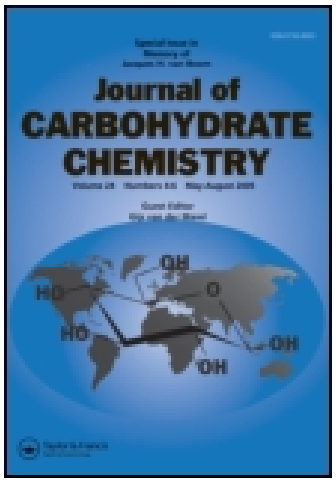
This article was downloaded by: [Tufts University]
On: 22 October 2014, At: 07:35
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

IMPROVEMENT ON LIPASE CATALYSED REGIOSELECTIVE O-ACYLATION OF LACTOSE:A CONVENIENT RUOTE TO 2′-O- FUCOSYLLACTOSE

Anna Rencurosi [a] , Laura Poletti [a] , Luigi Panza [b] & Luigi Lay [c]

[a] Dipartimento di Chimica Organicae Industriale , Centro di Studio sulle Sostanze Organiche Naturali del CNR , Università degli Studi di Milano , via G. Venezian, 21-20133, Milano, Italy

[b] Facoltà di Farmacia, Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche (DISCAFF),Viale Ferrucci, 33-28100 , Università degli Studi del Piemonte Orientale , Novara, Italy

[c] Facoltà di Farmacia, Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche (DISCAFF),Viale Ferrucci, 33-28100 , Università degli Studi del Piemonte Orientale , Novara, Italy

Published online: 16 Aug 2006.

To cite this article: Anna Rencurosi , Laura Poletti , Luigi Panza & Luigi Lay (2001) IMPROVEMENT ON LIPASE CATALYSED REGIOSELECTIVE O-ACYLATION OF LACTOSE:A CONVENIENT RUOTE TO 2′-O- FUCOSYLLACTOSE , Journal of Carbohydrate Chemistry, 20:7-8, 761-765

To link to this article: http://dx.doi.org/10.1081/CAR-100108289

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the “Content”) contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

J. CARBOHYDRATE CHEMISTRY, 20(7&8), 761–765 (2001)

COMMUNICATION

IMPROVEMENT ON LIPASE CATALYSED REGIOSELECTIVE *O*-ACYLATION OF LACTOSE: A CONVENIENT ROUTE TO 2′-*O*-FUCOSYLLACTOSE[1]

Anna Rencurosi,[1] Laura Poletti,[1] Luigi Panza,[2] and Luigi Lay,[2,*]

[1]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
[2]Università degli Studi del Piemonte Orientale, Facoltà di Farmacia, Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche (DISCAFF), Viale Ferrucci, 33–28100 Novara, Italy

Downloaded by [Tufts University] at 07:35 22 October 2014

Although enormous progress has been made in the past ten years, the synthesis of complex carbohydrates still remains a severe problem. Conventional syntheses of these molecules often dictate multiple protections and deprotections of various hydroxyl groups, dramatically increasing the number of steps required. On the other hand, enzymes offer the opportunity to carry out cheap, highly chemo and regioselective transformations, providing new approaches to tackle many synthetic problems encountered in carbohydrate synthesis. In particular, lipases allow highly regioselective *O*-acylations. Therefore, a growing interest in enzymatic manipulation of protecting groups for the synthesis of oligosaccharides has been developed during recent years.[2]

Recently, we reported on the use of lipase catalysed acylation for designing useful building blocks in oligosaccharide synthesis.[3,4] Excellent selectivity for 6′-*O*-acylation was observed on various disaccharidic substrates (such as lactose, maltose and cellobiose) using different acylating agents,[3] *Candida antarctica* lipase being the best biocatalyst. However, our procedure contained a main drawback: the high polarity of the sugar substrates required the use of *tert*-amyl alcohol as a solvent, whose high boiling point made the work up of the reaction mixture quite laborious, thus rendering the method impractical. An important improvement

Copyright © 2001 by Marcel Dekker, Inc. www.dekker.com

ORDER REPRINTS

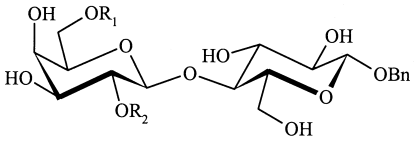
Table 1. Experimental Conditions and Yields of Enzymatic Acylation Reactions

