Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 3647–3651

TETRAHEDRON: *ASYMMETRY*

Regioselective lipase acylation as a useful tool for separation and selective protection of β-D-Gal(1→4)-D-GlcNAc and β-D-Gal(1→3)-D-GlcNAc disaccharides†

Barbara La Ferla,[a,*] Luigi Lay,[a] Giovanni Russo[a] and Luigi Panza[b]

[a] *Università degli Studi di Milano, Dipartimento di Chimica Organica e Industriale, Centro di Studio sulle Sostanze Organiche Naturali del CNR, via G. Venezian, 21–20133 Milano, Italy*
[b] *Università degli Studi del Piemonte Orientale, Facoltà di Farmacia, Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche (DISCAFF), Viale Ferrucci, 33–28100 Novara, Italy*

Received 21 July 2000; accepted 28 August 2000

Abstract

Supported lipase from *Candida antarctica* (Chirazyme®) was employed for a regioselective protection of the 2-azido derivatives **1** and **2**, synthetic equivalents of β-D-Gal(1→3)-D-GlcNAc and β-D-Gal(1→4)-D-GlcNAc (*N*-acetyl lactosamine), respectively. The selectivity of the enzyme towards **1** and **2** was also exploited for an easy separation of the mixture of the two compounds obtained from a straightforward synthetic approach.

A convenient approach to the preparation of complex carbohydrates is based on convergent block synthesis. Therefore, it is particularly interesting to have access to selectively protected building blocks to be used as donors or acceptors for assembly into more complex structures. The disaccharides β-D-Gal(1→4)-D-GlcNAc (*N*-Acetyl lactosamine) and β-D-Gal(1→3)-D-GlcNAc are among the most important components of glycoproteins and glycolipids, which play a central role in many cellular recognition phenomena. Moreover, they are contained in human milk oligosaccharides which exert a protective action on breast fed infants towards many infectious diseases. The great biological relevance of these disaccharides, combined with their scarce availability and extremely high cost, has stimulated many groups in the search of convenient and easy strategies for their synthesis.[1–8] Among other approaches, there has been a growing interest in the use of lipases and proteases, in both hydrolysis and transesterification reactions, as a tool for selective protection and deprotection of carbohydrates.[9] We recently

* Corresponding author.

† In memory of Prof. A. Verbert.

0957-4166/00/$ - see front matter
PII: S0957-4166(00)00342-6

achieved the regioselective enzymatic acylation of lactose building blocks, which were used for the synthesis of higher oligosaccharides.[10]

Our interest towards both human milk oligosaccharides and enzymatic acylation of carbohydrates, induced us to exploit our chemo-enzymatic approach for the preparation of a variety of differently protected building blocks related to the title disaccharides in order to have access to a family of higher oligosaccharides for biological testing.

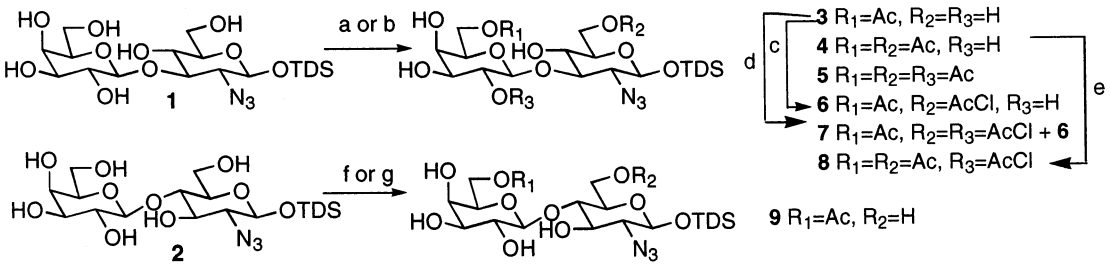
Previous observations revealed that 2-acetamido sugars are poor substrates for lipases. Therefore the behavior of these two disaccharides towards enzymatic acylation was explored on the corresponding 2-azido derivatives. The 2-azido derivatives **1** and **2**, which are synthetic equivalents of β-D-Gal(1→3)-D-GlcNAc and β-D-Gal(1→4)-D-GlcNAc, respectively, were separately submitted to selective enzymatic acetylation using lipase from *Candida antarctica* and vinyl acetate as acylating agent in different solvents. Interesting and unexpected results are reported in Table 1.

