Journal of Molecular Catalysis B: Enzymatic 59 (2009) 197–200

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

A short-step chemo-enzymatic synthesis of a precursor for L-nucleosides from D-lyxose

Kazunori Kitsuda, Jordi Calveras, Yasuhito Nagai, Toshinori Higashi, Takeshi Sugai*

Faculty of Pharmacy, Keio University, 1-5-30, Shibakoen, Tokyo 105-8512, Japan

ARTICLE INFO

Article history:
Received 2 February 2009
Received in revised form 20 February 2009
Accepted 20 February 2009
Available online 5 March 2009

Keywords:
L-Ribose
L-Nucleoside
D-Lyxose
Lipase
Regioselective reaction
Stereochemical inversion

ABSTRACT

A new chemo-enzymatic route to tetra-*O*-acetyl-L-ribofuranose from D-lyxose is described. Lipase-catalyzed regioselective transesterification of acetate proceeded on C-4 of the D-lyxopyranoside. Subsequently, stereochemistry of liberated secondary alcohol was inverted by way of oxidation and reduction by IBX and $NaBH(OAc)_3$ to give L-ribopyranoside. After deprotection, the furanose–pyranose isomeric mixture was converged to the target molecule, taking advantage of lipase-catalyzed preferential acetylation of primary alcohol on C-5.

1. Introduction

L-Nucleosides, the enantiomeric forms of D-nucleosides have recently been gaining interest as antiviral drugs [1,2]. Among synthetic precursors, peracylated form of L-ribofuranose is a good glycosyl donor [3] to control the stereochemistry in β-orientation. So far, many synthetic efforts toward the parent pentose, L-ribose have been devoted [4–7]. In this paper, we describe a chemo-enzymatic short-step synthesis of peracetylated form **1a*f*** from D-lyxose **2a**. As shown in Scheme 1, if we compare the pyranose forms of L-ribose and D-lyxose, only a simple inversion of the absolute configuration at C-4 is enough. The transformation, however, requires the regioselective protection of hydroxyl groups at C-1, C-2, and C-3 on the pyranose form of D-lyxose.

2. Experimental

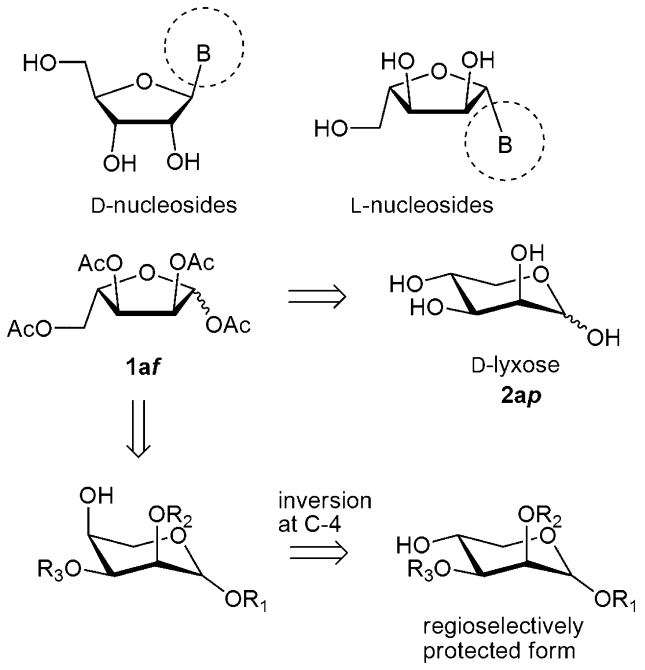
IR spectra were measured as thin films for oils or ATR for solid on a Jeol FT-IR SPX60 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were measured at 400 and 100 MHz respectively, on a VARIAN 400-MR spectrometer. Optical rotation values were recorded on a Jasco P-1010 polarimeter. Silica gel 60 (spherical, 100–210 μm, 37558-79) from Kanto Chemical Co. and florisil (100–200 mesh) from Nakarai Chemical were used for column chromatography.

2.1. 4-Methoxyphenyl 2,3-di-O-acetyl-α-D-lyxopyranoside (*2d*)

