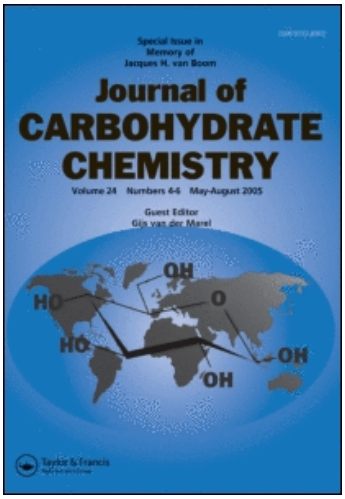

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IMPROVEMENT ON LIPASE CATALYSED REGIOSELECTIVE *O*-ACYLATION OF LACTOSE:A CONVENIENT RUOTE TO 2'-*O*-FUCOSYLLACTOSE[1]

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COMMUNICATION

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

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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

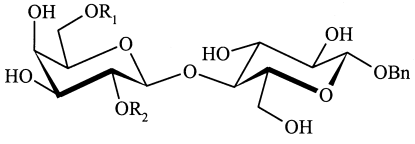
Table 1. Experimental Conditions and Yields of Enzymatic Acylation Reactions

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.

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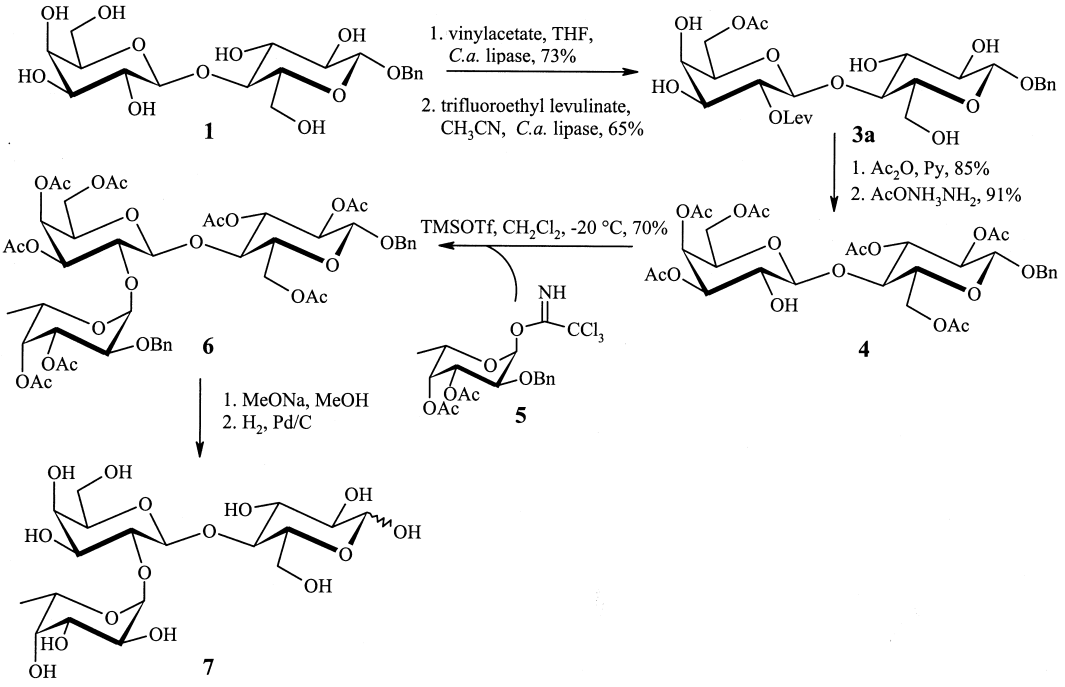
in 77 % of Caucasian women classified as secretors.[9] It seems reasonable that 2′-*O*-fucosyllactose may play an important role in determining the antiadhesive properties of human milk oligosaccharides, which have been demonstrated to represent a protection against infections for breast-fed infants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

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(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

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