JOURNAL OF combinatorial CHEMISTRY

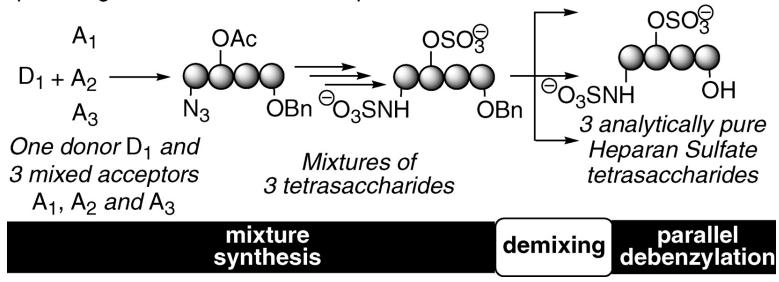
Report

Mixture Synthesis and "Charge Tagging" Based Demixing: An Efficient Strategy for the Preparation of Heparan Sulfate Libraries

Anna Dilhas, Ricardo Lucas, Latino Loureiro-Morais, Yaël Hersant, and David Bonnaffé

J. Comb. Chem., **2008**, 10 (2), 166-169• DOI: 10.1021/cc8000019 • Publication Date (Web): 16 February 2008

Mixture synthesis is used for the first time as efficient tool to speed up the synthesis of Heparan Sulfate tetrasaccharides. Demixing, based on "charge tagging" of the library members, allowed isolation of analytically pure fragments at the end of the process.



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

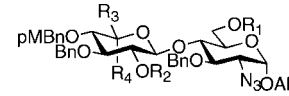
Mixture Synthesis and "Charge Tagging" Based Demixing: An Efficient Strategy for the Preparation of Heparan Sulfate Libraries

Anna Dilhas, Ricardo Lucas, Latino Loureiro-Morais, Yaël Hersant, and David Bonnaffé*

Univ Paris-Sud, Laboratoire de Chimie Organique Multifonctionnelle, Equipe de Glycochimie Moléculaire et Macromoléculaire, Institut de Chimie Moléculaire et des Matériaux d'Orsay, UMR CNRS-UPS 8182, Bat. 420, UPS, 91405 Orsay Cedex, France

Received January 4, 2008

Heparan sulfate (HS), a member of the glycosaminoglycan family, is a linear sulfated polysaccharide interacting with and regulating the activity of numerous proteins.[1] HS is composed of an alternation of uronic acids units (1→4) linked to α-(1→4)-linked 2-deoxy-2-aminoglucosyl units. It is one of the most heterogeneous biopolymers because various epimerization and sulfation patterns (sulfoforms) may occur along the chain. The uronic acid may be either D-glucuronic or L-iduronic, while *O*-sulfation may occur on position 2 of the uronic acid and 3 or 6 of the amino sugar. The glucosamine nitrogen may be sulfated, acetylated, or less frequently, unmodified, leading to 48 possible disaccharides. The diversity grows exponentially with the polymer length, leading to 2304 possible tetrasaccharides, 110592 hexasaccharides, and more than 5×10^6 octasaccharides.[2] After the commercialization of Arixtra,[3] a synthetic HS-like pentasaccharide that catalyzes the activity of antithrombin III, it is anticipated that new drugs will emerge from the identification of HS fragments able to control the activity of other therapeutic targets, such as cytokines, chemokines, FGFs, or other growth factors.[1,2,4,5] The development of methodologies allowing access to this huge molecular diversity, that is, the preparation of HS fragments with defined structures, is thus at the forefront of current research in glycochemistry.[3–16] The combinatorial nature of the HS polymer has struck several glycochemists and various modular synthesis schemes have been proposed,[7–11,15] but to our knowledge, these strategies have not yet been implemented into multiparallel or mixture syntheses. In a domain close to HS fragment synthesis, we have shown that solution-phase split-pool synthesis was an ideal tool to generate all the disaccharide sulfoforms of chondroitin sulfate (another glycosaminoglycan).[17] However, although this work, as well as studies performed in others fields of glycochemistry, have shown, in the late 1990s, that mixture synthesis could be efficiently used to prepare oligosaccharide libraries,[18] there have been few further developments of such methodologies, mainly because oligosaccharide mixtures are not really suitable for biological tests. Recently, the use of mixture synthesis followed by final demixing has been introduced in other area of organic chemistry, and this is beneficial both from the advantages of mixture synthesis and from the delivery of pure compounds for biological tests.[19] We thus decided to test whether such an approach could be used to speed up the preparation of HS fragment libraries. On the basis of chemistry we developed in the past decade in the synthesis of individual HS oligosaccharides,[4a,8,10,14,20] we planned to perform mixture syntheses of HS fragments by oligomerizing the suitably protected disaccharide building blocks **1**–**3**[8,10] (Figure 1). For the demixing step, we decided to rely on the synthetic scheme being designed to generate, prior to the final hydrogenolysis step, mixtures of oligosaccharides containing different sulfate/benzyl groups ratios, which are thus "charge tagged". As example, tetrasaccharide **11** contains eight charges/five Bn groups; tetrasaccharide **12** has seven charges/six Bn groups, and tetrasaccharide **13** has six charges/seven Bn groups. A mixture of those three compounds should thus be easily demixed by chromatography on a RP-C18 stationary phase. In this approach, charge tagging is used in a way similar to the fluorous tagging recently introduced by the group of D. P. Curran.[19]



