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A Synthetic Pentasaccharide with GTPase Activity

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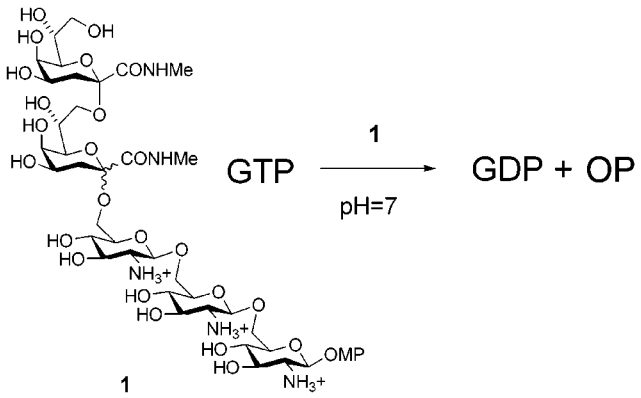
ABSTRACT



The design, synthesis, and preliminary evaluation of the first example of synthetic pentasaccharide (1) that shows marked rate enhancement and specificity for the hydrolysis of GTP to GDP and orthophosphate (OP) are reported. At the concentration ratios of GTP/1 = 3.6 and GTP/Mg^{2+} = 1 (pH 7.1, 50 °C), a rate enhancement of about 500-fold was obtained.

The discoveries of catalytic RNA (ribozyme)[1] and, more recently, the catalytic DNA (deoxyribozyme)[2] have immediately posed the question about the possible catalytic potency of oligosaccharides/polysaccharides, the third most abundant biopolymers in nature. However, even today while the complexity of macromolecular carbohydrate structures is well documented[3] and it is clear that they offer most of the structural and chemical diversity necessary for generating the broad range of specific catalysts, no such catalytic natural saccharide has yet been found.[4]

We assumed that in the framework of systematic research, it would be possible to either rationally design and synthesize or find in nature oligo- or polysaccharide structures capable of catalyzing certain chemical transformations. For this purpose, we formulated two complementary strategies. The first strategy utilizes a screening approach to search for catalytic activities among natural oligo- and polysaccharides, and the second one focuses on the chemical synthesis of carbohydrate-based catalysts. We report here the design, synthesis, and preliminary evaluation of the first example of synthetic pentasaccharide (**1**) that shows marked rate enhancement and specificity for the hydrolysis of GTP to GDP and orthophosphate (OP).

The design of pentasaccharide **1** as a first model structure is largely based on the earlier observations that several linear[5a] and cyclic polyamines[5b] are capable of producing a significant rate enhancement on ATP hydrolysis. Therefore,

(1) (a) Gurrier-Takada, C.; Altman, S. *Science* **1983**, *223*, 285. (b) Zang, A. J.; Cech, T. R. *Science* **1986**, *231*, 470.

(2) Breaker, R. R.; Joyce, G. F. *Chem. Biol.* **1994**, *1*, 223.

(3) For recent reviews in this field see the following special issues: (a) "Frontiers in Carbohydrate Research" *Chem. Rev.* **2000**, *291*, 4265−4712. (b) "Carbohydrates and Glycobiology" *Science* **2001**, *291*, 2337−2378.

(4) For cyclodextrin-based catalytic systems, see: (a) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997. (b) Han, M. J.; Yoo, K. S.; Chang, J. Y.; Ha, T.-K. *Angew. Chem., Int. Ed.* **2000**, *39*, 347. For ribose-containing catalytic polymers, see: (c) Han, M. J.; Yoo, K. S.; Cho, T. J.; Chang, J. Y.; Cha, Y. J.; Nam, S. H. *Chem. Commun.* **1997**, 163. (d) Han, M. J.; Yoo, K.; Kim, K. H.; Lee, G. H. S.; Chang, J. Y. *Macromolecules* **1997**, *30*, 5408.

