Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 681–686
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Note

Efficient selective preparation of methyl-1,2,4-tri-*O*-acetyl-3-*O*-benzyl-β-L-idopyranuronate from methyl 3-*O*-benzyl-L-iduronate

Anna Dilhas, David Bonnaffé*

Laboratoire de Chimie Organique Multifonctionnelle, UMR 8614 'Molecular Glycochemistry', Bat. 420 Université Paris Sud, F-91405 Orsay, France

Received 4 November 2002; accepted 27 November 2002

Abstract

Methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-L-idopyranuronate **6β**/**6α**, prepared from methyl 3-*O*-benzyl-L-iduronate (**4**), is a key synthon in heparin/heparan sulfate synthesis. The ^{1}H and ^{13}C NMR spectra of the furanose–pyranose mixture of **4**, after dissolution and equilibration in d_4-methanol, were fully assigned allowing to expect that **4** could crystallise in the β-pyranose form. New acetylation conditions able to trap this form were subsequently devised, allowing the isolation of 83% of pure **6β** by simple crystallisation, along with 9% of the **6β**/**6α** mixture. This represents a major advantage over the previously published procedure, especially on multigram scales.

Keywords: L-Idose; L-Iduronic acid; Pyranose form equilibration; Acetylation; Heparin; Heparan sulfate

1. Introduction

L-Iduronic acid (L-IdoUA) is a key component of dermatan sulfate (DS), heparin (HP) and heparan sulfate (HS). These linear sulfated oligosaccharides are members of the glycosaminoglycan family and play a crucial role in all organisms, not only through their structural functions but also by selectively binding and regulating the activity of numerous proteins. This is especially true for HS[1,2] which is present at the cell surface, as proteoglycan,[3] where it may interact with growth factors, such as FGFs,[4] interleukins and interferons,[5] chemokines,[6] viral proteins,[7] prion proteins[8] and adhesion molecules.[9] HS thus plays a key role in many biological processes such as development,[10] tumour growth and metastasis.[11] Among the biopolymer family, HS shows one of the highest level of molecular diversity. A current challenge in glycochemistry is thus the preparation of HS fragment libraries in order to find sequences responsible for the selective binding of a protein. In the total synthesis of HS/HP fragments, activated uronic acids have been shown to be efficient donors in glycosylation reactions.[12,13] However, efficient access to L-IdoUA synthons suitable for activation as L-iduronyl donors is often a serious challenge. The α/β mixture of methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-L-idopyranuronate **6α**/**6β**, that may easily be converted into a bromide donor, has been a key synthon in earlier heparin fragment syntheses.[13] However, its preparation by acetylation of methyl 3-*O*-benzyl-L-iduronate **4** (Scheme 1) with Ac_2O in pyridine[13a] leads to 40% of furanose derivatives **5α**/**5β** that have to be separated from **6α**/**6β** by chromatography. This is probably one of the reasons for the use of L-idose synthons that are more prone to cyclise in the pyranose form. However, in these cases C-6 oxidation has to be performed at a later stage on elaborated material.[13b,14] We have recently shown that the addition of tris(phenylthio)methyllithium on 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-dialdose (**1**) is a totally stereoselective and high yielding route to the L-IdoUA synthon **2** easily scalable up to 100 g.[15] This reaction is now a key step for the preparation of HS/HP fragments that we are currently synthe-

