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Synthesis of Nitrogen-Containing Furanose Sugar Nucleotides for Use as Enzymatic Probes

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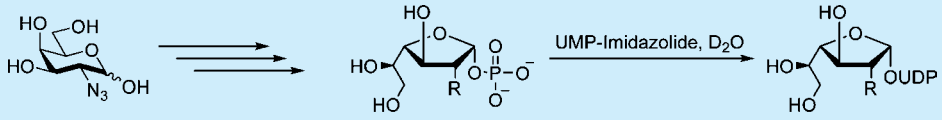
Supporting Information



ABSTRACT: The sugar nucleotides UDP-2-acetamido-2-deoxy-α-D-galactofuranose (UDP-Gal*f*NAc) and UDP-2-azido-2-deoxy-α-D-galactofuranose (UDP-Gal*f*N_3) have been synthesized in preparative scale for the first time. These compounds are useful probes for studying the biosynthesis of glycans containing galactofuranose and/or 2-acetamido-2-deoxy-α-D-galactofuranose residues.

Sugar nucleotides are widespread in nature, being the substrates for many glycosyltransferase enzymes.[1] The six-membered ring pyranose forms of sugar nucleotides predominate in most biological systems. In contrast, the five-membered ring furanose sugar nucleotides are less common and are absent in mammals.[2] As a result, glycosyltransferases from pathogenic organisms that use furanose sugar nucleotides for cell wall biosynthesis have been proposed as potential drug targets.[2b]

Campylobacter jejuni, one of the leading causes of bacterial gastroenteritis worldwide,[3] features a 2-acetamido-2-deoxy-α-D-galactofuranose (Gal*f*NAc) moiety within its capsular polysaccharide (CPS), occasionally modified with a methyl phosphoramidate group at C-3.[4] To understand the pathways that *C. jejuni* uses to construct its CPS, and potentially study the inhibition of CPS formation, milligram quantities of the sugar nucleotide precursors are required. We describe here the first preparative-scale synthesis of UDP-2-acetamido-2-deoxy-α-D-galactofuranose (UDP-Gal*f*NAc, **1**) (Figure 1), the donor species for Gal*f*NAc transferases. The only previously reported preparation of **1** is an enzyme-mediated reaction that interconverts the pyranose and furanose forms of this sugar nucleotide.[5] At equilibrium, the enzymatic reaction produces a 93:7 pyranose/furanose mixture, necessitating a more viable way of producing **1**. We also report the synthesis of an azido analogue of **1**, UDP-2-azido-2-deoxy-α-D-galactofuranose (UDP-Gal*f*N_3, **2**). We expect **2** to be an efficient probe of both Gal*f*NAc and Gal*f* biosynthesis, similar to how other azido-substituted carbohydrate derivatives have been useful in probing biosynthetic pathways by which complex glycans are assembled.[6]

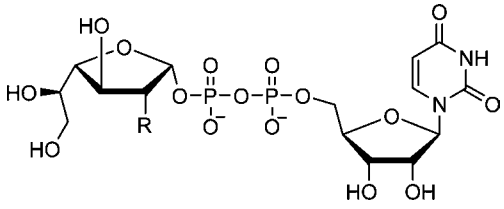


Figure 1. Structure of UDP-Gal*f*NAc (**1**, R = NHAc) and UDP-Gal*f*N_3 (**2**, R = N_3).

The synthetic precursor to **1**, 2-acetamido-2-deoxy-α-D-galactofuranose-1-phosphate (Gal*f*NAc-1-P, **3**), was synthesized as shown in Scheme 1. 2-Azido-2-deoxy-D-galactopyranose (**4**), obtained through either the azidonitration of 3,4,6-tri-*O*-acetyl-D-galactal[7] or diazo transfer with galactosamine and TfN_3,[8] was converted to its furanose form in the presence of 2,2-dimethoxypropane and *p*-TsOH,[9] followed by deprotection and acetylation to produce **5** in 78% overall yield. Acetolysis yielded a fully acetylated compound **6** in 67% yield, suitable for phosphorylation.