1: $R_1 = R_2 = OAc$, $R_3 = H$, $R_4 = COOMe$
2: $R_1 = OBn$, $R_2 = OAc$, $R_3 = H$, $R_4 = COOMe$
3: $R_1 = R_2 = OBn$, $R_3 = COOMe$, $R_4 = H$

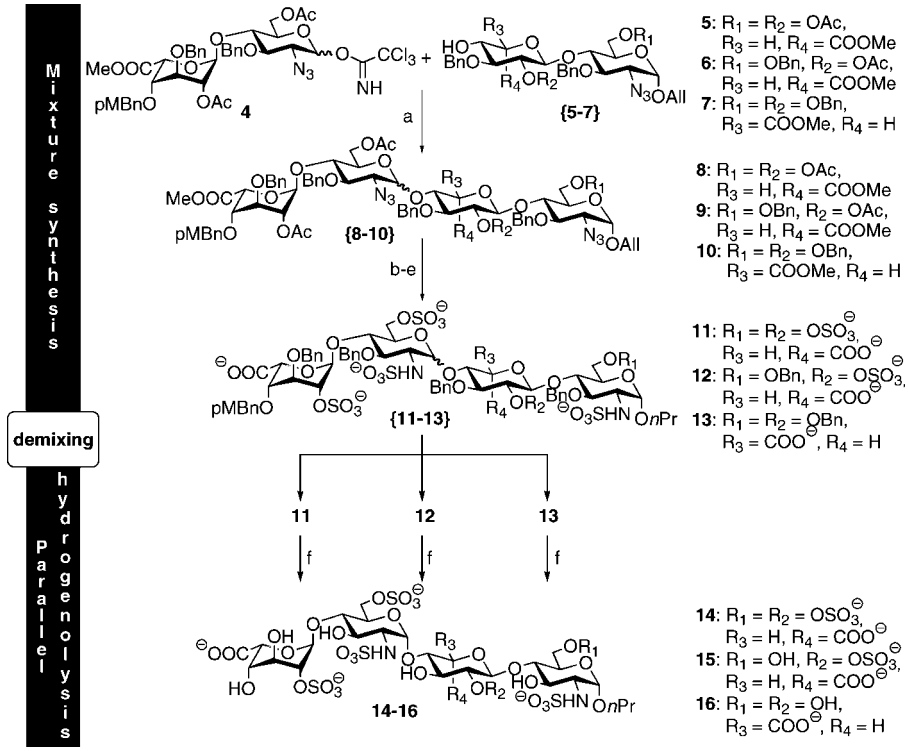
Figure 1. Set of disaccharide building blocks for the combinatorial synthesis of HS fragments.

