz, H-6$^{I}$b), 2.06−1.93 (7 s, 21H, 7MeCO). $^{13}$C NMR (125.7 MHz, $CDCl_3$, 313 K): $\delta$ (ppm) 170.3−169.4 (7Me*C*O), 156.4 (CN guanidinium), 135.9−127.6 (Ph), 96.8 (C-1$^{I}$), 81.0 (C-1$^{II}$), 73.4 (C-5$^{I}$), 72.5 (C-3$^{II}$), 70.7 (C-2$^{I}$), 70.4 (C-2$^{II}$), 69.7 (C-4$^{I}$), 69.1 (C-3$^{I}$), 68.9 (C-5$^{II}$), 68.0 (C-4$^{II}$), 61.6 (C-6$^{II}$), 55.7 (OMe), 45.8 ($CH_2$), 43.1 (C-6$^{I}$), 20.7−20.4 (7*Me*CO). FABMS: *m*/*z* 782 ([M − Cl]$^+$). Anal. Calcd for $C_{35}H_{48}ClN_3O_{17}$: C, 51.38; H, 5.91; N, 5.14. Found: C, 51.01; H, 5.72; N, 5.08.

**Methyl 6-Deoxy-6-[*N*′-(*β*-D-glucopyranosyl)-*N*″-benzylguanidinio]-α-D-glucopyranoside Hydrochloride (3).** To a solution of **13** (46 mg, 0.055 mmol) in MeOH (1 mL) was added NaOMe (1.5 m, 27.5 $\mu$mol); the reaction mixture was stirred at room temperature for 1 h and then neutralized with solid $CO_2$ and concentrated. The residue was dissolved in 0.4 M aqueous HCl (1 mL), freeze-dried, and purified by gel permeation chromatography (GPC, Sephadex G-10, 1:1 MeOH/$H_2O$) to give **3** (31 mg, 97%). $R_f$ 0.17 (4:1:1 MeCN/$H_2O$/$NH_4OH$). $[\alpha]_D$ +9.0 (*c* 1.0, $H_2O$). IR (KBr): 3314, 2926, 1643, 1454, 1370, 1101, 1045 cm$^{-1}$. $^{1}$H NMR (500 MHz, $D_2O$, 298 K): $\delta$ (ppm) 7.32−7.23 (m, 5H, Ph), 4.60 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1$^{II}$), 4.55 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1$^{I}$), 4.47(d, 1H, $^{2}J_{H,H} = 16.0$ Hz, C$H_a$H$_b$Ph), 4.41 (d, 1H, CH$_a$$H_b$Ph), 3.78 (dd, 1H, $J_{6a,6b} = 12.3$ Hz, $J_{5,6a} = 2.0$ Hz, H-6$^{II}$a), 3.61 (dd, 1H, $J_{5,6b} = 5.6$ Hz, H-6$^{II}$b), 3.59 (d, 1H, $J_{6a,6b} = 11.5$ Hz, H-6$^{I}$a), 3.54 (dd, 1H, $J_{4,5} = 9.3$ Hz, $J_{5,6b} = 6.9$ Hz, H-5$^{I}$), 3.49 (t, 1H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3$^{I}$), 3.44 (m, 1H, H-5$^{II}$), 3.43 (t, 1H, $J_{2,3} = J_{3,4} = 8.5$ Hz, H-3$^{II}$), 3.43 (dd, 1H, H-6$^{I}$b), 3.42 (s, 3H, OMe), 3.38 (t, 1H, H-2$^{II}$), 3.32 (dd, 1H, H-2$^{I}$), 3.31 (t, 1H, $J_{4,5} = 8.5$ Hz, H-4$^{II}$), 3.08 (t, 1H, $J_{4,5} = 9.3$ Hz, H-4$^{I}$). $^{13}$C NMR (125.7 MHz, $D_2O$, 298 K): $\delta$ (ppm) 156.6 (CN guanidinium), 135.9−127.1 (Ph), 99.2 (C-1$^{I}$), 82.4 (C-1$^{II}$), 77.3 (C-5$^{II}$), 76.2 (C-3$^{II}$), 72.7 (C-3$^{I}$), 71.9 (C-2$^{II}$), 71.0 (C-2$^{I}$), 70.5 (C-4$^{I}$), 70.5 (C-5$^{I}$), 69.0 (C-4$^{II}$), 60.5 (C-6$^{II}$), 55.1 (OMe), 45.1 ($CH_2$), 42.7 (C-6$^{I}$). FABMS: *m*/*z* 510 ([M − HCl + Na]$^+$), 488 ([M − Cl]$^+$). Anal. Calcd for $C_{21}H_{34}ClN_3O_{10}\cdot H_2O$: C, 46.54; H, 6.70; N, 7.75. Found: C, 46.46; H, 6.72; N, 7.60.