Phosphorylation was accomplished by first converting the fully acetylated compound **6** into the corresponding bromide, followed by reaction with dibenzyl phosphate under basic conditions to yield **7** in 61% yield as a 5:1 α/β mixture. For the first step in this transformation, due to the incompatibility of the azido group with HBr, a previously described method[10] employed successfully on the corresponding galactofuranose derivative could not be used. Instead, $TiBr_4$ was used to form the glycosyl bromide.[11] With the target compound **7** in hand, debenzylation, azide reduction, and amine acetylation were carried out in one step through hydrogenation over Pd/C with acetic anhydride as the solvent. Subsequent removal of the ester protecting groups in a methanol−water−triethylamine solution produced Gal*f*NAc-1-P (**3**) as a triethylammonium salt in 74% yield.

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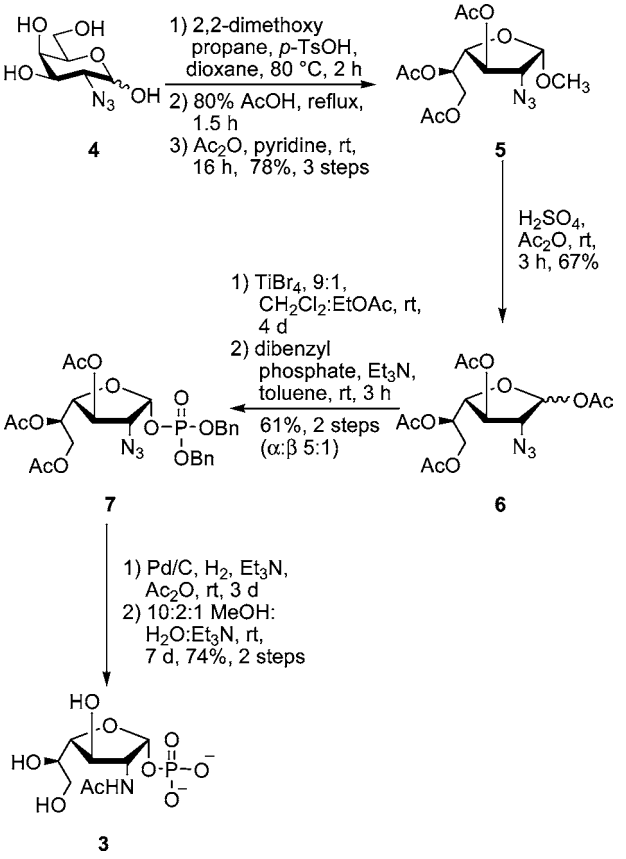
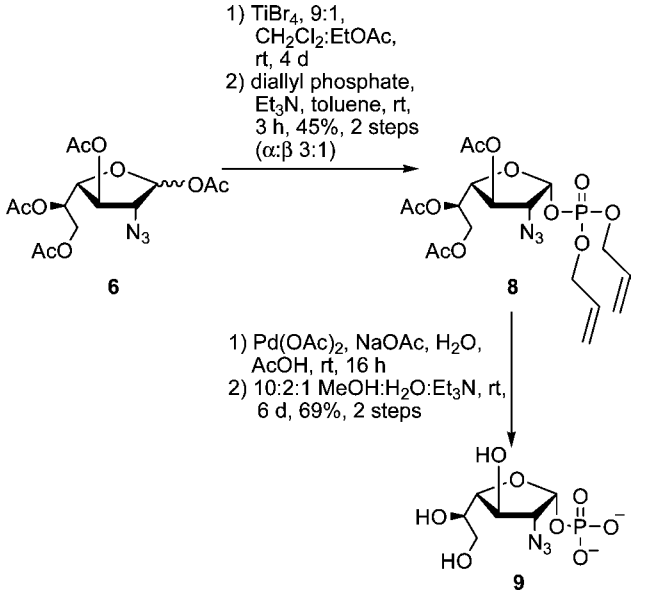
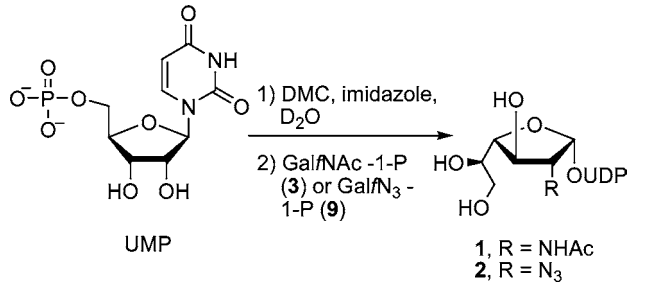
Scheme 1. Synthesis of 2-Acetamido-2-deoxy-α-D-galactofuranose-1-phosphate (Gal*f*NAc-1-P, 3)