As a poof of concept for this "mixture synthesis/demix" approach, we decided to prepare the small library of isolated tetrasaccharides **14**–**16** (scheme 1). Donor **4** can be obtained in high yield from disaccharide **1**.[4a] On the other hand, treatment of disaccharides **1**–**3** with TFA gave acceptors **5**–**7** in nearly quantitative yields.[20] We first wanted to perform some model experiments on small scale to check whether a glycosylation reaction involving one donor and three acceptors could be performed with enough synthetic efficiency for a mixture synthesis. It is indeed known that fragments from the repetitive sequence of heparin, may be assembled with high stereoselectivities and yields from building block similar to **1**.[4a,5b,c,16,21] However, when working on more heterogeneous structures than the repeating unit of heparin, glycosylation reactions become less predictable especially, but not only, when working with glucuronic acceptors.[14,20,22] For these model experiments, the three acceptors **5**–**7** were mixed in an equimolar ratio and condensed with 1.3 equiv of imidate **4**. The reactions were performed in CH_2Cl_2 at −30 °C in the presence of TMSOTf (Scheme 1). After three hours at this temperature, the reaction was quenched with NEt_3, warmed to room temperature (RT), and directly purified by exclusion chromatography on Sephadex LH-20. The fractions containing the highest molecular weight compounds were pooled and analyzed by RP-C18 HPLC.

* To whom correspondence should be addressed. Phone: 33 (0)1 69 15 72 33. Fax: 33 (0)1 69 15 47 15 . E-mail: david.bonnaffe@icmo.u-psud.fr.

10.1021/cc8000019 CCC: $40.75
Published on Web 02/16/2008

Scheme 1. Mixture Synthesis and Parallel Debenzylation of an HS Tetrasaccharide Library[a]



[a] Reaction conditionts: (a) TMSOTf (0.1 equiv % **4**), 1.3 equiv **4**, CH_2Cl_2, −30 °C, 3 h; (b) K_2CO_3, MeOH, RT, 18 h; (c) H_2, Pd/$BaSO_4$, 0.1 M pyridine, MeOH/THF 2/1, 24 h; (d) pyridine·SO_3, pyridine, 24 h RT, then 16 h 50 °C; (e) LiOH, H_2O_2, *n*BuOH/THF 1/1, 0 °C, 3 h, then KOH, RT 57 h (yields over the five steps after RP-C18 semi preparative HPLC 20% (**11**), 22% (**12**), and 4% (**13**)); (f) H_2, $Pd(OH)_2$/C, 100 mM phosphate buffer pH 7.0/*t*BuOH 6/4, 73% (**16**) to quant. (**14** and **15**).

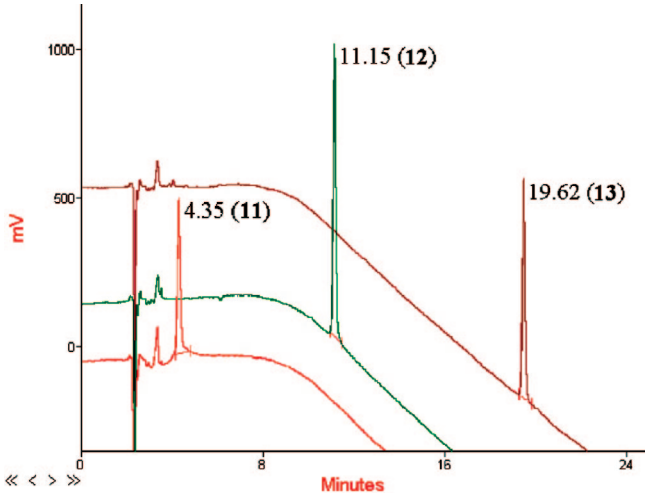


Figure 2. HPLC chromatograms of compounds **11**, **12**, and **13** after demixing (UV detection at 220 nm; see Supporting Information for chromatographic conditions).