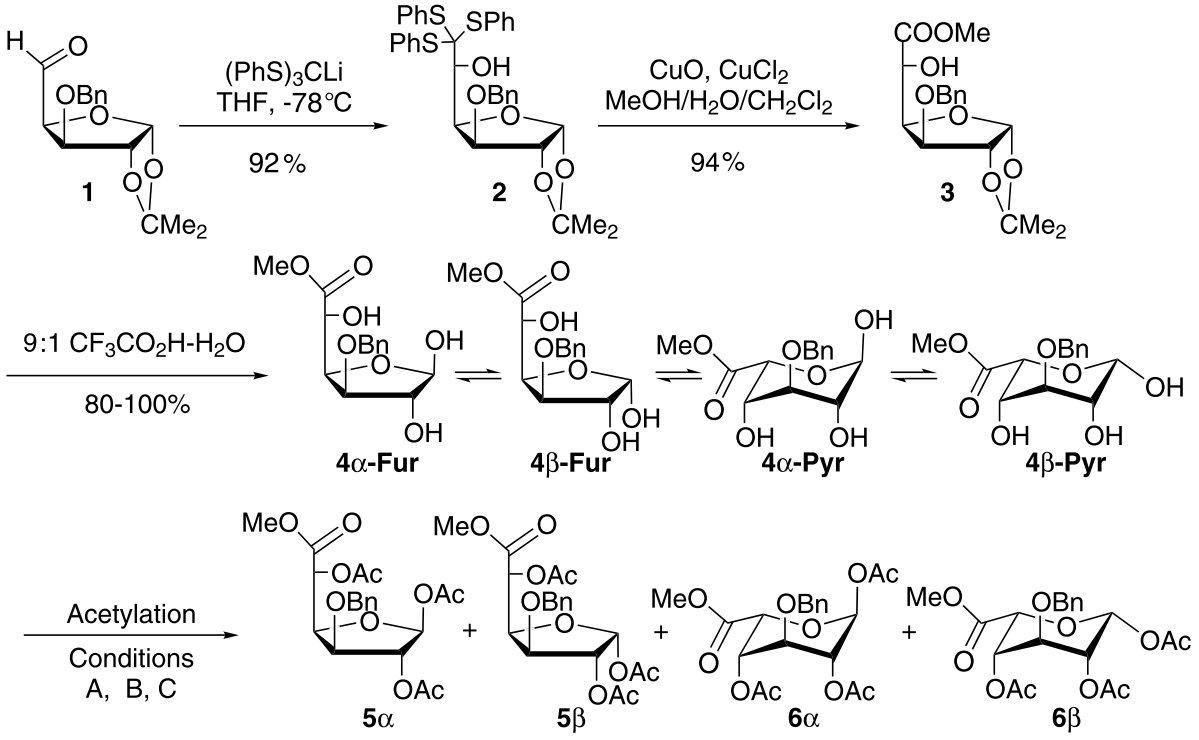
* Corresponding author. Tel.: +33-1-69154719; fax: +33-1-69154715
E-mail address: david@icmo.psud.fr (D. Bonnaffé).

0008-6215/03/$ - see front matter
doi:10.1016/S0008-6215(02)00525-6

sising in our laboratory. In order to avoid tedious chromatography resulting from unselective acetylation of methyl 3-*O*-benzyl-L-iduronate **4** in large-scale synthesis of **6α**/**6β** from **1**, we undertook a study of this step in order to reduce the amount of furanose derivatives **5α**/**5β**.