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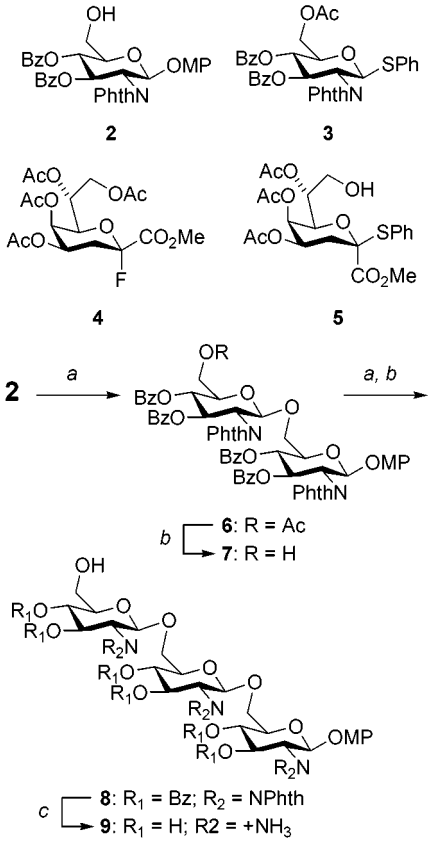
1 was designed so that its building blocks are derived from natural monosaccharides, 2-deoxy-2-amino glucose (GlcN) and 2-keto-3-deoxy-octulosonate (KDO), as precursors of base and acid functions, presumably required for catalysis. To allow high conformational flexibility, the glycosidic bonds between three GlcN moieties and between two KDO moieties were designed to be β-(**1**−**6**) and α-(**2**−**8**), respectively.

The key steps for the assembly of **1** are presented in Schemes 1 and 2. The protecting groups used on the

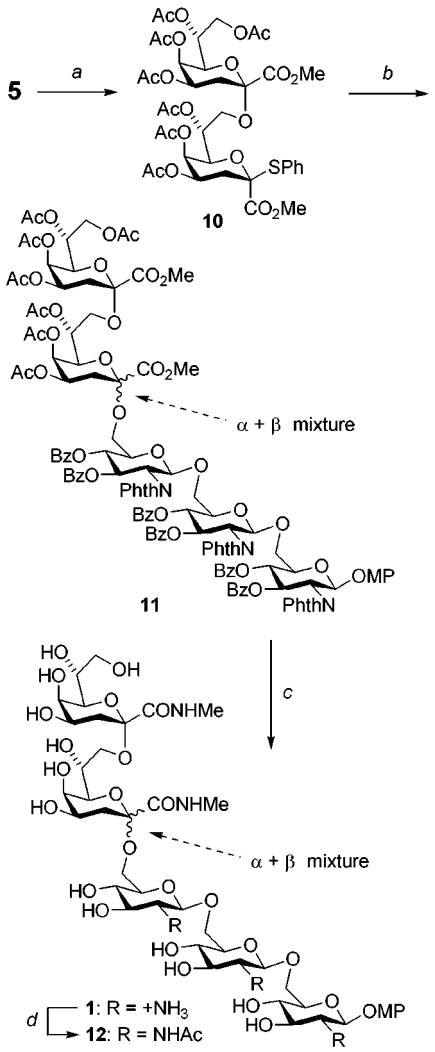
Scheme 1[a]



[a] Reagents and conditions: (a) **3** (1.2 equiv), NIS (2.5 equiv), 4 Å MS, TfOH (cat.), CH_2Cl_2, 0 °C, 1 h; (b) AcCl/MeOH 1:25, 0 °C, 3−7 h; (c) 33% $MeNH_2$ in EtOH, 25 °C, 65 h.

monosaccharide building blocks **2**−**5**[6] served admirably in terms of ease of attachment and removal, survivability under the reaction conditions, and orthogonality, whereas the thioglycoside-NIS glycosidation method[7] proved to be both rapid and efficient. The desired GlcN building blocks **2** and **3** were designed to allow, through neighboring group participation, selective β-glycoside bond formation at position

Scheme 2[a]



[a] Reagents and conditions: (a) **4** (1.7 equiv), Et_3N (1.2 equiv), $BF_3{\cdot}Et_2O$ (2.0 equiv), CH_2Cl_2, 0 °C, 24 h, 65%; (b) **8** (0.7 equiv), NIS (1.2 equiv), 4 Å MS, TfOH (1.3 equiv), MeCN, 0−25 °C, 44.8%; (c) 33% $MeNH_2$ in EtOH, 25 °C, 30 h, 97%; (d) Ac_2O (4 equiv), MeOH, 0 °C, 3 h, 87%.