Scheme 2. Synthesis of 2-Azido-2-deoxy-α-D-galactofuranose-1-phosphate (Gal*f*N$_3$-1-P, 9)



Scheme 3. Chemical Coupling of Sugar-1-phosphates 3 and 9 to UMP



It was thought that preserving the azide moiety could produce an analogue of **1** that would be a useful probe of Gal*f*/Gal*f*NAc biosynthesis. To protect the phosphate moiety with groups that could be removed without concomitant azide reduction, diallyl phosphate was prepared from methyl dichlorophosphate[12] and then reacted with **6** to produce **8** (Scheme 2) in a yield of 45%. Deallylation of **8** with $Pd(OAc)_2$ in acetic acid, followed by removal of the ester protecting groups, gave the desired azide analogue **9** in 69% yield.

With furanose-1-phosphates **3** and **9** in hand, their coupling with uridine-5′-monophosphate (UMP) was investigated. Several methods exist for achieving similar transformations, summarized by Wagner and co-workers in 2009;[1b] however, we chose to use a recent method developed by Tanaka et al.[13] (Scheme 3). This method relies on the initial formation of an imidazole-activated nucleotide monophosphate (NMP) through the action of imidazole and 2-chloro-1,3-dimethylimidazolinium chloride (DMC) on a NMP in D_2O. Subsequent addition of a sugar-1-phosphate and overnight reaction produces the desired sugar nucleotide. Using UMP and **3** or **9**, both UDP-Gal*f*NAc **1** (16% yield) and UDP-Gal*f*N$_3$ **2** (23% yield) were formed and isolated using this method. Although modest, the isolated yields of these compounds are comparable to those reported with similar substrates.[1b] Analysis of the ^{1}H and ^{31}P NMR spectra indicated that, upon complete consumption of UMP-imidazole, 50% of the starting material remained in solution (see the Supporting Information). Nevertheless, as noted by Tanaka et al., the reaction mixture for the UDP coupling may be used directly without purification as a source of UDP-Gal*f*NAc (**1**) for enzyme-catalyzed reactions.[13]

It is well established that the enzymatic synthesis of sugar nucleotides can, in many cases, give better yields, selectivity, and shorter reaction times as compared to chemical syntheses.[2c,14,15] For example, the highest yielding chemical synthesis of UDP-galactofuranose (UDP-Gal*f*, **10**, Scheme 4) gives the desired product in 35% yield,[16] whereas the enzymatic synthesis of this compound carried out by Errey et al. produces UDP-Gal*f* in 79% yield.[17]

We explored the possibility of enzymatically synthesizing **1** and **2** using an optimized procedure similar to that reported by Errey et al.[18,19] and outlined in Scheme 4. Unfortunately, this three-enzyme system failed to produce any of the desired sugar nucleotides when incubated with either Gal*f*NAc-1-P (**3**) or Gal*f*N$_3$-1-P (**9**). GalPUT is known to possess relaxed substrate specificity,[17] and it is somewhat surprising that **11** is a substrate whereas **3** and **9** are not. In previous studies, a 2-methoxy derivative of **11** could be converted to the corresponding UDP-Gal*f* derivative, albeit in low yield.[20]

To further probe the effect that the C-2 substituent has on the ability of these furanose-1-phosphates to serve as GalPUT substrates, Gal*f*N$_3$-1-P (**9**) was reduced using Pd/C under an H_2 atmosphere to produce 2-amino-2-deoxy-α-D-galactofuranose-1-phosphate (Gal*f*NH$_2$-1-P, see the Supporting Information). This compound was tested as a GalPUT substrate as