2. Results and discussion

Methyl 3-*O*-benzyl-L-iduronate (**4**) is a crystalline compound.[13a,15] We reasoned that if **4** could crystallise in a pyranose form and if acetylation could be performed in a solvent in which the crystals would be only sparingly soluble, we could trap this pyranose form and thus enhance the proportion of **6α** and/or **6β**. In order to address the first question, we dissolved crystals of **4** in d_4-methanol and followed the evolution of its ^{1}H NMR spectrum over time. The first spectrum, taken 3 min after dissolution, shows the presence of a major compound with H-1 at 4.97 ppm. This compound has been previously shown to be in the α-pyranose form, based on the multiplicity of the anomeric proton (broad singlet).[13a] We decided to test this hypothesis by fully attributing the ^{1}H and ^{13}C spectra of the pyranose–furanose mixture once the equilibrium had been reached. After 120 min at room temperature, a mixture of four compounds with anomeric proton chemical shifts at 4.97, 5.08, 5.17 and 5.28 ppm was formed in the proportions 41:17:18:24. A COSY spectrum was then recorded which allowed the identification of the ^{1}H spin system (H-1 to H-5) of each compound, while a HSQC experiment allowed total attribution of the ^{13}C spectrum of the sugar carbon atom ring. A HMBC map was used to determine whether a component was in the furanose or pyranose form: for the pyranose compounds, cross peaks were found between H-1 and C-5 (4.97 and 75.5 ppm for **4-β-Pyr**; 5.17 and 70.6 ppm for **4-α-Pyr**) and H-5 and C-1 (4.54 and 94.4 ppm for **4-β-Pyr**; 4.80 and 96.7 for **4-α-Pyr**). On the other hand, for the furanose component there are cross peaks between H-1 and C-4 (5.28 and 79.0 ppm for **4-β-Fur**; 5.08 and 82.2 ppm for **4-α-Fur**) and H-4 and C-1 (4.52 and 104.2 ppm for **4-α-Fur**). For the pyranose derivatives, attribution of the anomeric configuration was based on the existence of J^4 W coupling constants of 1.0 Hz between H-3 and H-1 and H-2 and H-4 in the spectrum of the pyranose compound with H-1 chemical shift at 5.17 ppm. This is only possible for **4-α-Pyr** in a $^1C_4$ conformation. On the contrary, there is only one J^4 W coupling constant between H-2 and H-4 in the spectrum of the other pyranose component (δ H-1 4.97 ppm) as expected for **4-β-Pyr** in a $^1C_4$ conformation. This later assignment was confirmed by the measurement in the HMBC map of a H-1 C-1 coupling constant of 162 Hz, which is characteristic of the presence of an axial hydrogen at the anomeric position of a pyranose compound.[16] For the furanose derivatives, attribution of the anomeric configuration was based on the chemical shift of the anomeric carbon, since for



Scheme 1.

Table 1
^{1}H and ^{13}C NMR data for compounds **4-β-Pyr**, **4-α-Pyr**, **4-β-Fur** and **4-α-Fur**

Compound % [a]	H/C	1	2	3	4	5	CH_2(Bn)	C=O	Me
4-β-Pyr 41%	δ ^{1}H [b]	4.97	3.69	3.84	3.98	4.54	4.67; 4.64		3.75
	J (Hz)	$J_{1,2}$ 1.0	$J_{2,4}$ 1.0 $J_{2,3}$ 3.5	$J_{3,4}$ 3.5	$J_{4,5}$ 1.5		J_{gem} 12.0		
	δ ^{13}C [b]	94.4	69.7	77.8	68.9	75.5	73.3	171.5	52.5
4-α-Pyr 18%	δ ^{1}H [b]	5.17	3.63	3.73	4.00	4.80	4.74; 4.69		3.75
	J (Hz)	$J_{1,2}$ 3.0	$J_{2,4}$ 1.0 $J_{2,3}$ 4.5	$J_{1,3}$ 1.0 $J_{3,4}$ 4.5	$J_{4,5}$ 2.5		J_{gem} 12.0		
	δ ^{13}C [b]	96.7	70.0	78.4	69.9	70.6	73.7	172.4	52.5
4-β-Fur 24%	δ ^{1}H [b]	5.28	4.31	4.23	4.50	4.30	4.79; 4.59		3.66
	J (Hz)	$J_{1,2}$ 4.5	$J_{2,3}$ 6.5	$J_{3,4}$ 6.5	$J_{4,5}$ 3.5		J_{gem} 11.5		
	δ ^{13}C [b]	96.9	76.5	84.0	79.0	71.2	73.9	174.3	52.5
4-α-Fur 17%	δ ^{1}H [b]	5.08	4.21	4.10	4.52	4.36	4.72; 4.52		3.65
	J (Hz)	$J_{1,2}$ 2.5	$J_{2,3}$ 4.5	$J_{3,4}$ 7.0	$J_{4,5}$ 4.0		J_{gem} 12.0		
	δ ^{13}C [b]	104.2	81.1	85.3	82.2	71.4	73.7	174.1	52.5

[a] Proportion of each compound after equilibration for 120 min after dissolution of **4** in d_4-methanol.
[b] δ are in ppm relative to the d_4-methanol signal at 3.31 for ^{1}H and 49.0 for ^{13}C.