C-6 of GlcN. Thus, glycosidation of acceptor **2** with thioglycoside donor **3** in the presence of NIS proceeded in 86% yield to afford exclusively β-glycoside **6**.[8] Selective removal of the acetate group in **6** with dry HCl liberated the primary hydroxyl group to furnish disaccharide acceptor **7** in 87% isolated yield. Reiteration of the last two steps, coupling of **7** with monosaccharide donor **3** followed by HCl-induced deacetylation, allowed the growth of the oligosaccharide in the desired direction until trisaccharide acceptor **8** was reached.

The di-KDO unit **10** was prepared in an orthogonal manner by coupling glycosyl fluoride **4** with thioglycoside **5** in the presence of ethereal BF_3 (65%).[9] NIS-Promoted coupling of **10** with trisaccharide acceptor **8** furnished the desired

(5) (a) Suzuki, S.; Higashiama, T.; Nakahara, A. *Bioorg. Chem.* **1973**, *2*, 145. (b) Hosseini, M. W.; Lehn, J.-M.; Mertes, M. P. *Helv. Chim. Acta* **1983**, *66*, 2454. (c) Hosseini, M. W.; Lehn, J.-M.; Jones, K. C.; Plute, K. E.; Mertes, K. B.; Mertes, M. P. *J. Am. Chem. Soc.* **1989**, *111*, 6330.

(6) The monosaccharides **2**−**5** were prepared by standard methods. All new compounds exhibited satisfactory spectral and analytical data. Yields refer to spectroscopically and chromatographically homogeneous materials.

(7) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.

(8) The purity and exclusive β-stereochemistry of a new glycosidic bond in **6** was confirmed by ^{1}H NMR spectroscopy (H-1, δ 5.68, J = 8.4 Hz).

pentasaccharide **11** (45%) as a mixture (~1:1) of α- and *β*-anomers at the newly generated glycosidic bond. Finally, one-step removal of all the ester and phthalimido protecting groups by treatment of **11** with methylamine (33% solution in EtOH) furnished the targeted pentasaccharide **1** (α/*β* ~ 1:1) with 2.2% overall yield for the total of 31 chemical steps from GlcN·HCl and KDO·NH_4 as starting materials. The ^{1}H, ^{13}C, and 2D-COSY NMR and mass spectroscopic properties of the purified **1** were consistent with the expected structure.[10]

Initially, the pure **1** (mixture of α- and *β*-anomers) was tested for DNAse, phosphodiesterase, and GTPase activities.[11] Among these, the observed relatively high GTPase activity was particularly intriguing and was therefore more extensively investigated. The rates of GTP hydrolysis were measured at pH 7.1 and 50 °C by direct observation of the reaction course by ^{31}P NMR spectroscopy. Figure 1 illustrates