Table 1
Enzymatic selective acylation of disaccharides **1** and **2**

Entry	Compound	Conditions	°C	Time (h)	Products	Acylation position	Yield %
1	**1**	THF, vinylacetate	40	24	**3**	6′-OAc	90
2	**1**	CH_3CN, vinylacetate	40	24	**3**	6′-OAc	8
					4	6,6′-OAc	70
					5	6,2′,6′-OAc	16
3	**2**	THF, vinylacetate	45	40	**9**	6′-OAc	90
4	**2**	CH_3CN, vinylacetate	45	40	**9**	6′-OAc	90
5	**3**	CH_3CN, vinylchloroacetate	28	4	**6**	6′-OAc, 6-$OCOCH_2Cl$	90
6	**3**	CH_3CN, vinylchloroacetate	35	1	**6**	6′-OAc, 6-$OCOCH_2Cl$	67
					7	6′-OAc, 6,2′-$OCOCH_2Cl$	29
7	**4**	CH_3CN, vinylchloroacetate	40	12	**8**	6′,6-OAc, 2′-$OCOCH_2Cl$	91

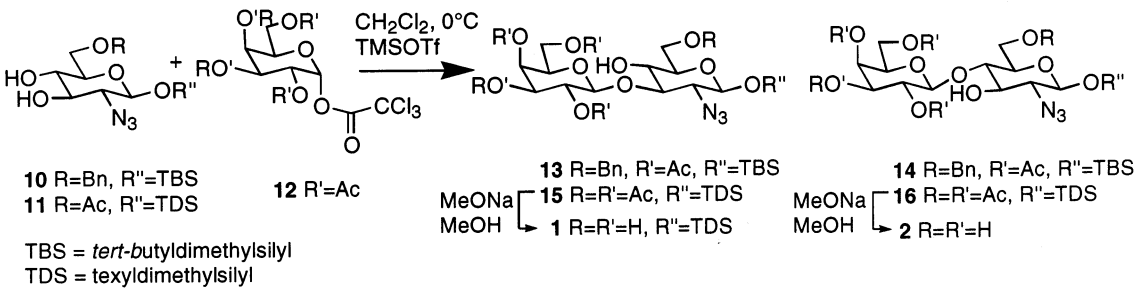
Compound **2** was selectively acetylated at the 6′-position, both in THF and in acetonitrile, in 90% yield, as already observed for the benzyl lactoside,[11] but only if the reaction was effected at 40–45°C; at lower temperatures it did not react. Compound **1** behaved differently to compound **2**. In fact, the acetylation reaction of **1** carried out in THF at 40°C (entry 1) afforded only the 6′-monoacetylated compound **3** in a shorter time than compound **2** (entry 3). Moreover, on switching the solvent from THF to CH_3CN (entry 2) the reaction did not stop after the expected acetylation of the 6′-position, but a second selective acetylation at the 6-position occurred, affording **4** as the main product. A small amount of the 6,6′,2′-tri-*O*-acetyl derivative **5** was also recovered. This surprising behavior has not previously been observed, as lipases usually acylate only the primary hydroxyl on the non-reducing end on a disaccharide. This result was exploited for a straightforward selective differential protection of the two primary hydroxyl groups, which can be chemically achieved only in many steps. By effecting the first acetylation in THF we obtained the 6′-*O*-acetyl derivative **3** (Scheme 1). Compound **3** was then allowed to react with a different acylating agent (vinylchloroacetate) in acetonitrile affording **6** through a second selective acylation at position 6. The temperature control was crucial for a successful

reaction. In fact, when the temperature is kept around 28–29°C only compound **6** (entry 5) was formed, while at higher temperature (35°C) (entry 6) a partial third acylation at position 2′ provides compound **6** along with compound **7**. Taking advantage of these observations, we were able to introduce the chloroacetyl group selectively at position 2′ of compound **4** to obtain **8** in high yield (entry 7).