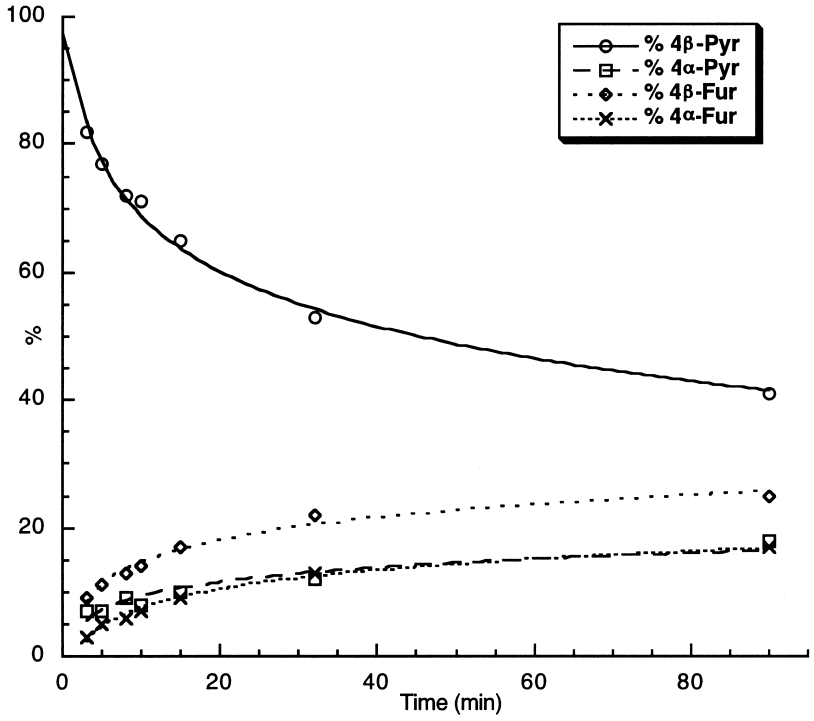


Fig. 1. Evolution of the composition in **4-β-Pyr**, **4-α-Pyr**, **4-β-Fur** and **4-α-Fur** after dissolution of crystalline **4** in d_4-methanol, based upon the integration of the anomeric proton signals.

furanoses in which the substituents at C-1 and C-2 are *trans* oriented, the signals of the anomeric carbon atoms are always found at a lower field as compared to those of the corresponding *cis* isomer.[17] Thus the furanose anomeric ^{13}C atom with chemical shift at 104.2 ppm was assigned to **4-α-Fur** and that at δ 96.9 ppm to **4-β-Fur**. Further attributions of the benzylic protons and carbons, carbonyls and methoxy groups were performed using HMBC and HSQC maps, allowing complete assignment of the ^{1}H and ^{13}C spectra (Table 1).

In order to establish the nature of the crystalline form of **4**, we followed the evolution of its ^{1}H NMR spectrum after dissolution in d_4-methanol. Fig. 1 show the course of the integration of each anomeric proton

over time. The major compound present 3 min after dissolution is **4-β-Pyr** (83%). Fig. 1 shows that its concentration decreases steadily over time, while the α-pyranose anomer and the furanose derivatives are formed. These results show unambiguously that the crystalline form of **4** is the β-pyranose form.