Entry	Substrate	Acylating Agent	Conditions	Product	Yield (%)
1	**1**	Vinyl acetate	THF, 40°C, 3 days	**2a**	73
2	**1**	Vinyl acetate	CH_3CN, 40°C, 4 days	**2a**	64
3	**1**	Trifluoroethyl levulinate	THF, 40°C, 4 days	**2b**	83
4	**1**	Vinyl chloroacetate	THF, 40°C, 2 days	**2c**	78
5	**2a**	Trifluoroethyl levulinate	CH_3CN, 45°C, 4 days	**3a**	65
6	**2a**	Vinyl chloroacetate	CH_3CN, 45°C, 5 days	**3b**	62

was achieved when we observed that when using relatively low boiling solvents such as THF or CH_3CN, the enzymatic acylations proceeded with equal efficiency, despite the poor solubility of the substrates. For example, employing THF as a solvent, we could introduce a chloroacetyl group at the 6′-OH of lactose with very high regioselectivity and chemical yield (78%) and with a much easier product recovery.[4] This finding prompted us to explore in more detail the behaviour of the *Candida antarctica* lipase in different solvents and on various biologically relevant substrates.[5] In the present communication we show further achievements obtained in the functionalization of lactose and a new straightforward synthesis of 2′-*O*-fucosyllactose **7**.[6]

In a preliminary screening of different organic solvents, THF and CH_3CN gave the best results. In THF, 6′-*O*-acylated lactosides were obtained regioselectively from **1**[7] at 40 °C in 73–83 % yields from different acylating agents with *Candida antarctica* lipase[8] (Table 1). The same reaction performed in CH_3CN (Table 1, entry 2) showed less selectivity, giving, besides the 6′-*O*-monoacylated derivative as the main product, fair amounts (16%) of diacylated disaccharides. However, when compound **2a** (Scheme 1) was submitted to a second acylation with lipase from *Candida antarctica* in CH_3CN at 45 °C, an excellent regioselectivity for the 2′-OH was observed with both the acylating agents employed (Table 1, entries 5 and 6). All the reactions were very clean, without formation of by-products, allowing full recovery of the unreacted substrate. Thus, a double sequential acylation, changing the solvent and using orthogonal protecting groups, provides an easy access to a family of lactose building blocks.

We took advantage from our chemo-enzymatic approach to design a new convenient route to the synthesis of 2′-*O*-fucosyllactose **7**, which, after lactose, was found to be the most abundant component of the human milk oligosaccharides



1 R_1=R_2=H
2a R_1=Ac, R_2=H
2b R_1=Lev, R_2=H
2c R_1= ClAc, R_2=H

3a R_1=Ac, R_2=Lev
3b R_1=Ac, R_2=ClAc

Scheme 1.

Downloaded by [Tufts University] at 07:35 22 October 2014

Copyright © Marcel Dekker, Inc. All rights reserved.