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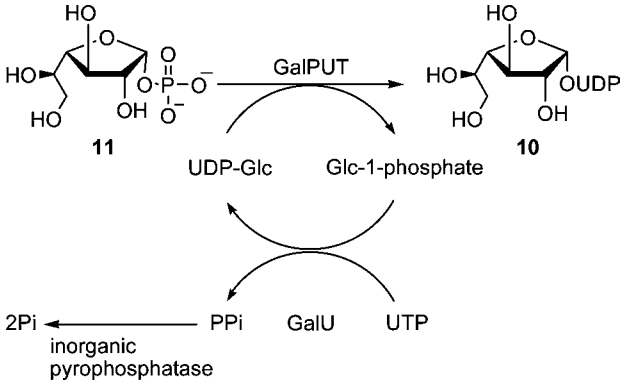
Scheme 4. Enzymatic Synthesis of UDP-Gal*f*, 10[a]



[a]Reactions performed at rt on an 11 μmol scale, using 1 equiv of UTP, 5 U GalU, 1.25 U inorganic pyrophosphatase, and 300 μL of immobilized GalPUT containing 5 U of enzyme buffered with 50 mM HEPES (pH = 8.0) containing 10 mM $MgCl_2$ and 5 mM KCl. Total reaction volume was 500 μL. Reaction initiated by the addition of 0.05 μmol UDP-Glc and analyzed after 24 h incubation. PPi = inorganic pyrophosphate; Pi = inorganic phosphate.

described above but was similarly found not to be a substrate. This suggests that the enzyme, although generally promiscuous, is sensitive to the nature of the C-2 group in these furanose phosphate derivatives. Further studies on the origin of this specificity are underway.

Both **1** and **2** could prove useful for a number of biological investigations. For example, **1** is a substrate for UDP-*N*-acetylgalactopyranose mutase (UNGM), an enzyme from *C. jejuni* that catalyzes the interconversion of UDP-Gal*p*NAc and UDP-Gal*f*NAc (**1**).[5] Additionally, incorporation of the sugar moiety of UDP-Gal*f*N_3 (**2**) into *C. jejuni* CPS, or into other molecules containing Gal*f* or Gal*f*NAc residues, would present attractive opportunities for labeling these glycans with a fluorescent tag via "click" chemistry[21] or Staudinger ligation.[22] However, to date the transferase that carries out this transformation has not been identified.

To demonstrate the potential utility of **2**, we incubated it with two different glycosyltransferases: GlfT2, a galactofuranosyltransferase that synthesizes the cell wall galactan in *Mycobacterium tuberculosis*,[20,23] and WbbI, a β-(1→6)-Gal*f* transferase from *Escherichia coli* K-12 that contributes to O-antigen biosynthesis.[24] Although no product formation was observed when **2** was incubated with WbbI and a model acceptor, GlfT2 did attach **2** to the trisaccharide acceptor **12** to form the tetrasaccharide **13**, shown in Scheme 5. No product formation was observed in similar experiments using **1** as the donor substrate. Despite the fact that GlfT2 is a processive polymerase,[25] the resulting product contains only a single Gal*f*N_3 residue, suggesting that it could be useful as a chain terminating substrate in biochemical studies of this enzyme.[20,26]

In summary, we report here the first preparative-scale synthesis of UDP-Gal*f*NAc (**1**) and its azido analogue **2** using a chemical approach. A previously described enzymatic method, which could be used to produce UDP-Gal*f* (**10**) from Gal*f*-1-P (**11**), failed to convert either Gal*f*NAc-1-P (**3**) or Gal*f*N_3-1-P (**9**) into the corresponding sugar nucleotides. The glycosyltransferase GlfT2 accepts **2** as a substrate, raising the possibility of using this compound for in vitro labeling studies of Gal*f*-containing glycans. Access to milligram quantities of **1** will greatly facilitate investigations of *C. jejuni* CPS biosynthesis.[5,27]