Evaporative light scattering (ELS) detection and LC-MS were respectively used for quantification and peak identification.[23] As expected, the pooled fractions contained only tetrasaccharides, confirming that LH-20 chromatography can be efficiently used to separate tetrasaccharides from disaccharide side products derived from **4** and from unreacted acceptors after a mixture glycosylation. Quantitative analysis of the data, obtained from three independent competitive glycosylations, revealed that tetrasaccharides **8** and **9** were obtained in quantitative yields and full α-stereoselectivities.[24] For tetrasaccharide **10**, lower yields (18−12%) and diastereoselectivities[24] (90/10 to 86/14 α/β ratios) were obtained. Such results are consistent with data obtained in this and other laboratories, showing that glycosylations on position 4 of L-iduronyl acceptors, in a $^1C_4$ conformation, proceed generally in high yields and full α-stereoselectivity, while glycosylations on position 4 of D-glucuronyl acceptors, in a $^4C_1$ conformation, proceed in lower yields and stereoselectivities.[14,20,22] Although tetrasaccharides **8**−**10** were not present in equimolar ratios after competitive glycosylations, we decided to capitalize on these results and perform, on a preparative scale, glycosylation, followed by the functionalization/deprotection steps, leading to tetrasaccharides **14**–**16** to test the efficiency of the charge tagging-based demixing (Scheme 1).

During the preparative mixture synthesis of HS library, all the reactions were followed by RP-C18 HPLC to ascertain a full conversion of the starting materials and the presence of only three major components in the library. It should be noted that a key point in the success of the mixture synthesis part of this work was the careful optimization of an efficient deprotection/sulfation scheme allowing, in each step, the transformation of each disaccharide in high yields. All purifications following glycosylation, functionalization, or deprotection reactions were performed by LH-20 chromatography. IR spectra were also recorded on the purified tetrasaccharide mixtures to follow the appearance or removal of functional groups. We first performed a preparative mixture glycosylation, as described above, starting from 0.3 mmol of each acceptor **5**–**7** (21–24 mg) and 1.17 mmol of donor **4** (108 mg). After purification, the library of tetrasaccharides **8**–**10** was deacetylated by transesterification with MeOH using K_2CO_3 as base. After neutralization with ion-

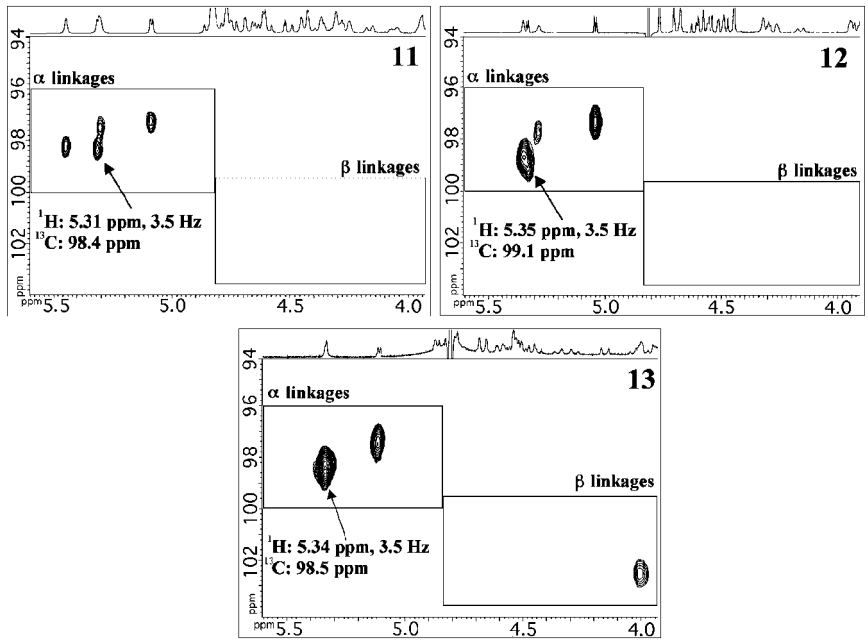


Figure 3. ^{1}H−^{13}C HSQC spectra of compounds **11**−**13** in the anomeric proton and carbon regions. The arrows point the crosspeaks corresponding to the three anomeric centers involved in the mixture glycosylation. Chemical shifts and J_{H1-H2} coupling constants are also given for these centers.