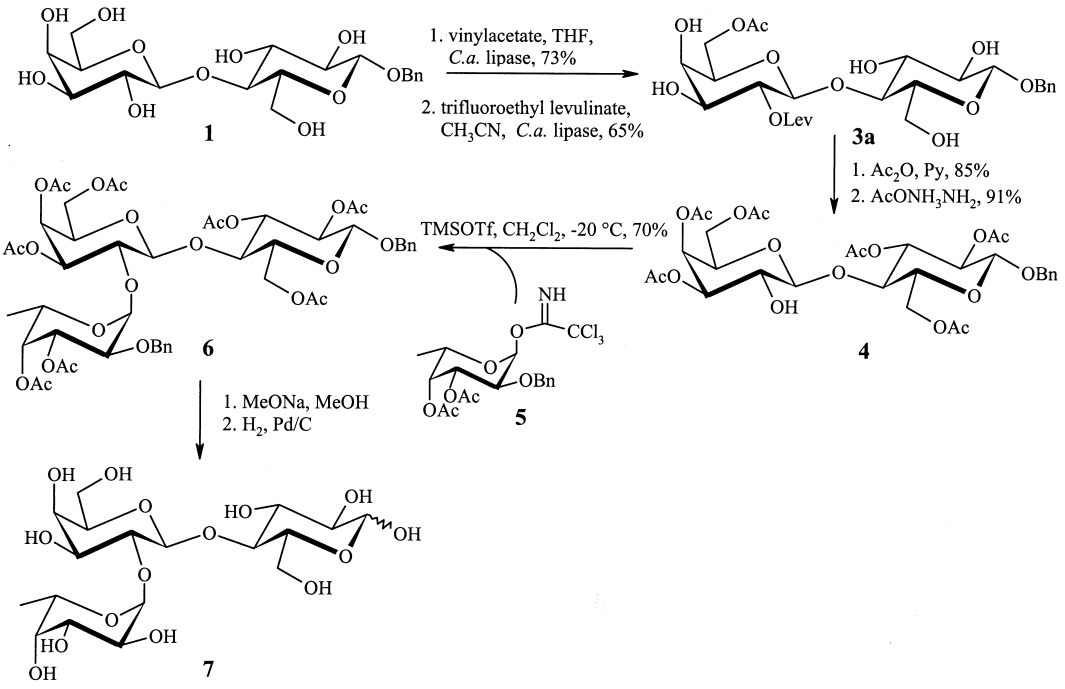
Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

in 77 % of Caucasian women classified as secretors.[9] It seems reasonable that 2′-*O*-fucosyllactose may play an important role in determining the antiadhesive properties of human milk oligosaccharides, which have been demonstrated to represent a protection against infections for breast-fed infants during the lactation period.[10]

To the best of our knowledge, only three chemical syntheses of 2′-*O*-fucosyllactose have been reported so far, all of them relying on a tri-*O*-isopropylidene lactose derivative as a key intermediate.[11] However, our procedure offers some advantages, such as better overall yield and easier removal of the protective groups. Our synthesis of the 2′-*O*-fucosyllactose was accomplished as follows. An acetyl and a levulinoyl group were sequentially introduced on the benzyl lactoside **1**[7] to give compound **3a** in 47% overall yield (95% conversion). After conventional acetylation of the remaining hydroxyl groups and selective removal of levulinoyl ester, acceptor **4** was glycosylated with the α-trichloroacetimidate of the 3,4 di-*O*-acetyl-2-*O*-benzyl-L-fucopyranose **5**[12] to give the protected 2′-*O*-fucosyllactose **6**[13] in 70 % yield (Scheme 2).

The deprotection to 2′-*O*-fucosyllactose was very cleanly and easily accomplished by standard Zemplèn deacetylation and hydrogenolysis (Pd/C, H_2, MeOH/H_2O, 24 h) of the remaining benzyls leading to 2′-*O*- fucosyllactose **7** in quantitative yield (Scheme 2). The optical rotation ($[\alpha]_D$ −52.3, *c* 0.6, H_2O, after three days) as well as ^1H and ^{13}C NMR data from compound **7** are in agreement with those reported in the literature;[6,14] ^1H NMR (300 MHz, D_2O): δ 5.51, H-1″



Scheme 2.

Downloaded by [Tufts University] at 07:35 22 October 2014

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

(α/β), J = 2.7 Hz, 5.43, H-1α, J = 3.7 Hz, 4.72, H-1′ (α/β), J = 7.7 Hz, (the signal of H-1β is overlapped with the solvent peak at δ 4.80–4.85); ^{13}C NMR (75.44 MHz, D_2O): δ 103.11 (C-1′), 102.15 (C-1″), 98.72 (C-1β), 94.64 (C-1α).

In conclusion, we described a new and easy chemo-enzymatic synthesis of the biologically relevant 2′-*O*-fucosyllactose exploiting a versatile lactose building block, obtained by a double sequential acylation at 6′-OH and 2′-OH with two orthogonal protecting groups catalysed by lipase from *Candida antarctica*.