We began our study by investigating the reaction conditions required to trap the β-pyranose form of the crystals of **4** by acetylation. As a reference, a reaction with acetic anhydride in pyridine at room temperature was performed as described.[13a] After the reaction had reached completion, an aqueous work-up was carried out and the crude reaction mixture was analysed by ^{13}C NMR spectroscopy in order to determine the relative proportion of **5α**- and **5β**-furanose and **6α**- and **6β**-pyranose derivatives. ^{1}H NMR spectroscopy was also used as control, but could not be used to determine precisely the relative proportions of **5α** and **6β** due to signal overlapping (see Table 2 for chemical shifts). We found a 8:29:18:45 ratio for **5α**–**5β**–**6α**–**6β** (Entry 1, Table 2) similar to that reported previously.[13a] We then suspended the crystalline **4β-Pyr** compound in methylene chloride, and added pyridine, 2,4-dimethylaminopyridine and acetyl chloride after lowering the temperature to −20 °C. As expected, we observed a dramatic increase in the pyranose forms and obtained 80% of **6β** (Entry 3). Since we were working in a 4:1 CH_2Cl_2–pyridine mixture, we considered that dissolution and equilibration may occur at −20 °C, which would allow the formation of the acetylated furanose derivatives. We thus lowered the temperature to −40 and −50 °C and obtained, in both cases, a 94% selectivity for the formation of the pyranose compound **6β**, along with 3–4% of **6α** and only 2–3% of the **6β** furanose derivative (Entries 4 and 5). In order to determine the influence of the temperature on this excellent selectivity, we dissolved crystalline **4-β-Pyr** in pyridine and performed the reactions at −40 °C either in the classical conditions with Ac_2O[13a] or under our conditions with AcCl, DMAP after dilution with CH_2Cl_2. The results are similar for both experiments (Entries 2 and 6) resulting in an acetylated furanose–pyranose ratio similar to that observed at room temperature and a slight increase of **5α** at the detriment of **5β**. We have thus shown that the dramatic increase of acetylated derivative **6β**-pyranose in our conditions was linked to the trapping of the β-pyranose crystalline form and not to a temperature or solvent effect. These results also rationalise a similar selectivity observed in the anomeric silylation of **4-β-Pyr**.[18] Once the reaction conditions have been optimised, the reaction was tested on multigram scales and allowed to reproductively isolate, after extraction and crystallisation from Et_2O, 83% of pure **6β** without the need for chromatography. From the mother liquors, a further amount of **6α**–**6β** mixture could be isolated by chromatography giving a total isolated yield in pyranose derivatives of 92%. Moreover, we have also shown that a careful crystallisation of **4** is not essential for the selectivity. The crude crystals obtained after the evaporation of the trifluoroacetic–water mixture used for the removal of the 1,2-isopropylidene group in **2**[13a] may be used in the acetylation step without any loss of selectivity (Entry 7).

Table 2

Entry	Conditions [a]	Temperature (°C)	Compounds (%) [b]				Isolated yields (%) [c]	
			5α	**5β**	**6α**	**6β**	Crist. **6β**	**6α**+**6β**
1	A	20	8	29	15	48	nd.	nd.
2	A	−40	18	22	20	40	nd.	nd.
3	B	−20	8	6	6	80	nd.	nd.
4	B	−40		2	4	94	83	92
5	B	−50		3	3	94	nd.	nd.
6	C	−40	15	18	18	49	nd.	nd.
7	B [d]	−40		2	4	94	60	70

[a] A: acetylation using Ac_2O in pyridine as described.[13a] B: acetylation in CH_2Cl_2 with 6.0 equiv AcCl, 10 equiv pyridine and 0.1 equiv DMAP. C: crystalline **4β-Pyr** was first dissolved in 10 equiv pyridine, then CH_2Cl_2 was added and the rest of the reaction was performed as for B.

[b] Product ratio was determined by NMR: ^{13}C: δ (ppm): 98.7 (C-1 **5α**); 92.7 (C-1 **5β**); 91.2 (C-1 **6α**); 89.8 (C-1 **6β**) and ^{1}H: δ (ppm): 6.11 (d, J 3.0 Hz, H-1 **5α**); 6.43 (d, J 4.5 Hz, H-1 **5β**), 6.24 (t, J 1.5 Hz, H-1 **6α**), 6.09 (d, J 1.5 Hz, H-1 **6β**).

[c] Crystalline **6β** was obtained after extraction and crystallisation from diethyl ether–petroleum ether; remaining **6α**+**6β** mixture was obtained after flash chromatography of the mother liquor.

[d] Conditions B were used on the crude crystals obtained after removal of the 1,2-*O*-isopropylidene group of **3**.

3. Conclusion

We have thus shown that methyl 3-*O*-benzyl-L-iduronate **4** crystallises in the β-pyranose form by careful assignment of its ^{1}H and ^{13}C NMR spectra in d_4-methanol after equilibration into α- and β-pyranose and furanose forms. This allowed us to define reaction conditions in which the acetylated pyranose compound **6β** could be isolated in high yield by simple crystallization, a major improvement over the previously described conditions.[13a] This strategy for the enhancement of the pyranose content, after derivatisation of the anomeric position, should be applicable to reactions other than acylation or silylation as long as it is possible to find reactions conditions where **4β-Pyr** could be only sparingly soluble.