exchange resin (H^+) and purification, a strong ν_{OH} signal in the IR spectrum of the mixture ascertained the unmasking of hydroxyl groups. The azido moieties were then reduced under H_2 atmosphere, using $Pd/BaSO_4$ as catalyst and 0.1 M pyridine to prevent hydrogenolysis of the benzyl groups. In this step, the allyl groups were also converted into *n*-propyl moieties. After purification, the IR spectra of the mixture showed complete disappearance of the ν_{N3} signal confirming the completion of the azido group reduction. *O* and *N*-sulfation were then performed at 50 °C using pyridine·SO_3 complex as sulfating agent and pyridine as the solvent.[4a] Saponification of the methyl esters was performed subsequently using lithium hydroperoxide followed by potassium hydroperoxide[5c] to give tetrasaccharides **11**–**13** as a three member library. The HPLC analysis of this mixture confirmed the presence of three major components along with minor impurities. As expected, compounds **11**−**13** have very different retention times on RP-C18 (see Figure 2), and we decided, as planned, to demix the library members at this stage of the synthesis. A simple RP-C18 flash chromatography allowed us to isolate 21 mg of **11**, 17 mg of **12**, and 4 mg of **13** already highly pure as determined by ^{1}H NMR (see Supporting Information). Semipreparative HPLC was then used to further purify the three tetrasaccharides to full homogeneity. The high purity of each isolated tetrasaccharide, **11**−**13**, was confirmed by HPLC analyses with each of them giving a single peak as shown in Figure 2. After this second purification, we obtained 11 mg of **11** (20% over the 5 mixture synthesis steps), 12 mg of **12** (22%), and 2 mg of **13** (4%). The lower yield of the glycosylation reaction on acceptor **3** (16−11% for the α-stereoisomer) explains by itself the lower global yield obtained for **13**. To confirm the structures of each compound, MS and NMR analyses were performed. Molecular ions corresponding to the expected mass[25] were obtained using LC-MS analysis. The α-anomericity of the glycosidic linkages created at the first step of the mixture synthesis was then confirmed using ^{1}H and ^{1}H−^{13}C HSQC experiments; as shown in Figure 3, representing an expansion at the anomeric proton and carbon regions of the HSQC of each compound, no correlation peak corresponding to a β-linkage can be found in the spectra of **11** and **12**, while a single cross peak in the β-region can be found for compound **13**. This last crosspeak corresponds to the anomeric center involved in the linkage between the GlcUA and reducing end unit of tetrasaccharide **13**. Moreover, the ^{1}H and ^{13}C chemical shifts as well as J_{H1-H2} coupling constants were measured for the newly created anomeric centers from ^{1}H spectra and ^{1}H−^{13}C HSQCs. The obtained values (δ_{1H} = 5.31–5.35 ppm and δ_{13C} = 98.4–99.1 ppm, with J_{H1-H2} = 3.5 Hz) confirm, as expected, the exclusive or major formation of α linkages in the glycosylation step and thus the occurrence of natural linkages in the synthesized tetrasaccharides.

Finally, parallel hydrogenolyses (Scheme 1) were performed in standard conditions using $Pd(OH)_2$ as catalyst in a 6/4 mixture of 100 mM phosphate buffer pH 7.0/*t*BuOH. Yields ranging from quantitative (**14** and **15**) to 73% (**16**) were obtained in this last step.

We have thus shown that multistep mixture synthesis, followed by charge tagging-based demixing, is an appealing methodology to increase the speed of preparation of HS fragments with different structures. This strategy allowed us to save 10 steps and 10 purifications with respect to a multiparallel synthesis, while still allowing the isolation of pure oligosaccharides at the end of the process. It is anticipated that such a mixture synthesis/demix strategy will also be applicable to the synthesis of longer HS fragments as long as the synthesis is designed to obtain library members having different sulfate/benzyl ratios. It may thus represent a promising alternative or complementary tool to solid-phase synthesis[26] for the preparation of HS fragments libraries. The

preparation of other tetrasaccharides, as well as longer fragments, is underway and will be reported in due course.