ACKNOWLEDGMENTS

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

REFERENCES

1. Dedicated to Prof. Joachim Thiem on the occasion of his 60th anniversary.
2. a) Khan, R.; Gropen, L.; Konowicz, P.A.; Matulovà, M.; Paoletti, S. Enzymic regioselective hydrolysis of peracetylated reducing disaccharides, specifically at the anomeric center: intermediates for the synthesis of oligosaccharides. Tetrahedron Lett. **1993**, *34*, 7767–7770.
   b) Palmer, D. C.; Terradas, F. Regioselective enzymatic deacetylation of sucrose octaacetate in organic solvents. Tetrahedron Lett. **1994**, *35*, 1673–1676.
   c) Cai, S.; Hakomori, S.; Toyokuni, T. Application of protease-catalyzed regioselective esterification in synthesis of 6′-deoxy-6′-fluoro- and 6-deoxy-6-fluorolactosides. J. Org. Chem. **1992**, *57*, 3431–3437.
3. Lay, L.; Panza, L.; Riva, S.; Khitri, M.; Tirendi, S. Regioselective acylation of disaccharides by enzymatic transesterification. Carbohydr. Res. **1996**, *291*, 197–204.
4. La Ferla, B.; Lay, L.; Poletti, L.; Russo, G.; Panza, L. Easy chemo-enzymatic synthesis of human milk trisaccharides from a common selectively protected lactose building block. J. Carbohydr. Chem. **2000**, *19*, 331–343.
5. La Ferla, B.; Lay, L.; Russo, G.; Panza, L. 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. Tetrahedron: Asymmetry **2000**, *11*, 3647–3651.
6. a) Kuhn, R.; Baer, H.H.; Gauhe, A. Fucosido-lactose, das Trisaccharid der Frauenmilch. Chem. Ber. **1955**, *88*, 1135–1146.
   b) Kuhn, R.; Baer, H.H.; Gauhe, A. Kristallisierte Fucosido-lactose. Chem. Ber. **1956**, *89*, 2513.
7. Jung, K-H.; Hoch, M.; Schmidt, R.R. Selectively protected lactose and 2-azido lactose, building blocks for glycolipid synthesis. Liebigs Ann. Chem. **1989**, 1099–1106.
8. General procedure for enzymatic acylation reaction: The substrate (**1** or **2a**) (100 mg) suspended in the solvent (10 mL) and the activated ester (2 mL) were shaken in the presence of *Candida antarctica* lipase (300 mg), immobilized on macroporus acrylic resin (CHIRAZYME® L-2, c.-f. C2, lyo; purchased from Roche Diagnostics), at the temperature reported in Table1. The enzyme was filtered off and the solvent removed at reduced pressure. The acylated product and the unreacted substrate were isolated by chromatography on silica gel with ethyl acetate/methanol 9/1.
9. Kunz, C.; Rudloff, S. Biological functions of oligosaccharides in human milk. Acta Pediatr. **1993**, *82*, 903–912.

Downloaded by [Tufts University] at 07:35 22 October 2014

Copyright © Marcel Dekker, Inc. All rights reserved.