**Methyl 6-Deoxy-6-[*N*′-(2,3,4-tri-O-acetyl-6-azido-6-deoxy-*β*-D-glucopyranosyl)thioureido]-α-D-glucopyranoside (15).** A solution of amine **8** (83 mg, 0.43 mmol) and isothiocyanate **14**[19] (176

mg, 0.47 mmol, 1.1 equiv) in pyridine (4 mL) was stirred at room temperature for 5 h and then concentrated and coevaporated with toluene. The resulting residue was purified by column chromatography (gradient from EtOAc to 45:5:3 EtOAc/EtOH/$H_2O$) to give **15** (241 mg, 98%) having $^1$H and $^{13}$C NMR data in full agreement with those reported in the literature.[19]

**Methyl 6-Deoxy-6-[*N*′-(6-azido-6-deoxy-*β*-D-glucopyranosyl)-thioureido]-α-D-glucopyranoside (16).** Conventional deacetylation of **15** (440 mg, 0.78 mmol), as described above for **2**, yielded **16** (344 mg, 99%) having $^1$H and $^{13}$C NMR data in full agreement with those reported in the literature.[19]

**Methyl 6-Deoxy-6-[*N*′-(6-amino-6-deoxy-*β*-D-glucopyranosyl)-thioureido]-α-D-glucopyranoside (17).** To a solution of azide **16** (381 mg, 0.68 mmol) in MeOH (20 mL) under Ar were added 1,3-propanedithiol (141 $\mu$L, 1.4 mmol) and triethylamine (192 $\mu$L, 1.4 mmol). The reaction mixture was stirred at room temperature for 16 h, diluted with water, and extracted with $CH_2Cl_2$. Freeze-drying of the aqueous solution afforded **17** (354 mg, 99%) as a hygroscopic white foam that was used directly in the next coupling reaction without further purification. The $^1$H and $^{13}$C NMR data were in full agreement with those reported in the literature.[19]

**Methyl 6-Deoxy-6-[*N*′-[6-deoxy-6-[*N*′-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)thioureido]-*β*-D-glucopyranosyl]thioureido]-α-D-glucopyranoside (18).** A solution of amine **17** (0.25 g, 0.61 mmol) and isothiocyanate **7** (0.26 g, 0.67 mmol) in pyridine (5 mL) was stirred at room temperature for 1 h. Evaporation of the solvent and column chromatography of the resulting residue (45:5:3 EtOAc/EtOH/$H_2O$) yielded **18** (0.49 g, 99%). $R_f$ 0.14 (45:5:3 EtOAc/EtOH/$H_2O$). $[\alpha]_D$ +17.2 (*c* 1.0, $H_2O$). UV ($H_2O$): 245 nm ($\epsilon_{mM}$ 26.2). IR (KBr): 3507, 2913, 2108, 1755, 1578, 1375, 1240, 1072 cm$^{-1}$. $^1$H NMR (500 MHz, DMSO-$d_6$, 333 K): $\delta$ (ppm) 8.10 (d, 1H, $J_{NH,1}$ = 9.0 Hz, NH′$^{II}$), 8.03 (bd, 1H, NH$^{II}$), 7.53 (bs, 1H, NH′$^{I}$), 7.37 (bt, 1H, NH$^{I}$), 5.73 (bt, 1H, $J_{1,2}$ = 9.5 Hz, H-1$^{III}$), 5.23 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3$^{III}$), 5.01 (bs, 1H, H-1$^{II}$), 4.86 (t, 1H, $J_{4,5}$ = 9.5 Hz, H-4$^{III}$), 4.85 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3$^{I}$), 4.84 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3$^{II}$), 4.82 (t, 1H, H-2$^{III}$), 4.56 (d, 1H, $J_{OH,4}$ = 4.5 Hz, OH-4$^{I}$), 4.52 (d, 2H, $J_{OH,4}$ = $J_{OH,2}$ = 3.5 Hz, OH-4$^{II}$, OH-2$^{I}$), 4.45 (d, 1H, $J_{1,2}$ = 6.5 Hz, H-1$^{I}$), 4.11 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 5.0 Hz, H-6$^{III}$a), 3.95 (dd, 1H, $J_{5,6b}$ = 1.2 Hz, H-6$^{III}$b), 3.93 (ddd, 1H, H-5$^{III}$), 3.85 (m, 2H, H-6$^{I}$a, H-6$^{II}$a), 3.40 (m, 2H, H-6$^{I}$b, H-6$^{II}$b), 3.35 (t, 1H, $J_{4,5}$ = 9.7 Hz, H-4$^{I}$), 3.26 (s, 3H, OMe), 3.21 (m, 2H, H-2$^{I}$, H-4$^{II}$), 3.05 (m, 1H, H-2$^{II}$), 2.97 (m, 2H, H-5$^{I}$, H5$^{II}$), 1.95−1.89 (4 s, 12H, 4 MeCO). $^{13}$C NMR (125.7 MHz, DMSO-$d_6$, 323 K): $\delta$ (ppm) 183.4 (2CS), 173.4−169.7 (4Me*CO*), 100.4 (C-1$^{I}$), 84.5 (C-1$^{II}$), 82.1 (C-1$^{III}$), 77.5 (C-2$^{I}$), 75.8 (C-2$^{II}$), 73.5 (C-3$^{III}$), 73.4 (C-4$^{I}$), 73.2, 72.4 (C-3$^{I}$, C-3$^{II}$), 72.6 (C-5$^{III}$), 72.4 (C-4$^{II}$), 71.9 (C-5$^{II}$), 71.1 (C-2$^{III}$), 70.3 (C-5$^{I}$), 68.6 (C-4$^{III}$), 62.2 (C-6$^{III}$), 55.3 (OMe), 46.3 (C-6$^{I}$, C-6$^{II}$), 20.9−20.7 (4*Me*CO). FABMS: *m/z* 825 (15%, [M + Na]$^+$), 803 (5%, [M + H]$^+$). Anal. Calcd for $C_{29}H_{46}N_4O_{18}S_2$: C, 43.39; H, 5.78; N, 6.98. Found: C, 43.26; H, 5.66; N, 6.79.