4-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-D-lyxopyranoside **2c** [8] (1.043 g, 2.73 mmol) was dissolved in toluene (20 mL). Cyclopentanol (0.8 mL) and *Candida antarctica* lipase B (Roche Diagnostics, Chirazyme L-2, 0.994 g) were added to the solution with stirring. The mixture was stirred for 24 h at 60 °C. The reaction was monitored by silica gel TLC (hexane/AcOEt = 2:3). After cooling, the mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (46 g). Elution with hexane/AcOEt = 1:1 afforded **2d** (0.898 g, 97%) as a colorless oil. $[\alpha]_D^{25}$: +46.4 (c 1.52, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.10 (s, 3H, Ac), 2.13 (s, 3H, Ac), 3.71 (dd, $J_{4,5b}$ = 10.0 Hz, $J_{5a,5b}$ = 11.4 Hz, 1H, H5b), 3.75 (s, 3H, ArOCH_3), 3.87 (dd, $J_{4,5a}$ = 5.5 Hz, 1H, H5a), 4.13 (ddd, $J_{3,4}$ = 9.6 Hz, 1H, H4), 5.31 (d, $J_{1,2}$ = 2.2 Hz, 1H, H1), 5.31 (dd, $J_{2,3}$ = 3.3 Hz, 1H, H3), 5.38 (dd, 1H, H2), 6.78–6.99 (4H, Ar-*H*); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.8, 20.9, 55.6, 63.1, 65.6, 69.6, 72.1, 96.8, 114.6, 117.9, 149.9, 155.2, 170.0, 171.1; the signal 114.6 and 117.9 included totally four carbons; IR: 3460, 2945, 2837, 1743, 1506 cm^{-1}; anal. calcd for $C_{16}H_{20}O_8$: C 56.47, H 5.92; found: C 56.07, H 5.96. When the same triacetate **2c** was treated with *C. antarctica* lipase in buffer solution, the reaction resulted in the highly polar mixture. Instead, hydrolysis of **2c** with another lipase from *Burkholderia cepacia* (Amano PS-IM) provided **2d**, which was contaminated with an impurity whose proton chemical shifts at C-2 and C-3 appeared upfileded: δ 4.19 (dd, $J_{1,2}$ = 3.2 Hz, $J_{2,3}$ = 3.2 Hz, 1H, H2), 4.27 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H3), respectively.

* Corresponding author. Fax: +81 3 5400 2665.
E-mail address: sugai-tk@pha.keio.ac.jp (T. Sugai).

1381-1177/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2009.02.014



Scheme 1.

2.2. 4-Methoxyphenyl 2,3-di-O-acetyl-4-deoxy-4-oxo-α-D-lyxopyranoside (*3*)

Diacetate **2d** (444 mg, 1.31 mmol) was dissolved in AcOEt (10 mL). IBX (1.14 g, 4.07 mmol, 3 eq.) was added to the solution with stirring. The mixture was stirred for 32 h at 75 °C. The reaction was monitored by silica gel TLC (hexane/AcOEt = 2:3). After cooling, the mixture was filtered and concentrated *in vacuo* to afford **3** (435 mg, 99%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13 (s, 3H, Ac), 2.18 (s, 3H, Ac), 3.77 (s, 3H, ArOC*H*$_3$), 4.11 (d, $J_{5a,5b}$ = 15.3 Hz, 1H, H5a), 4.40 (d, 1H, H5b), 5.57 (d, $J_{1,2}$ = 2.4 Hz, 1H, H1), 5.65 (dd, $J_{2,3}$ = 3.9 Hz, 1H, H2), 6.01 (d, 1H, H3), 6.82–7.03 (4H, Ar-*H*). This was employed for the next step without further purification.

2.3. 4-Methoxyphenyl 2,3-di-O-acetyl-β-L-ribopyranoside (*1b*) and 4-methoxyphenyl 2,3-di-O-acetyl-α-D-lyxopyranoside (*2d*)

Ketone **3** (435 mg, 1.29 mmol) was dissolved in a mixture of CH_3CN (6.5 mL) and AcOH (6.5 mL). After stirring at −45 °C, $NaBH(OAc)_3$ (826 mg, 3.90 mmol, 3 eq.) was added to the solution. The mixture was stirred for 7 h, by slowly raising to 10 °C, and quenched by adding saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with AcOEt. The combined extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford a mixture (10/1) of **1b** and **2d** (430 mg, 98%). ^{1}H NMR spectrum (400 MHz, $CDCl_3$) attributed to **1b** is as follows: δ 2.13 (s, 3H, Ac), 2.17 (s, 3H, Ac), 3.75 (s, 3H, ArOC*H*$_3$), 3.84 (dd, $J_{4,5a}$ = 2.7 Hz, $J_{5a,5b}$ = 12.3 Hz, 1H, H5a), 4.00 (m, 1H, H4), 4.07 (dd, $J_{4,5b}$ = 1.2 Hz, 1H, H5b), 5.29–5.40 (3H, H1-3), 6.80–6.99 (4H, Ar-*H*). This was employed for the next step without further purification.