ORDER REPRINTS

10. a) Coppa, G.V.; Gabrielli, O.; Giorgi, P.; Catassi, C.; Montanari, M. P.; Varaldo, P. E.; Nichols, B. L. Preliminary study of breastfeeding and bacterial adhesion to uroepithelial cells. The Lancet **1990**, *335*, 569–571.
b) Cravioto, A.; Tello, A.; Villafan, H.; Ruiz, J.; Del Vedovo, S.; Neeser, J-R. Inhibition of localized adhesion of enteropathogenic Escherichia coli to HEp-2 cells by immunoglobulin and oligosaccharide fractions of human colostrum and breast milk. J. Infect. Dis. **1991**, 1*63*, 1247–1255.
c) Laegreid, A.; Otnaess, A. B. K.; Fuglesang, J. Human and bovine milk: comparison of ganglioside composition and enterotoxin-inhibitory activity. Pediatr. Res. **1986**, *20*, 416–421.
d) Newburg, D. S.; Pickering, L. K; McCluer, R. H.; Cleary, T. G. Fucosylated oligosaccharides of human milk protect suckling mice from heat-stable enterotoxin of Escherichia coli. J. Infect. Dis. **1990**, *162*, 1075–1080.
11. a) Abbas, S.A.; Barlow, J.J.; Matta, K. L. Synthetic studies in carboydrates. Part XIV. Synthesis of *O*-α-L-fucopyranosyl-(1–2)-*O*-β-D-galactopyranosyl-(1–4)-D-glucopyranose (2′-*O*-α-L-fucopyranosyllactose). Carbohydr. Res. **1981**, *88*, 51–60.
b) Jain, R. K.; Locke, R.D.; Matta, K. L. Synthetic studies in carboydrates. Part LXXVII. A convenient synthesis of *O*-α-L-fucopyranosyl-(1–2)-*O*-β-D-galactopyranosyl-(1–4)-D-glucopyranose (2′-*O*-α-L-fucopyranosyllactose). Carbohydr. Res. **1991**, *212*, c1-c3.
c) Izumi, M.; Tsuruta, O.; Harayama, S.;. Hashimoto, H. Synthesis of 5-thio-L-fucose-containing disaccharides, as sequence-specific inhibitors, and 2′-fucosyllactose, as substrate of α-L-fucosidases. J. Org. Chem. **1997**, *62*, 992–998.
d) Matta, K. L.; Jain, R. K.; Locke, R.D. US Patent 5,438,124; 1 Aug 1995; (CA, **124**, 30256j, 1996).
12. Manzoni, L.; Lay, L.; Schmidt, R.R. Synthesis of Lewis a and Lewis x pentasaccharides based on *N*-trichloroethoxycarbonyl protection. J. Carbohydr. Chem. **1998**, *17*, 739–758 and references therein.
13. Selected analytical data for trisaccharide **6**: $[\alpha]_D$ − 47.7° (*c* 1.05, $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39−7.21 (m, 10H, H_{Ar}), 5.32 (d, 1H, $J_{3',4'}$ = 3.3 Hz, H-4′), 5.26 (d, 1H, $J_{1'',2''}$ = 3.9 Hz, H-1″), 5.25 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 4.6 Hz, H-4″), 5.15 (dd, 1H, $J_{2'',3''}$ = 10.6 Hz, H-3″), 5.10 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.4 Hz, H-3), 5.01 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, H-3′), 5.00 (t, 1H, H-2), 4.86 (d, 1H, J = 12.4 Hz, C*H*Ph), 4.59 (d, 1H, J = 12.4 Hz, C*H*Ph), 4.59 (unresolved, 1H, H-6a), 4.58 (d, 1H, C*H*Ph), 4.53 (d, 1H, C*H*Ph), 4.51 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.43 (d, 1H, $J_{1',2'}$ = 7.6 Hz, H-1′), 4.37 (qd, 1H, H-5″), 4.30 (dd, 1H, $J_{6a,5}$ = 4.7 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6b), 4.15−4.02 (m, 2H, H-6′a, H-6′b), 3.88 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.0 Hz, H-4), 3.85−3.79 (m, 3H, H-2′, H-5′, H-2″), 3.62 (ddd, H-5), 2.12−1.86 (6s, 24 H, CH_3CO), 1.13 (d, 3H, $J_{5'',6''}$ = 6.6 Hz, H-6″); ^{13}C-NMR (75.44 MHz, $CDCl_3$): δ 170−169 (8 *C*O), 136−127 (C_{Ar}), 100.71, 99.41, 97.14 (C-1, C-1′, C-1″), 73.99, 73.70, 73.19, 73.18, 72.89, 72.30, 72.15, 71.63, 71.29, 70.69, 69.72 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″), 73.18, 70.69 (2 *C*H_2Ph), 20.74−20.46 (*C*H_3CO), 15.65 (C-6″) Anal. Calcd for $C_{48}H_{60}O_{23}$ (1004.80): C, 57.37; H, 6.02. Found: C, 57.42; H, 6.05.
14. Ishizuka, Y.; Nemoto, T.; Fujiwara, M.; Fujita, K.; Nakanishi, H. Three-dimensional structure of fucosyllactoses in an aqueous solution. J. Carbohydr. Chem. **1999**, *18*, 523–533.

Received March 19, 2001
Accepted September 5, 2001

Downloaded by [Tufts University] at 07:35 22 October 2014

Copyright © Marcel Dekker, Inc. All rights reserved.

Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the U.S. Copyright Office for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on Fair Use in the Classroom.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our Website User Agreement for more details.

Order now!

Reprints of this article can also be ordered at
http://www.dekker.com/servlet/product/DOI/101081CAR100108289

Downloaded by [Tufts University] at 07:35 22 October 2014