**Methyl 6-Deoxy-6-[*N*′-[6-deoxy-6-(*N*′-*β*-D-glucopyranosyl-thioureido)-*β*-D-glucopyranosyl]thioureido]-α-D-glucopyranoside (5).** Conventional deacetylation of the hemiacetylated pseudotrisaccharide **18** (160 mg, 0.20 mmol), as previously described for **2**, yielded **5** (110 mg, 89%); $R_f$ 0.37 (6:3:1 MeCN/$H_2O$/$NH_4OH$). $[\alpha]_D$ +1.5 (*c* 1, $H_2O$). UV (MeOH): 244 nm ($\epsilon_{mM}$ 33.4). IR (KBr): 3412, 2907, 1560, 1408, 1018 cm$^{-1}$. $^1$H NMR (500 MHz, $D_2O$, 343 K): $\delta$ (ppm) 5.66 (bd, 1H, $J_{1,2}$ = 9.4 Hz, H-1$^{II}$), 5.63 (bd, 1H, $J_{1,2}$ = 9.2 Hz, H-1$^{III}$), 5.15 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1$^{I}$), 4.38 (m, 2H, H-6$^{II}$a, H-6$^{II}$b), 4.31 (m, 2H, H-6$^{I}$a, H-6$^{I}$b), 4.21 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 2.0 Hz, H-6$^{III}$a), 4.12 (m, 1H, H-5$^{I}$), 4.08 (dd, 1H, $J_{5,6b}$ = 5.0 Hz, H6$^{III}$b), 4.00 (m, 1H, H-5$^{II}$), 3.99 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3$^{I}$), 3.93 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3$^{III}$), 3.92 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3$^{II}$), 3.91 (dd, 1H, H-2$^{I}$), 3.90 (ddd, 1H, $J_{4,5}$ = 9.2 Hz, H-5$^{III}$), 3.81 (t, 1H, H-2$^{II}$), 3.78 (t, 1H, H-2$^{III}$), 3.77 (t, 1H, H-4$^{III}$), 3.75 (s, 3H, OMe), 3.71 (t, 1H, $J_{4,5}$ = 9.5 Hz, H-4$^{II}$), 3.67 (t, 1H, $J_{4,5}$ = 9.5 Hz, H-4$^{I}$). $^{13}$C NMR (125.7 MHz, $D_2O$, 343 K) $\delta$ (ppm) 183.7 (2 CS), 99.9 (C-1$^{I}$), 84.2 (C-1$^{III}$), 84.0 (C-1$^{II}$), 77.8 (C-5$^{III}$), 77.1 (C-3$^{II}$), 76.9 (C-3$^{III}$), 75.8 (C-5$^{II}$), 73.5 (C-3$^{I}$), 72.7 (C-2$^{III}$), 72.6 (C-2$^{II}$), 71.9 (C-2$^{I}$), 71.8 (C-4$^{I}$), 71.5 (C-4$^{II}$), 70.4 (C-5$^{I}$), 70.0 (C-4$^{III}$), 61.3 (C-6$^{III}$), 55.7 (OMe), 45.8 (C-6$^{I}$, C-6$^{II}$). FABMS: *m/z* 657 (10%, [M + Na]$^+$). Anal. Calcd for $C_{21}H_{38}N_4O_{14}S_2$: C, 39.74; H, 6.03; N, 8.83. Found: C, 39.62; H, 5.96; N, 8.68.