2.4. 2,3-Di-O-acetyl-L-ribose (*1c*) and 2,3-di-O-acetyl-D-lyxose (*2e*)

The mixture of **1b** and **2d** (391 mg, 1.15 mmol) were dissolved in a mixture of CH_3CN (54 mL) and H_2O (13.5 mL). CAN [$(NH_4)_2[Ce(NO_3)_6]$] (4.42 g, 8.06 mmol, 7 eq.) was added to the solution at room temperature. The reaction was monitored by silica gel TLC (hexane/AcOEt = 1:2). After stirring for 1 h at room temperature, the reaction was quenched by adding Dowex® 1X8 resin (Cl^- form). After adjusting its pH to 7–8, the mixture was filtered and concentrated *in vacuo*. The residue was refined through a short column of florisil (7.8 g). Elution with hexane/THF = 2:1 afforded a mixture of **1c** and **2e**. This was employed for the next step without further purification.

2.5. 1,2,3,5-Tetra-O-acetyl-L-ribofuranose (*1af*)

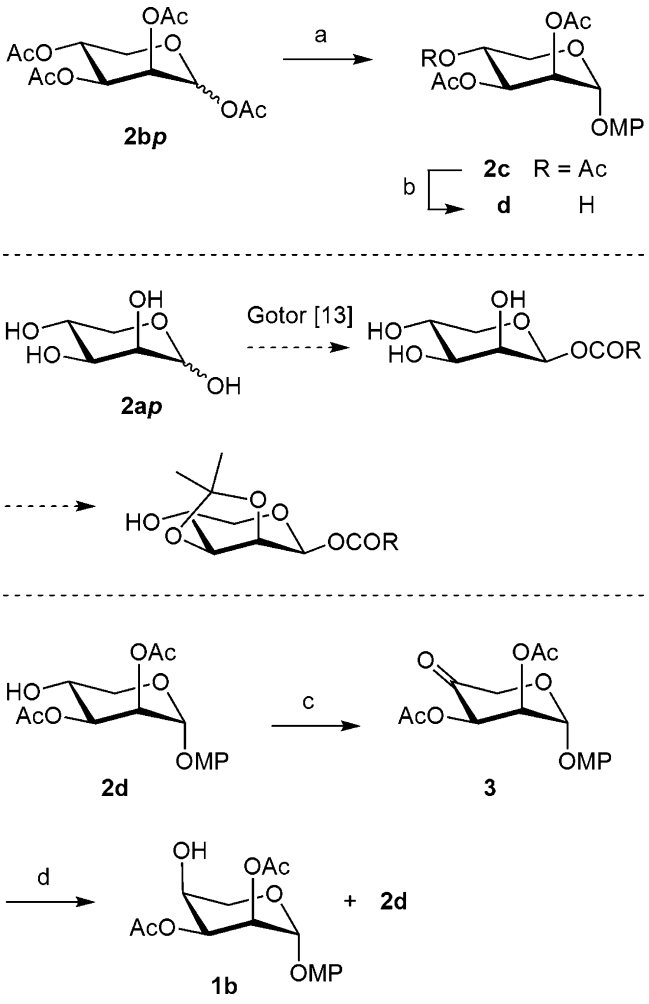
The mixture of **1c** and **2e** as above was dissolved in vinyl acetate (8 mL). *C. antarctica* lipase (576 mg) was added to the solution with stirring. The mixture was stirred for 24 h at 30 °C. The reaction was monitored by silica gel TLC (hexane/THF = 1:1). The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (13 g). Elution with hexane/THF = 1:2 afforded **1d*f*** (142.9 mg, 45%). This was acetylated by a conventional manner to give **1a*f*** (153.7 mg, 93%) as an inseparable mixture of α- and β-anomers (1/6). ^{1}H NMR (400 MHz, $CDCl_3$) signals attributed to **1a*f***(β): δ 2.05 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.10 (s, 3H, Ac), 4.12 (dd, $J_{4,5a}$ = 5.3 Hz, $J_{5a,5b}$ = 11.9 Hz, 1H, H5a), 4.31 (dd, $J_{4,5b}$ = 3.5 Hz, 1H, H5b), 4.35 (ddd, $J_{3,4}$ = 6.3 Hz, 1H, H4), 5.31–5.34 (2H, H2, H3), 6.14 (s, 1H, H1); signals attributed to **1a*f***(α): 4.18 (dd, $J_{4,5a}$ = 3.9 Hz, $J_{5a,5b}$ = 12.1 Hz, 1H, H5a), 4.28–4.33 (1H, H5b), 4.41 (ddd, $J_{3,4}$ = 2.7 Hz, $J_{4,5a}$ = 3.3 Hz, 1H, H4), 5.20 (dd, $J_{1,2}$ = 4.1 Hz, $J_{2,3}$ = 6.7 Hz, 1H, H2), 5.23 (dd, 1H, H3), 6.40 (d, 1H, H1); ^{13}C NMR ($CDCl_3$, 100 MHz) signals attributed to **1a*f***(β): δ 20.4, 20.5, 20.7, 21.0, 63.6, 70.5, 74.1, 79.3, 98.2, 169.0, 169.4, 169.7, 170.4; signals attributed to **1a*f***(α): 63.3, 69.7, 70.0, 81.6, 94.0; IR: 2958, 2926, 1741, 1367, 1211 cm^{-1}. Its NMR spectrum was identical with that reported previously [7].