Acknowledgment. We thank Pr. A. Lubineau for continuous support and fruitful discussion, the CNRS, the Ministère de l'Education Nationale et de la Recherche (A.D.), the National Agency for AIDS and HCV research (A.N.R.S.), and Sidaction (R.L.) for funding and grants. Julie Hemez and Isabelle Afonso (ICSN, Gif sur Yvette) are also greatly acknowledged for their kindness and skills in performing the LC-MS analyses.

Supporting Information Available. Conditions for HPLC and LC-MS analyses of the library of compounds **8–10** and **11–13**, NMR data for **11–13** before and after semipreparative HPLC, and NMR data for **15–16**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Capila, I.; Linhardt, R. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 390–412. (b) Imberty, A.; Lortat-Jacob, H.; Perez, S. *Carbohydr. Res.* **2007**, *342*, 430–439.

(2) (a) Lindahl, U.; Kjellen, L. *Thromb. Haemostasis* **1991**, *66*, 44–48. (b) Turnbull, J. E.; Gallagher, J. T. *Biochem. J.* **1991**, *273*, 553–559.

(3) Petitou, M.; van Boeckel, C. A. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 3118–3133.

(4) (a) Lubineau, A.; Lortat-Jacob, H.; Gavard, O.; Sarrazin, S.; Bonnaffé, D. *Chem.—Eur. J.* **2004**, *10*, 4265–4282. (b) Sarrazin, S.; Bonnaffé, D.; Lubineau, A.; Lortat-Jacob, H. *J. Biol. Chem.* **2005**, *280*, 37558–37564.

(5) (a) Kovensky, J.; Duchaussoy, P.; Bono, F.; Salmivirta, M.; Sizun, P.; Herbert, J.-M.; Petitou, M.; Sinaÿ, P. *Bioorg. Med. Chem.* **1999**, *7*, 1567–1580. (b) Tabeur, C.; Mallet, J.-M.; Bono, F.; Herbert, J.-M.; Petitou, M.; Sinaÿ, P. *Bioorg. Med. Chem.* **1999**, *7*, 2003–2012. (c) de Paz, J.-L.; Martin-Lomas, M. *Eur. J. Org. Chem.* **2005**, 1849–1858.

(6) Poletti, L.; Lay, L. *Eur. J. Org. Chem.* **2003**, 2999–3024.

(7) de Paz, J.-L.; Ojeda, R.; Reichardt, N.; Martin-Lomas, M. *Eur. J. Org. Chem.* **2003**, 3308–3324.

(8) Gavard, O.; Alais, J.; Bascou, A.; Hersant, Y.; Duverger, V.; Dilhas, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **2003**, 3603–3620.

(9) Prabhu, A.; Venot, A.; Boons, G.-J. *Org. Lett.* **2003**, *5*, 4875–4978.

(10) Dilhas, A.; Bonnaffé, D. *Tetrahedron Lett.* **2004**, *45*, 3643–3645.

(11) Fan, R.-H.; Achkar, J.; Hernandez-Torres, M.; Wei, A. *Org. Lett.* **2005**, *7*, 5095–5098.

(12) Codée, J. D. C.; Stubba, B.; Schiattarella, M.; Overkleeft, H. S.; van Boeckel, C. A. A.; van Boom, J. A.; van der Marel, G. A. *J. Am. Chem. Soc.* **2005**, *127*, 3767–3773.

(13) Huang, L.; Teumelsan, N.; Huang, X. *Chem.—Eur. J.* **2006**, *12*, 5246–5252.

(14) Hamza, D.; Lucas, R.; Feizi, T.; Chai, W.; Bonnaffé, D.; Lubineau, A. *ChemBioChem* **2006**, *7*, 1856–1858.

(15) Lu, L.-D.; Shie, C.-R.; Kulkarni, S. S.; Pan, G.-R.; Lu, X.-A.; Hung, S.-C. *Org. Lett.* **2006**, *8*, 5995–5998.