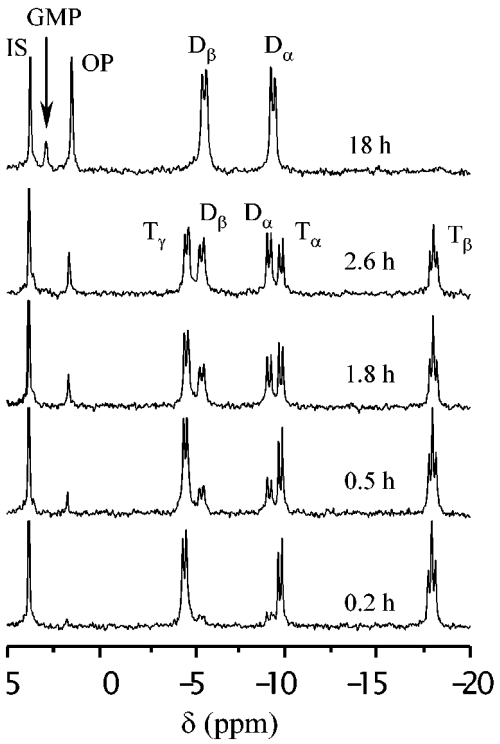


Figure 1. Time course of ^{31}P NMR spectra (proton decoupled) showing the conversion of GTP to GDP and OP (1.5 ppm) in the presence of **1** (4.2 mM), GTP (15.0 mM), and Mg^{2+} (15.0 mM) at an apparent pH of 7.1 in D_2O at 50 °C. Spectra were acquired on a Bruker WH-200 operating at 81.03 MHz (referenced to 200 mM D_3PO_4 at 0.0 ppm). T_α (−9.8 ppm), T_β (−17.9 ppm), and T_γ (−4.5 ppm) refer to the α-, *β*-, and *γ*-phosphate signals of GTP, respectively; D_α (−9.3 ppm) and D_β (−5.6 ppm) refer to α- and *β*-phosphate signals of GDP, respectively; IS refers to trimethyl phosphate (4.7 ppm), used as an internal standard.

the typical time course of ^{31}P NMR spectra for the conversion of GTP to GDP and OP in the presence of **1** (4.2 mM), GTP (15 mM), and Mg^{2+} ions (15 mM). No other phosphate peaks, except those shown in Figure 1, were detected in the region between −40 and 40 ppm,[12] suggesting that no side reaction other than the hydrolysis of GTP occurred. Only a small amount of GMP formation (2.9 ppm) was detected after prolonged incubation of the reaction products (spectrum after 18 h in Figure 1), indicating that **1** has a substantial selectivity to GTP as a substrate in comparison to that to GDP as a substrate.

The pseudo-first-order rate constant k_{obs} for the hydrolysis of GTP in the presence of **1** was determined from plots of ln[GTP] vs time (Figure 2) and found to be 3.98×10^{-3}

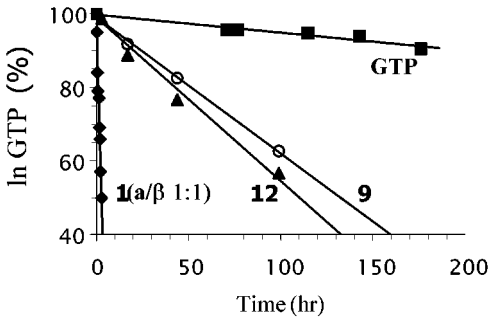


Figure 2. Rate profiles of GTP hydrolysis in the presence of different oligosaccharides as indicated. The ln of the remaining GTP normalized as a percentage as a function of time is plotted for each oligosaccharide. The data were obtained from ^{31}P NMR experiments conducted in the presence of GTP (15.0 mM), Mg^{2+} (15.0 mM), and the particular oligosaccharide (4.2 mM) as indicated at an apparent pH of 7.1 in D_2O at 50 °C. The curve referred to as GTP was obtained in the presence of GTP (15.0 mM) and Mg^{2+} (15.0 mM) under the same conditions.

min^{-1}. This corresponds to a rate acceleration of about 500-fold compared with that of the same reaction in the absence of **1** ($k_{obs} = 8.3 \times 10^{-6}$ min^{-1}). It turns out that Mg^{2+} ions

(9) The α- or *β*-stereochemistry at the glycosidic linkages of KDO was determined either by the chemical shift (*δ*) of H-4 proton (*δ* > 5 for α-anomers and *δ* < 5 for *β*-anomers: Kosma, P.; Strobl, M.; Allmaier, G.; Schmid, E.; Brade, H. *Carbohydr. Res.* **1994**, *254*, 105) or by the chemical shift difference (Δ*δ*) between H-3_{ax} and H-3_{eq} (small Δ*δ* for α-anomers and larger Δ*δ* for *β*-anomers: Imoto, M.; Kusunose, N.; Matsuura, Y.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1987**, *28*, 6277). Complementary results were obtained by both methods in all molecules containing KDO (see Supporting Information).

(10) Complete NMR assignments for the monosaccharides **2**−**5** along with the protected oligosaccharides **7**−**8**, **10**, **11**α, **11***β* and selected data for **1**α and **1***β* are given in Supporting Information.