**Methyl 2,3,4-Tri-*O*-acetyl-6-deoxy-6-[*N*′-[2,3,4-tri-*O*-acetyl-6-deoxy-6-[*N*′-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)thioureido]-*β*-D-glucopyranosyl]thioureido]-α-D-glucopyranoside (19).** A solution of **18** (160 mg, 0.20 mmol) in 1:1 $Ac_2O$/pyridine (5 mL) was stirred at 4 °C for 2 h. Evaporation of the solvents and purification by column chromatography (gradient from 1:1 to 2:1 EtOAc/petroleum ether) yielded **19** (160 mg, 77%). $R_f$ 0.28 (2:1 EtOAc/petroleum ether). $[\alpha]_D$ +44.0 (*c* 1.0, $CH_2Cl_2$). UV ($CH_2Cl_2$): 248 nm ($\epsilon_{mM}$ 36.9). IR (film): 3362, 2943, 1763, 1554, 1364, 1198, 984 cm$^{-1}$. $^1$H NMR (500 MHz, $CD_3OD$, 313 K): $\delta$ (ppm) 5.86 (bd, 1H, $J_{1,2}$ = 10.0 Hz, H-1$^{II}$), 5.82 (bd, $J_{1,2}$ = 10.0 Hz, H-1$^{III}$), 5.41 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3$^{I}$), 5.37 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3$^{III}$), 5.35 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3$^{II}$), 5.05 (t, 1H, $J_{4,5}$ = 10.0 Hz, H-4$^{III}$), 4.02 (bt, 1H, H-2$^{II}$), 5.01 (t, 1H, H-2$^{III}$), 4.99 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1$^{I}$), 4.89 (dd, 1H, H-2$^{I}$), 4.88 (t, 2H, $J_{4,5}$ =10.0 Hz, $J_{4,5}$ = 10.0 Hz, H-4$^{I}$, H-4$^{II}$), 4.34 (dd, 1H, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 4.6 Hz, H-6$^{III}$a), 4.17 (dt, 1H, $J_{5,6b}$ = 4.6 Hz, H-5$^{III}$), 4.04 (m, 1H, H-5$^{I}$), 3.97 (m, 2H, H-6$^{I}$a, H-6$^{II}$a), 3.96 (dd, 1H, H-6$^{III}$b), 3.85 (bt, 1H, H-5$^{II}$), 3.82 (m, 1H, H-6$^{I}$b), 3.62 (m, 1H, H-6$^{II}$b), 3.43 (s, 3H, OMe), 2.08−1.98 (10 s, 30H, MeCO). $^{13}$C NMR (125.7 MHz, $CD_3OD$, 313 K): $\delta$ (ppm) 184.7, 184.3 (2CS), 173.2−172.2 (10Me*C*O), 98.9 (C-1$^{I}$), 84.3 (C-1$^{II}$, C-1$^{III}$), 76.1 (C-5$^{II}$), 75.6 (C-3$^{II}$, C-3$^{III}$), 75.1 (C-5$^{I}$), 72.9 (C-4$^{I}$), 72.8 (C-2$^{II}$, C-2$^{III}$), 72.4 (C-3$^{I}$), 71.7 (C-4$^{III}$), 71.5 (C-2$^{I}$), 70.6 (C-4$^{II}$), 69.8 (C-5$^{III}$), 64.0 (C-6$^{III}$), 56.9 (OMe), 47.0 (C-6$^{II}$), 46.4 (C-6$^{I}$), 21.8−21.4 (10*Me*CO). FABMS: *m/z* 1077 (70%, [M + Na]$^+$), 1055 (100%, [M + H]$^+$). Anal. Calcd for $C_{41}H_{58}N_4O_{24}S_2$: C, 46.67; H, 5.54; N, 5.31. Found: C, 46.37; H, 5.48; N, 5.20.