■ ASSOCIATED CONTENT

Supporting Information

Spectroscopic data and experimental details of all new compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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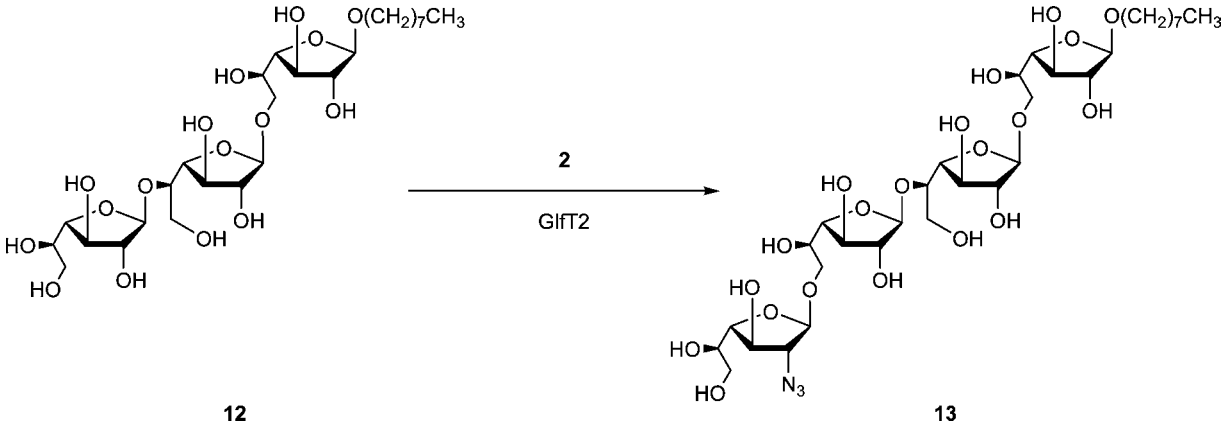
Scheme 5. Coupling of Acceptor 12 with UDP-Gal*f*N_3 Donor 2 by the Glycosyltransferase Enzyme GlfT2[a]



[a]Reaction performed at rt over 3 days using final concentrations of 0.5 mM **12**, 4 mM **2**, 150 μg of GlfT2, and 2 U alkaline phosphatase in MOPS (pH = 7.6) containing 20 mM $MgCl_2$ and 5 mM β-mercaptoethanol.

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REFERENCES

(1) Recent reviews: (a) Thibodeaux, C. J.; Melancon, C. E.; Liu, H. W. *Angew. Chem., Int. Ed.* **2008**, *47*, 9814−9859. (b) Wagner, G. K.; Pesnot, T.; Field, R. A. *Nat. Prod. Rep.* **2009**, *26*, 1172−1194. (c) Singh, S.; Phillips, G. N., Jr.; Thorson, J. S. *Nat. Prod. Rep.* **2012**, *29*, 1201−1237. (d) Gantt, R. W.; Peltier-Pain, P.; Thorson, J. S. *Nat. Prod. Rep.* **2011**, *28*, 1811−1853.

(2) (a) Brennan, P. J. *Tuberculosis* **2003**, *83*, 91−97. (b) Richards, M. R.; Lowary, T. L. *ChemBioChem* **2009**, *10*, 1920−1938. (c) Chlubnova, I.; Legentil, L.; Dureau, R.; Pennec, A.; Almendros, M.; Daniellou, R.; Nugier-Chauvin, C.; Ferrieres, V. *Carbohydr. Res.* **2012**, *356*, 44−61.

(3) Young, K. T.; Davis, L. M.; DiRita, V. J. *Nat. Rev. Microbiol.* **2007**, *5*, 665−679.

(4) (a) St. Michael, F.; Szymanski, C. M.; Li, J.; Chan, K. H.; Khieu, N. H.; Larocque, S.; Wakarchuk, W. W.; Brisson, J. R.; Monteiro, M. A. *Eur. J. Biochem.* **2002**, *269*, 5119−5136. (b) McNally, D. J.; Lamoureux, M. P.; Karlyshev, A. V.; Fiori, L. M.; Li, J.; Thacker, G.; Coleman, R. A.; Khieu, N. H.; Wren, B. W.; Brisson, J.-R.; Jarrell, H. C.; Szymanski, C. M. *J. Biol. Chem.* **2007**, *282*, 28566−28576.

(5) Poulin, M. B.; Nothaft, H.; Hug, I.; Feldman, M. F.; Szymanski, C. M.; Lowary, T. L. *J. Biol. Chem.* **2010**, *285*, 493−501.

(6) Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Discov.* **2005**, *4*, 477−488.