(11) DNAse activity was examined by using DNA plasmid pSL301 (Brosius, J. *DNA* **1989**, *8*, 759) as a substrate. The extent of DNA hydrolysis was monitored [in Tris/HCl (50 mM), DNA (16.7 *μ*g/mL), **1** (6.2 mM), pH 7.5, 2 h, 55 °C] by measuring the relative quantities of the supercoiled (ccc), open-circular (oc), and linear (l) DNA forms by using agarose gel electrophoresis. The bis(*p*-nitrophenyl)phosphate was used as a substrate to determine the phosphodiesterase activity; the reaction progress was followed [in Tris/HCl (50 mM), bis (*p*-nitrophenyl) phosphate (0−20 mM), **1** (1.9 mM), pH 7.2, 50 °C] by UV spectroscopy (400 nm). In both cases, only moderate activities were determined (see Figures 1S and 2S in Supporting Information).

(12) We note that the integral of the inorganic phosphate signal in Figure 1 (the spectra were acquired at a relaxation delay time of 0.5 s, 320 scans) appears to be less than that for either of the phosphate groups of GDP. This is attributed to the longer spin lattice relaxation time (T_1) of inorganic phosphate than that for either phosphate group of GDP. Indeed, when the same spectra were recorded at the relaxation delay time of 10 s, 320 scans, integration of 1:1 was observed.

have a very important role in the catalysis of this reaction. Indeed, when the same reaction [**1** (4.2 mM), GTP (15 mM), pH 7.1, 50 °C] was performed in the absence of Mg^{2+} ions, a rate acceleration of only 4.2-fold was observed (Table 1).

Table 1. Kinetic Constants for GTP Hydrolysis as Determined from the Plots in Figure 2[a]

compd	$k_{obs} \times 10^3$ (min^{-1})	$t_{1/2}$ (hr)	rate enhancement
GTP	0.008 (0.008)	1386 (1386)	
1 (α/β 1:1)	3.98 (0.035)	2.9 (330)	498 (4.2)
12	0.097 (0.017)	119.5 (693)	12.1 (2.0)
9	0.078 (0.008)	147.5 (1386)	9.8 (1.0)

[a] Values in parentheses refer to the kinetic constants that were determined from the same experiments but in the absence of Mg^{2+} ions (data not shown in Figure 2).

In attempts to understand the structural and functional requirements for the observed GTPase activity by **1**, triglucosamine **9** and a tri-NAc derivative of **1** (**12**) were synthesized and their ability for GTP hydrolysis was examined under the same conditions (Figure 2, Table 1). Rate enhancements of 9.8-fold and 12.1-fold were observed in the presence of **9** and **12**, respectively.

These data indicate that neither the positively charged triglucosamine fragment of **1** alone nor the entire structure of **1** in the absence of positive charges can reach the catalytic efficiency observed by **1**. It seems that the whole structure of **1** as a trication creates with magnesium ion(s) and GTP an appropriate three-dimensional organization that leads to the catalysis. In addition, Mg^{2+} can assist in the displacement of the leaving group, GDP, by electrostatic catalysis.[13] Nevertheless, it is obvious that the exact role of Mg^{2+} as well as the catalytic mechanism of this reaction is not clear yet and requires further intensive investigation.[14]

In summary, here we show for the first time the design, synthesis, and catalytic potential of a small oligosaccharide under ambient reaction conditions. Although the study on the structure−function relationship of GTP hydrolysis by **1** is only at its beginning, we believe that such efforts should lead to a new era in our understanding of biocatalysis and catalysis required for design.

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Supporting Information Available: Selected procedures and data for compounds **1**−**5**, **7**−**8**, and **10**−**11**, along with the figures showing DNAse (Figure 1S) and phosphodiesterase (Figure 2S) activities in the presence of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) (a) Yohannes, P. G.; Plute, K. E.; Mertes, M. P.; Mertes, K. B. *Inorg. Chem.* **1987**, *26*, 1751. (b) Maegley, K. A.; Admiraal, S. J.; Herschlag, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8160.

(14) We note that the preliminary experiments with pure diastereomers indicated that **1**β is considerably more active than **1**α (Scheme 2). Further kinetic and mechanistic studies are in progress and will be reported in due course.