**Methyl 2,3,4-Tri-*O*-acetyl-6-deoxy-6-[*N*′-[2,3,4-tri-*O*-acetyl-6-deoxy-6-[*N*′-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)carbodiimido]-*β*-D-glucopyranosyl]carbodiimido]-α-D-glucopyranoside (20).** Desulfurization of **19** (380 mg, 0.36 mmol) with yellow HgO, as described above for **11**, followed by purification by column chromatography (1:1 EtOAc/petroleum ether) yielded **20** (184 mg, 52%). $R_f$ 0.26 (2:1 EtOAc/petroleum ether). $[\alpha]_D$ +54.0 (*c* 1.0, $CH_2Cl_2$). IR (KBr): 2944, 2151, 1755, 1431, 1371, 1223, 1038 cm$^{-1}$. $^1$H NMR (500 MHz, $CDCl_3$, 298 K): $\delta$ (ppm) 5.48 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.9 Hz, H-3$^{I}$), 5.21 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3$^{II}$), 5.18 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.1 Hz, H-3$^{III}$), 5.10 (t, 1H, $J_{4,5}$ = 9.1 Hz, H-4$^{III}$), 5.09 (dd, 1H, $J_{1,2}$ = 8.6 Hz, H-2$^{II}$), 5.01 (t, 1H, $J_{4,5}$ = 9.9 Hz, H-4$^{I}$), 4.98 (dd, 1H, $J_{1,2}$ = 8.8 Hz, H-2$^{III}$), 4.97 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1$^{I}$), 4.94 (t, 1H, $J_{4,5}$ = 9.5 Hz, H-4$^{II}$), 4.90 (dd, 1H, H-2$^{I}$), 4.78 (d, 1H, H-1$^{III}$), 4.75 (d, 1H, H-1$^{II}$), 4.31 (dd, 1H, $J_{6a,6b}$ = 12.7 Hz, $J_{5,6a}$ = 4.9 Hz, H-6$^{III}$a), 4.22 (dd, 1H, $J_{5,6b}$ = 2.3 Hz, H-6$^{III}$b), 3.89 (ddd, 1H, $J_{5,6b}$ = 5.5 Hz, $J_{5,6a}$ = 2.8 Hz, H-5$^{I}$), 3.84 (ddd, 1H, H-5$^{III}$), 3.68 (m, 1H, H-5$^{II}$), 3.48 (m, 2H, H-6$^{I}$a, H-6$^{I}$b), 3.47 (m, 2H, H-6$^{II}$a, H-6$^{II}$b), 3.46 (s, 3H, OMe), 2.08−1.97 (10 s, 30H, 10MeCO). $^{13}$C NMR (125.7 MHz, $CDCl_3$, 298 K): $\delta$ (ppm) 170.7−169.4 (10Me*C*O), 138.0, 137.3 (2 NCN), 99.8 (C-1$^{I}$), 84.6 (C-1$^{II}$, C-1$^{III}$), 74.6 (C-5$^{II}$), 73.8 (C-5$^{III}$), 73.0 (C-3$^{III}$), 72.9 (C-4$^{I}$), 72.7 (C-4$^{II}$), 70.7 (C-2$^{I}$), 70.0 (C-3$^{II}$), 69.7 (C-2$^{III}$), 69.2 (C-2$^{II}$, C-4$^{III}$), 68.2 (C-3$^{I}$), 68.0 (C-5$^{I}$), 61.8 (C-6$^{III}$), 55.7 (OMe), 46.4 (C-6$^{I}$, C-6$^{II}$), 20.7−20.6 (10*Me*CO). FABMS: *m/z* 1009 (30%, [M + Na]$^+$), 987 (3%, [M + H]$^+$). Anal. Calcd for $C_{41}H_{54}N_4O_{24}$: C, 49.90; H, 5.52; N, 5.68. Found: C, 49.86; H, 5.41; N, 5.61.

**Methyl 2,3,4-Tri-*O*-acetyl-6-deoxy-6-[*N*′-[2,3,4-tri-*O*-acetyl-6-deoxy-6-[*N*′-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)ureido]-*β*-D-glucopyranosyl]ureido]-α-D-glucopyranoside (21).** To a solution of **20** (130 mg, 0.13 mmol) in $H_2O$/acetone (1:2, 3 mL) was added TFA (50 $\mu$L), and the reaction mixture was stirred at room temperature for 30 min. The solvent was removed under vacuum, and the resulting residue was purified by column chromatography (gradient from 4:1 EtOAc/petroleum ether to EtOAc) to give **21**