Scheme 1. Selective enzymatic acylation. Reagents and conditions: (a) THF, vinyl acetate, 40°C, 24 h; (b) CH_3CN, vinyl acetate, 40°C, 24 h; (c) CH_3CN, vinyl chloroacetate, 28°C, 4 h; (d) CH_3CN, vinyl chloroacetate, 25°C, 1 h; (e) CH_3CN, vinyl chloroacetate, 40°C, 12 h; (f) THF, vinyl acetate, 45°C, 40 h; (g) CH_3CN, vinyl acetate, 45°C, 40 h

We also exploited the different reactivity of lipase from *Candida antarctica* to develop a practical route to the 2-azido derivatives **1** and **2**. A quick approach to the synthesis of compounds differentially protected at the anomeric position, described by Schmidt et al.,[12] was based on the glycosylation of diol **10** with the galactosyl trichloroacetamidate **12**. The reaction (Scheme 2) afforded a 4/5 mixture of compounds **13** and **14**, which were separated only by tedious medium pressure chromatography. Applying a similar synthetic strategy with acceptor **11**, in which we put an acetyl group on position 6 in place of the benzyl group used by Schmidt, we obtained compounds **15** and **16** in a ratio of 85/15, and a 76% yield (based on the recovered acceptor). This mixture was submitted to a first crude chromatographic separation, which afforded 32% of pure **15** and 44% of a 2.8/1 mixture of **15** and **16** as estimated by NMR. The mixture was deacetylated and submitted to enzymatic acetylation using vinyl acetate as acylating agent in THF at 35°C for 5 days. Using the above conditions, the more reactive compound **1** was acetylated, whereas compound **2** remained unreacted.[13] The reaction mixture was now easily separated using standard flash chromatography: from compound **1** the 6′-monoacetylated **3**[14] was isolated in 72% yield together with a little amount of 6,6′-diacetylated **4** (25%), whereas pure compound **2** was recovered unreacted (Table 2).



Scheme 2. Synthesis of compounds **1** and **2**

Table 2
Separation of a mixture of disaccharides **1** and **2** by selective acylation

Compound	Conditions	Product	Yield %[a]
1+**2**	THF, vinylacetate,	6,6′-OAc **4**	25
(2.8:1)	*Candida antartica*,	6′-OAc **3**	72
	35°C, 5 days	Unreacted **2**	90

[a] The yield is calculated considering only the relevant starting compound.

In conclusion the above technique allows the regioselective introduction of different acyl protecting groups on the title disaccharides. In particular compound **1** showed to be highly versatile allowing the regioselective protection at position 6′, 6,6′ or 6,6′,2′ by tuning the reaction conditions through the reactivity of the acylating agent and the proper choice of the solvent. Moreover, exploiting the intrinsic different reactivity of the disaccharides **1** and **2** it was possible to selectively acylate only compound **1**, thus allowing their otherwise difficult separation. Such disaccharidic building blocks, regioselectively protected in positions 6′, 6,6′ or 6,6′,2′ by lipase catalyzed acylation, will be further elaborated and used for complex oligosaccharide synthesis.

Acknowledgements

This work was supported by EU-NOFA project (grant FAIR CT973142).