(7) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244−1251.

(8) Liu, Q.; Tor, Y. *Org. Lett.* **2003**, *5*, 2571−2572.

(9) Tanahashi, E.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1983**, *2*, 129−137.

(10) de Lederkremer, R. M.; Nahmad, V. B.; Varela, O. *J. Org. Chem.* **1994**, *59*, 690−692.

(11) Paulsen, H.; Stenzel, W. *Chem. Ber.* **1978**, *111*, 2348−2357.

(12) Rubinstein, M.; Patchornik, A. *Tetrahedron* **1975**, *31*, 2107−2110.

(13) Tanaka, H.; Yoshimura, Y.; Jørgensen, M. R.; Cuesta-Seijo, J. A.; Hindsgaul, O. *Angew. Chem., Int. Ed.* **2012**, *51*, 11531−11534.

(14) Recent reviews: (a) Cai, L. *J. Carbohydr. Chem.* **2012**, *31*, 535−552. (b) Engels, L.; Elling, E. In *Carbohydrate-Modifying Biocatalysts*; Grunwald, P., Ed.; Pan Stanford Publishing: Boca Raton, FL, 2012; pp 237−254.

(15) (a) Timmons, S. C.; Hui, J. P. M.; Pearson, J. L.; Peltier, P.; Daniellou, R.; Nugier-Chauvin, C.; Soo, E. C.; Syvitski, R. T.; Ferrières, V.; Jakeman, D. L. *Org. Lett.* **2008**, *10*, 161−163. (b) Timmons, S. C.; Mosher, R. H.; Knowles, S. A.; Jakeman, D. L. *Org. Lett.* **2007**, *9*, 857−860.

(16) Marlow, A. L.; Kiessling, L. L. *Org. Lett.* **2001**, *3*, 2517−2519.

(17) Errey, J. C.; Mukhopadhyay, B.; Kartha, K. P. R.; Field, R. A. *Chem. Commun.* **2004**, *23*, 2706−2707.

(18) Rose, N. L.; Zheng, R. B.; Pearcey, J.; Zhou, R.; Completo, G. C.; Lowary, T. L. *Carbohydr. Res.* **2008**, *343*, 2130−2139.

(19) Poulin, M. B.; Lowary, T. L. *Methods Enzymol.* **2010**, *478*, 389−411.

(20) Poulin, M. B.; Zhou, R.; Lowary, T. L. *Org. Biomol. Chem.* **2012**, *10*, 4074−4087.

(21) Anderson, C. T.; Wallace, I. S.; Somerville, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 1329−1324.

(22) Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. *Nature* **2004**, *430*, 873−877.

(23) (a) Wheatley, R. W.; Zheng, R. B.; Richards, M. R.; Lowary, T. L.; Ng, K. K. S. *J. Biol. Chem.* **2012**, *287*, 28132−28143. (b) May, J. F.; Levengood, M. R.; Splain, R. A.; Brown, C. D.; Kiessling, L. L. *Biochemistry* **2012**, *51*, 1148−1159. (c) Rose, N. L.; Completo, G. C.; Lin, S.-J.; McNeil, M.; Palcic, M. M.; Lowary, T. L. *J. Am. Chem. Soc.* **2006**, *128*, 6721−6729.

(24) Wing, C.; Errey, J. C.; Mukhopadhyay, B.; Blanchard, J. S.; Field, R. A. *Org. Biomol. Chem.* **2006**, *4*, 3945−3950.

(25) Levengood, M. R.; Splain, R. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2011**, *133*, 12758−12766.

(26) Brown, C. D.; Rusek, M. S.; Kiessling, L. L. *J. Am. Chem. Soc.* **2012**, *134*, 6552−6555.

(27) Sternberg, M. J. E.; Tamaddoni-Nezhad, A.; Lesk, V. I.; Kay, E.; Hitchen, P. G.; Cootes, A.; van Alphen, L. B.; Lamoureux, M. P.; Jarrell, H. C.; Rawlings, C. J.; Soo, E. C.; Szymanski, C. M.; Dell, A.; Wren, B. W.; Muggleton, S. H. *J. Mol. Biol.* **2013**, *425*, 186−197.