4. Experimental

4.1. General methods

All moisture-sensitive reactions were performed under an Ar atmosphere using oven-dried glassware. All solvents were dried over standard drying agents and freshly distilled prior to use. Flash column chromatography was performed on silica gel 60 A C.C. (6–35 μm, SDS). Reactions were monitored by TLC on silica gel 60 F_{254} with detection by charring with H_2SO_4. Melting points were determined with a capillary apparatus and are uncorrected. All products have characteristics similar to those described in theLit. [13a] slight variations in ^{1}H chemical shifts (< 0.02 ppm) were found for **5α**, **5β**, **6α** and **6β** due to substitution of CD_2Cl_2 by $CDCl_3$.

4.2. NMR spectroscopy

NMR spectra were recorded at room temperature with Bruker AC 200, AC 250 or DRX 400, using Me_4Si as internal reference for spectra in $CDCl_3$ and solvent signal for d_4-methanol (3.31 for ^{1}H and 49.0 for ^{13}C). Gradient enhanced COSY, HMBC and HSQC were performed with standard Bruker software. 2D experiments were performed using 256 increment in t_1 and 16 scans for COSY and 32 for HMBC and HSQC, 1024 point were used in the t_2 dimension. Fourier transform was applied after zero filling to 1024 point of the t_1 domain and apodisation in both dimensions with respectively an unshifted sine function or $\pi/2$ shifted squared sinus function for COSY and HSQC and an unshifted sine function in the t_1 dimension and an exponential function (LB = 1 Hz) in the t_2 dimension for HMBC. The spectral widths used lead to digital resolutions in the t_2 dimension (^{1}H) of 2.1 Hz/pt for COSY and 2.6 Hz/pt for HSQC and HMBC.

4.3. Methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-β-L-idopyranuronate (6β)

Crystalline methyl 3-*O*-benzyl-L-iduronate **4**[13a] (4.2 g, 14.1 mmol) was suspended in anhyd CH_2Cl_2 (70 mL) at 0 °C. The mixture was brought to −40 °C and 2,4-dimethylaminopyridine (172 mg, 1.4 mmol, 0.1 equiv), Py (11.3 mL, 141 mmol, 10 equiv) and acetyl chloride (6.0 mL, 84.6 mmol, 6 equiv) were added. After stirring at this temperature for 10 h, the mixture was diluted with CH_2Cl_2 (200 mL) and the resulting organic phase was washed with satd $NaHCO_3$ solution (3 × 50 mL), water (2 × 50 mL), 1 M H_2SO_4 (3 × 50 mL) and water (3 × 50 mL). The organic layer was passed through a phase separator filter paper and concentrated under diminished pressure. The residue was crystallised from Et_2O giving 5.0 g **6β** (11.7 mmol, 83%). Evaporation of the mother liquor followed by flash chromatography gave an additional 0.5 g amount of a **6α**–**6β** (1/2) mixture (combined yield of **6α**/**6β**: 92%).

Acknowledgements

This work was supported by the ANRS (Agence Nationale pour la Recherche sur le SIDA), Sidaction and the CNRS 'Physique Chimie du Vivant' program. We thank Professor A. Lubineau for helpful discussions.