References

1. Paulsen, H.; Hölck, J. P. *Liebigs Ann. Chem.* **1982**, 1121.
2. Toepfer, A.; Schmidt, R. R. *Carbohydr. Res.* **1993**, *247*, 159–164.
3. Zhu, X. X.; Ding, P. Y.; Cai, M. S. *Tetrahedron Lett.* **1996**, *37*, 8549–8552.
4. Wrodnigg, T. M.; Stutz, A. E. *Angew. Chem., Int. Ed.* **2000**, *38*, 827–828.
5. Kimura, T.; Takayama, S.; Huang, H.; Wong, C. H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2348–2350.
6. Li, J.; Wang, P. G. *Tetrahedron Lett.* **1997**, *38*, 7967–7970.
7. Vic, G.; Tran, C. H.; Scigelova, M.; Crout, D. H. G. *Chem. Commun.* **1997**, 169–170.
8. Fang, J. W.; Xie, W. H.; Li, J.; Wang, P. G. *Tetrahedron Lett.* **1998**, *39*, 919–922.
9. Waldmann, H.; Sebastian, D. *Chem. Rev.* **1994**, *94*, 911.
10. La Ferla, B.; Lay, L.; Poletti, L.; Panza, L.; Russo, G. *J. Carb. Chem.* **2000**, *19*, 331–343.
11. Unpublished results.
12. Toepfer, A.; Kinzy, W.; Schmidt, R. R. *Liebigs Ann. Chem.* **1994**, 449–464.
13. Only traces of the 6′-monoacetylated compound **9**, derived from **2**, were detected by TLC.
14. ^{1}H NMR diagnostic data: **3** (MeOD, 300 MHz): δ (ppm) 4.58 (d, 1H, $J1,2=7.5$ Hz, H-1), 4.44 (d, 1H, $J1',2'=7.5$ Hz, H-1′), 4.31 (dd, 1H, $J6'a,6'b=11.6$, $J5',6'b=8.3$ Hz, H-6′b), 4.19 (dd, 1H, $J6'a,6'b=11.6$, $J5',6'a=4.5$ Hz, H-6′a), all other protons have a $\delta<3.90$ ppm. **4** (MeOD, 300 MHz): δ (ppm) 4.59 (d, 1H, $J1,2=7.1$ Hz, H-1), 4.44 (d, 1H, $J1',2'=7.1$ Hz, H-1′), 4.40 (dd, 1H, $J6'a,6'b=11.4$, $J5',6'b=1.4$ Hz, H-6′b), 4.31 (dd, 1H, $J6'a,6'b=11.4$, $J5',6'a=8.4$ Hz, H-6′a), 4.23 (dd, 1H, $J6a,6b=11.7$, $J5,6b=4.2$ Hz, H-6b), 4.17 (dd, 1H, $J6a,6b=11.7$, $J5,6a=7.2$ Hz, H-6a), all other protons have a $\delta<3.90$ ppm. **6** (MeOD, 300 MHz): δ (ppm) 4.61 (d, 1H, $J1,2=7.1$ Hz, H-1), 4.53 (dd, 1H, $J6'a,6'b=11.5$, $J5',6'b=1.6$ Hz, H-6′b), 4.44 (d, 1H, $J1',2'=7.0$ Hz, H-1′), 4.32 (dd, 1H, $J6'a,6'b=11.5$, $J5',6'a=7.6$ Hz, H-6′a), 4.27 (dd, 1H, $J6a,6b=11.7$, $J5,6b=5.3$ Hz, H-6b), 4.19 (dd, 1H, $J6a,6b=11.7$, $J5,6a=4.4$ Hz, H-6a), 4.18 (s, 2H, CH_2Cl), all other protons have a $\delta<3.90$ ppm. **7** (MeOD, 300

MHz): δ (ppm) 5.12 (dd, 1H, $J2',3'=9.4$, $J1',2'=8.3$ Hz, H-2′), 4.70–4.15 (m, 10H, H-1,1′,6a,6b,6′a,6′b, $2CH_2Cl$), all other protons have a $\delta<3.90$ ppm. **8** ($CDCl_3$, 300 MHz): δ (ppm) 5.12 (dd, 1H, $J2',3'=9.3$, $J1',2'=8.2$ Hz, H-2′), 4.53 (d, 1H, $J1,2=8.0$ Hz, H-1), 4.49 (d, 1H, $J1',2'=7.1$ Hz, H-1′), 4.44–4.27 (m, 3H, H-6′b,6′a,6b), 4.22–4.14 (m, 3H, H-6a, CH_2Cl), all other protons have a $\delta<4.0$ ppm. **9** (MeOD, 300 MHz): δ (ppm) 4.56 (d, 1H, $J1,2=7.7$ Hz, H-1), 4.37 (d, 1H, $J1',2'=7.0$ Hz, H-1′), 4.33 (dd, 1H, $J6'a,6'b=11.7$, $J5',6'b=8.6$ Hz, H-6′b), 4.23 (dd, 1H, $J6'a,6'b=11.7$, $J5',6'a=4.0$ Hz, H-6′a), all other protons have a $\delta<3.90$ ppm.