(16) Noti, C.; de Paz, J.-L.; Polito, L.; Seeberger, P. H. *Chem.—Eur. J.* **2006**, *12*, 8664–8686.

(17) Lubineau, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **1999**, 2523–2532.

(18) (a) Kanie, O.; Baresi, F.; Ding, Y.; Labbe, J.; Otter, A.; Forsberg, L. S.; Ernst, B.; Hindsgaul, O. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2720–2722. (b) Johnson, M.; Arles, C.; Boons, G.-J. *Tetrahedron Lett.* **1998**, *39*, 9801–9804. (c) Wong, C.-H.; Ye, X.-S.; Zhang, Z. *J. Am. Chem. Soc.* **1998**, *120*, 7137–7138.

(19) (a) Zhang, W.; Luo, Z.; Hiu-Tung Chen, C.; Curran, D. P. *J. Am. Chem. Soc.* **2002**, *124*, 10443–10450. (b) Yang, F.; Newsome, J. J.; Curran, D. P. *J. Am. Chem. Soc.* **2006**, *128*, 14200–14205.

(20) Lucas, R.; Hamza, D.; Lubineau, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **2004**, 2107–2117.

(21) (a) Poletti, L.; Fleischer, M.; Vogel, C.; Guerrini, M.; Torri, G.; Lay, L. *Eur. J. Org. Chem.* **2001**, 2727–2734. (b) Angulo, J.; Ojeda, R.; de Paz, J.-L.; Lucas, R.; Nieto, P. M.; Lozano, R. M.; Redondo-Horcajo, M.; Gimenez-Gallego, G.; Martin Lomas, M. *ChemBioChem* **2004**, *5*, 55–61. (c) Lee, J.-C.; Lu, X.-A.; Kulkami, S. S.; Wen, Y.-S.; Hung, S.-C. *J. Am. Chem. Soc.* **2004**, *126*, 476–477.

(22) (a) Duchaussoy, P.; Jaurand, G.; Driguez, P.-A.; Lederman, I.; Gourvenec, F.; Strassel, J.-M.; Sizun, P.; Petitou, M.; Herbert, J.-M. *Carbohydr. Res.* **1999**, *317*, 63–84. (b) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2128–2131.

(23) LC-MS data obtained for **8**-**10**: 7.12 min (*m/z* calcd for $C_{73}H_{84}N_6NaO_{26}$ [**8** + Na]$^+$ 1483.5, found 1483.4); 8.99 min (*m/z* calcd for $C_{78}H_{88}N_6NaO_{25}$ [**9** + Na]$^+$ 1531.6, found 1531.4); 12.37 min (*m/z* calcd for $C_{83}H_{92}N_6NaO_{24}$ [**10***β* + Na] 1579.6, found 1579.6); 13.14 min (*m/z* calcd for $C_{83}H_{92}N_6NaO_{24}$ [**10**α + Na]$^+$ 1579.6, found 1579.5).

(24) The assignment of α-configurations to the major components formed during the glycosylation reaction was anticipated from the presence of a non-participating azido group in position 2 of donor **4** and was further confirmed by the NMR data recorded on isolated tetrasaccharides **11**, **12**, and **13** (see text and Figure 3).

(25) LC-MS data obtained for **11**−**13**: 14.98 min (*m/z* calcd for $C_{63}H_{76}N_2O_{40}S_6^{2-}$[**11** + 6H]$^{2-}$ 846.12, found 846.09), 18.89 min (*m/z* calcd for $C_{70}H_{82}N_2O_{37}S_5^{2-}$[**12** + 5H]$^{2-}$ 851.16, found 851.14); **13** (*m/z* calcd for $C_{77}H_{88}N_2O_{34}S_4^{2-}$[**13** + 4H]$^{2-}$ 856.21, found 856.18).

(26) Ojeda, R.; de Paz, J.-L.; Martin-Lomas, M. *Chem. Commun.* **2003**, *7*, 2486–2487.

CC8000019