(101 mg, 76%). $R_f$ 0.37 (EtOAc). $[\alpha]_D$ +4.8 (*c* 1.0, $CH_2Cl_2$). IR (KBr): 3387, 2938, 2855, 1753, 1553, 1373, 1227, 1040 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$, 298 K): $\delta$ (ppm) 5.44 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3$^{I}$), 5.25 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3$^{II}$), 5.24 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3$^{III}$), 5.09 (t, 1H, $J_{1,2} = J_{NH,1}$ = 9.5 Hz, H-1$^{III}$), 5.06 (t, 1H, $J_{1,2} = J_{NH,1}$ = 9.5 Hz, H-1$^{II}$), 5.05 (t, 1H, $J_{4,5}$ = 9.5 Hz, H-4$^{III}$), 4.90 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1$^{I}$), 4.89 (t, 1H, H-2$^{III}$), 4.83 (t, 1H, $J_{4,5}$ = 9.5 Hz, H-4$^{I}$), 4.82 (t, 1H, H-2$^{II}$), 4.81 (t, 1H, $J_{4,5}$ = 9.5 Hz, H-4$^{II}$), 4.80 (dd, 1H, H-2$^{I}$), 4.29 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 4.0 Hz, H-6$^{III}$a), 4.04 (dd, 1H, $J_{5,6b}$ = 2.0 Hz, H-6$^{III}$b), 3.83 (dt, 1H, $J_{5,6b} = J_{5,6a}$ = 3.5 Hz, H-5$^{I}$), 3.79 (ddd, 1H, H-5$^{III}$), 3.61 (m, 2H, H-5$^{II}$, H-6$^{II}$a), 3.52 (m, 1H, H-6$^{I}$a), 3.38 (s, 3H, OMe), 3.33 (m, 1H, H-6$^{I}$b), 3.05 (m, 1H, H-6$^{II}$b), 2.10−1.98 (10 s, 30H, 10MeCO). $^{13}C$ NMR (125.7 MHz, $CDCl_3$, 298 K): $\delta$ (ppm) 171.2−169.6 (10Me*C*O), 157.1, 156.9 (2 CO urea), 96.8 (C-1$^{I}$), 80.1 (C-1$^{II}$, C-1$^{III}$), 74.9 (C-5$^{II}$), 73.3 (C-5$^{III}$), 73.0 (C-3$^{II}$, C-3$^{III}$), 70.8 (C-2$^{I}$, C-2$^{II}$), 70.7 (C-2$^{III}$), 70.0 (C-3$^{I}$), 69.3 (C-4$^{I}$), 69.1 (C-4$^{II}$), 68.2 (C-4$^{III}$), 67.9 (C-5$^{I}$), 61.7 (C-6$^{III}$), 55.5 (OMe), 40.9 (C-6$^{II}$), 39.8 (C-6$^{I}$), 20.7−20.6 (10*Me*CO). FABMS: *m/z* 1045 (40%, $[M + Na]^+$), 1023 (10%, $[M + H]^+$). Anal. Calcd for $C_{41}H_{58}N_4O_{26}$: C, 48.14; H, 5.72; N, 5.48. Found: C, 47.98; H, 6.03; N, 5.39.

**Methyl 6-Deoxy-6-[*N*′-[6-deoxy-6-(*N*′-*β*-D-glucopyranosylureido)-*β*-D-glucopyranosyl]ureido]-α-D-glucopyranoside (4).** Conventional deacetylation of **21** (95 mg, 0.09 mmol), as described above for **2**, gave **4** (49 mg, 87%). $R_f$ 0.20 (6:3:1 $CH_3CN/H_2O/NH_4OH$). $[\alpha]_D$ +25.0 (*c* 1.0, $H_2O$). IR (KBr): 3368, 2918, 1659, 1566, 1366, 1279, 1109, 1044 $cm^{-1}$. $^1H$ NMR (500 MHz, $D_2O$, 298 K): $\delta$ (ppm) 4.75 (d, 1H, $J_{1,2}$ = 9.0 Hz, H-1$^{III}$), 4.72 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1$^{II}$), 4.70 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1$^{I}$), 3.80 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 1.5 Hz, H6$^{III}$a), 3.63 (dd, 1H, $J_{5,6b}$ = 5.5 Hz, H-6$^{III}$b), 3.58 (ddd, 1H, $J_{4,5}$ = 9.0 Hz, $J_{5,6b}$ = 5.8 Hz, $J_{5,6a}$ = 2.4 Hz, H-5$^{I}$), 3.57 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3$^{I}$), 3.53 (dd, 1H, $J_{6a,6b}$ = 11.5 Hz, $J_{5,6a}$ = 3.0 Hz, H6$^{II}$a), 3.46 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3$^{III}$), 3.45 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3$^{II}$), 3.44 (m, 1H, H-5$^{II}$), 3.40 (m, 1H, H-5$^{III}$), 3.31 (s, 3H, OMe), 3.31 (t, 1H, $J_{4,5}$ = 9.0 Hz, H-4$^{III}$), 3.30 (m, 1H, H-6$^{II}$b), 3.29 (dd, 1H, $J_{6a,6b}$ = 14.6 Hz, H-6$^{I}$a), 3.27 (t, 1H, H-2$^{II}$), 3.27 (t, 1H, $J_{4,5}$ = 9.5 Hz, H-4$^{II}$), 3.26 (t, 1H, H-2$^{III}$), 3.22 (t, 1H, H-4$^{I}$), 3.22 (dd, 1H, H-6$^{I}$b), 3.21 (t, 1H, H-2$^{I}$). $^{13}C$ NMR (125.7 MHz, $D_2O$, 298 K): $\delta$ (ppm) 159.7 (2CO urea), 99.2 (C-1$^{I}$), 81.2 (C-1$^{II}$), 81.1 (C-1$^{III}$), 77.1 (C-5$^{III}$), 76.6 (C-3$^{III}$), 76.3 (C-3$^{II}$), 75.7 (C-5$^{II}$), 72.9 (C-3$^{I}$), 72.1, 72.0 (C-4$^{II}$, C-2$^{III}$), 71.2 (C-2$^{I}$), 71.0 (C-2$^{II}$), 70.8 (C-4$^{I}$), 70.4 (C-5$^{I}$), 69.4 (C-4$^{III}$), 60.7 (C-6$^{III}$), 55.0 (OMe), 40.8 (C-6$^{I}$), 40.4 (C-6$^{II}$). FABMS: *m/z* 625 (32%, $[M + Na]^+$). Anal. Calcd for $C_{21}H_{38}N_4O_{16}$: C, 41.86; H, 6.36; N, 9.30. Found: C, 41.55; H, 6.62; N, 9.23.