References

1. Esko, J. D.; Sellek, S. B. *Annu. Rev. Biochem.* **2002**, *71*, 435–471.
2. Capila, I.; Linhardt, R. J. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 390–412.
3. Iozzo, R. V. *J. Clin. Invest.* **2001**, *108*, 165–167.
4. Ye, S.; Luo, Y.; Lu, W.; Jones, R. B.; Linhardt, R. J.; Capila, I.; Toida, T.; Kan, M.; Pelletier, H.; McKeehan, W. L. *Biochemistry* **2001**, *40*, 14429–14439 and references cited therein.
5. Lortat-Jacob, H.; Turnbull, J. E.; Grimaud, J. A. *Biochem. J.* **1995**, *310*, 497–505.
6. (a) Lortat-Jacob, H.; Grosdidier, A.; Imberty, A. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 568–573;
   (b) Kuschert, G. S. V.; Coulin, F.; Power, C. A.; Proudfoot, A. E. I.; Hubbard, R. E.; Hoogewerf, A. J.; Wells, T. N. C. *Biochemistry* **1999**, *38*, 12959–12968.
7. (a) Germi, R.; Crance, J. M.; Garin, D.; Guimet, J.; Lortat-Jacob, H.; Ruigrok, R. W. H.; Zarski, J. P.; Drouet, E. *Virology* **2002**, *292*, 162–168;
   (b) Moulard, M.; Lortat-Jacob, H.; Mondor, I.; Roca, G.; Wyatt, R.; Sodroski, J.; Zhao, L.; Olson, W.; Kwong, P. D.; Sattentau, Q. J. *J. Virol.* **2000**, 1948–1960 and refs therein.
8. Warner, R.G.; Hundt, C.; Weiss, S.; Turnbull. *J. Biol. Chem.* **2002**, *271*, 18421–18430.
9. Wang, L.; Brown, J. R.; Varki, A.; Esko, J. D. *J. Clin. Invest.* **2002**, *110*, 127–136.

10. Perrimon, N.; Bernfield, M. *Nature* **2000**, *404*, 725–728.
11. Liu, D.; Shriver, Z.; El Shabrawi, Y.; Sasisekharan, R. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 1229–1234.
12. (a) De Paz, J. L.; Angulo, J.; Lassaletta, J. M.; Nieto, P. M.; Redondo-Horcajo, M.; Lozano, R. M.; Giménez-Gallego, G.; Martin-Lomas, M. *ChemBioChem* **2001**, *2*, 673–685;
(b) Poletti, L.; Fleischer, M.; Vogel, C.; Guerrini, M.; Torri, G.; Lay, L. *Eur. J. Org. Chem.* **2001**, 2727–2734;
(c) Rochepeau-Jobron, L.; Jacquinet, J. C. *Carbohydr. Res.* **1998**, *305*, 181–191;
(d) Tabeur, C.; Machetto, F.; Mallet, J. M.; Duchaussoy, P.; Petitou, M.; Sinaÿ, P. *Carbohydr. Res.* **1996**, *281*, 253–276;
(e) Nilson, M.; Svahn, C. M.; Westamn, J. *Carbohydr. Res.* **1993**, *246*, 161–172.
13. (a) Jacquinet, J. C.; Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J.; Torri, G.; Sinaÿ, P. *Carbohydr. Res.* **1984**, *130*, 221–241;
(b) Van Boeckel, C. A. A.; Petitou, M. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1671–1818.
14. (a) Barroca, N.; Jacquinet, J. C. *Carbohydr. Res.* **2000**, *329*, 667–679;
(b) Duchaussoy, P.; Jaurand, G.; Driguez, P. A.; Lederman, I.; Gourvenec, F.; Strassel, J. M.; Sizun, P.; Petitou, M.; Hebert, J. M. *Carbohydr. Res.* **1999**, *317*, 63–84;
(c) van Boeckel, C. A. A.; Beetz, T.; de Jong, A. J. M.; van Aelst, S. F.; van den Bosch, R. H.; Mertens, J. M. R.; van der Vlugt, F. A. *J. Carbohydr. Chem.* **1985**, *4*, 293–321.
15. Lubineau, A.; Gavard, O.; Bonnaffé, D. *Tetrahedron Lett.* **2000**, *41*, 307–311.
16. (a) Augé, J.; David, S. *New J. Chem.* **1976**, *1*, 57–60;
(b) Bock, K.; Lundt, I.; Pedersen, C. *Tetrahedron Lett.* **1973**, 1037–1040.
17. (a) Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66;
(b) Ritchien, R. G. S.; Cyr, N.; Korsch, B.; Perlin, A. S. *Can. J. Chem.* **1975**, *53*, 1424–1433.
18. Ojeda, R.; de Paz, J. L.; Martin-Lomas, M.; Lassaletta, J. M. *Synlett* **1999**, *8*, 1316–1318.