**Methyl 2,3,4-Tri-*O*-acetyl-6-deoxy-6-[*N*′-[2,3,4-tri-*O*-acetyl-6-deoxy-6-[*N*′-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-*N*″-benzyl)guanidinio]-*β*-D-glucopyranosyl-*N*″-benzyl]guanidinio]-α-D-glucopyranoside (22).** To a solution of **20** (85 mg, 86 μmol) in DMF (2 mL) was added benzylamine hydrochloride (145 mg, 1.01 mmol), and the reaction mixture was stirred at 60 °C for 2 h. The solvent was removed under vacuum; the residue was dissolved in $CH_2Cl_2$; and the organic solution was washed with water, dried ($MgSO_4$), and concentrated. The resulting residue was purified by column chromatography (gradient from 9:1 to 3:1 EtOAc/MeOH) to yield **22** (118 mg, 92%). $R_f$ 0.52 (85:15:1 $CH_2Cl_2$/MeOH/$H_2O$). $[\alpha]_D$ +16.0 (*c* 1.0, $CH_2Cl_2$). IR (KBr): 3290, 2940, 1753, 1649, 1611, 1370, 1225, 1042 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$, 313 K): $\delta$ (ppm) 7.60−7.30 (m, 16H, 2Ph, 6NH), 5.42 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.9 Hz, H-3$^{II}$), 5.35 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3$^{I}$), 5.34 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3$^{III}$), 5.32 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1$^{II}$), 5.20 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1$^{III}$), 5.16 (t, 1H, $J_{4,5}$ = 9.9 Hz, H-4$^{II}$), 5.15 (m, 2H, H-2$^{I}$, H-4$^{I}$), 5.15 (t, 1H, $J_{4,5}$ = 10.0 Hz, $J_{4,5}$ = 9.6 Hz, H-4$^{III}$), 5.09 (t, 1H, H-2$^{III}$), 4.95 (t, 1H, H-2$^{II}$), 4.77 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1$^{I}$), 4.58 (d, 2H, $^2J_{H,H}$ = 16.1 Hz, C$H_a$H$_b$-Ph), 4.52 (d, 2H, CH$_a$$H_b$Ph), 4.45 (m, 1H, H-5$^{II}$), 4.27 (dd, 1H, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 4.6 Hz, H-6$^{III}$a), 4.14 (bd, 1H, $J_{5,6b}$ = 1.0 Hz, H-6$^{III}$b), 4.06 (ddd, 1H, H-5$^{III}$), 3.84 (m, 1H, H-5$^{I}$), 3.73 (m, 1H, H-6$^{II}$a), 3.64 (m, 1H, H-6$^{I}$a), 3.40 (dd, 2H, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 6.9 Hz, H-6$^{I}$b, H-6$^{II}$b), 3.23 (s, 3H, OMe), 2.10−1.99 (5 s, 30H, 10MeCO). $^{13}C$ NMR (125.7 MHz, $CDCl_3$, 313 K) $\delta$ = 170.3−169.8 (10Me*C*O), 155.9, 155.8 (2CN guanidinium), 135.7−126.6 (Ph), 96.8 (C-1$^{I}$), 80.5 (C-1$^{II}$), 80.3 (C-1$^{III}$), 73.3 (C-5$^{III}$), 72.7 (C-3$^{I}$, C-4$^{III}$), 72.5 (C-5$^{II}$), 72.3 (C-3$^{II}$), 70.5 (C-2$^{I}$), 70.2 (C-4$^{I}$), 69.9 (C-4$^{II}$), 69.5 (C-2$^{II}$), 69.3 (C-3$^{III}$), 67.8 (C-5$^{I}$, C-2$^{III}$), 61.5 (C-6$^{III}$), 54.9 (OMe), 45.5 (2$CH_2$), 43.1 (C-6$^{II}$), 41.9 (C-6$^{I}$), 19.5−19.2 (10*Me*CO). FABMS *m/z* 1201 ($[M - 2Cl - H]^+$). Anal. Calcd for $C_{55}H_{74}Cl_2N_6O_{24}\cdot 2H_2O$: C, 50.42; H, 6.00; N, 6.41. Found: C, 50.62; H, 6.22; N, 6.34.

**Methyl 6-Deoxy-6-[*N*′-[6-deoxy-6-[(*N*′-*β*-D-glucopyranosyl-*N*″-benzyl)guanidinio]-*β*-D-glucopyranosyl-*N*″-benzyl]guanidinio]-α-D-glucopyranoside (6).** Deacetylation of **22** (110 mg, 85 μmol), as described above for **3**, and purification by GPC (Sephadex G-10, 1:1 MeOH/$H_2O$) afforded **6** (74 mg, 99%). $R_f$ 0.28 (5:3:5 $CH_3CN/H_2O/NH_4OH$). $[\alpha]_D$ −17.1 (*c* 1.0, MeOH). IR (KBr): 3312, 3065, 2922, 1645, 1362, 1044, 754 $cm^{-1}$. $^1H$ NMR (500 MHz, $D_2O$, 313 K): $\delta$ (ppm) 7.54−7.40 (m, 10H, 2Ph), 4.78 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1$^{II}$), 4.77 (d, 1H, $J_{1,2}$ = 8.7 Hz, H-1$^{III}$ ), 4.74 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1$^{I}$), 4.65−4.46 (m, 4H, 2C$H_2$Ph), 3.98 (dd, 1H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 1.7 Hz, H-6$^{III}$a), 3.91 (m, 1H, H-6$^{II}$a), 3.86 (m, 1H, H-6$^{II}$b), 3.80 (dd, 1H, $J_{5,6b}$ = 5.6 Hz, H-6$^{III}$b), 3.76 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3$^{II}$), 3.72 (m, 2H, H-5$^{I}$, H-6$^{I}$a), 3.71 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.9 Hz, H-3$^{I}$), 3.66 (m, 1H, H-5$^{II}$), 3.65 (t, 1H, H-2$^{II}$), 3.63 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.4 Hz, H-3$^{I}$), 3.61 (m, 1H, H-5$^{III}$), 3.56 (t, 1H, $J_{4,5}$ = 9.7 Hz, H-4$^{II}$), 3.53 (dd, 1H, $J_{5,6b}$ = 6.0 Hz, H-6$^{I}$b), 3.51 (t, 1H, H-2$^{III}$), 3.50 (dd, 1H, H-2$^{I}$), 3.49 (t, 1H, $J_{4,5}$ = 9.2 Hz, H-4$^{III}$), 3.34 (s, 3H, OMe), 3.27 (t, 1H, $J_{4,5}$ = 9.9 Hz, H-4$^{I}$). $^{13}C$ NMR (125.7 MHz, $D_3O$, 313 K) $\delta$ = 157.5, 157.4 (2CN guanidinium), 137.1−127.0 (Ph), 99.6 (C-1$^{I}$), 84.3 (C-1$^{III}$), 83.5 (C-1$^{II}$), 77.6 (C-3$^{II}$, C-3$^{III}$), 77.3 (C-3$^{I}$, C-5$^{II}$), 76.0 (C-5$^{III}$), 73.1 (C-5$^{I}$), 72.9 (C-2$^{II}$), 71.7 (C-4$^{III}$), 70.8 (C-4$^{I}$), 70.6, 70.5 (C-2$^{I}$, C-4$^{II}$), 69.8 (C-2$^{III}$), 61.0 (C-6$^{III}$), 55.4 (OMe), 45.6, 45.4 ($CH_2$), 43.7 (C-6$^{II}$), 42.8 (C-6$^{I}$). FABMS *m/z* 781 ($[M - 2Cl - H]^+$), 803 ($[M - 2HCl + Na]^+$). Anal. Calcd for $C_{35}H_{54}Cl_2N_6O_{14}\cdot 2H_2O$: C, 47.25; H, 6.57; N, 9.45. Found: C, 47.43; H, 6.21; N, 9.37.

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**Supporting Information Available:** General experimental procedures, NMR spectra of the final pseudodi- and pseudotrisaccharides **1**−**6**, and significant examples of titration curve fittings. This material is available free of charge via the Internet at http://pubs.acs.org.

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