3. Results and discussion

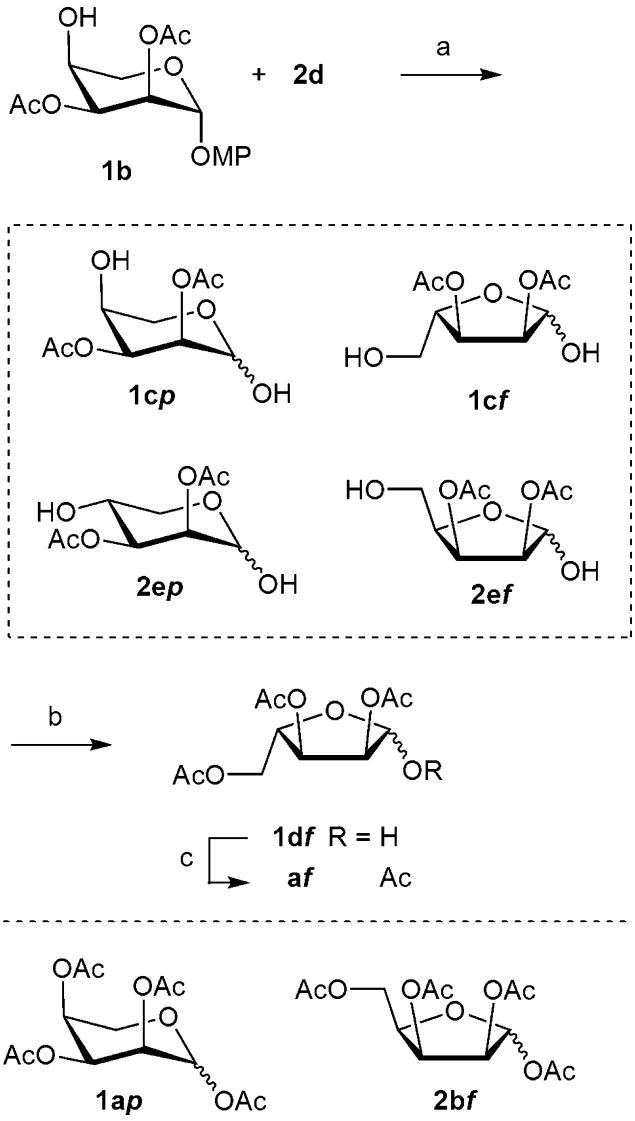
To acquire the key intermediate with a liberated hydroxyl group on C-4 of lyxose (**2d*p***), we focused our attention on the regioselective lipase-catalyzed ester exchange reaction, which was observed on *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-D-xylopyranoside by Mastihubovà and Biely [9].

The peracetylated pyranoside form of D-lyxose, **2b*p*** [10], was treated with *p*-methoxyphenol under acidic conditions for temporary blocking the hydroxyl group on C-1. The desired *p*-methoxyphenyl glycoside **2c** was obtained with an exclusive α-orientation at the anomeric position [8]. The first trial was the treatment with immobilized form of *B. cepacia* lipase (Amano PS-IM) and *n*-butanol, exactly followed by the reported procedure. The reaction was slow, and only as low as 12% of the desired product **2d** was obtained. Then, we turned our attention to the use of certain secondary alcohols as nucleophile [11,12]. In our case, transesterification between cyclopentanol worked well to give 50% yield of **2d**. Higher reactivity of cyclopentanol, compared with so far reported cyclohexanol (2%) [11] and 2-propanol (14%) [12], suggested the importance of steric hindrance as well as the conformational mobility of secondary alcohols. An enhanced yield by applying secondary alcohol is understandable, by suppressing reverse transesterification between the resulting cyclopentyl acetate and **2d** to regenerate **2c**. Moreover, it should be noted that *C. antarctica* lipase B (Chirazyme L-2), instead of *B. cepacia* lipase, improved the yield to be as high as 97%. Through above-mentioned elaboration, the yield became much higher than that (68%) under originally reported conditions for *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-D-xylopyranoside [9].

The ester exchange in hydrophobic organic solvent worked quite beneficially, since the hydrolysis by the lipases in buffer solution was accompanied with C-2 free hydroxyl contaminants, due to cer-



Scheme 2. Reagents and conditions: (a) *p*-methoxyphenol, TfOH, CH_2Cl_2 (76%), see Ref. [4]; (b) *C. antarctica* lipase B (Chirazyme L-2), cyclopentanol, toluene (97%); (c) IBX, AcOEt (99%); (d) $NaBH(OAc)_3$, CH_3CN, AcOH (98%).



Scheme 3. Reagents and conditions: (a) $(NH_4)_2[Ce(NO_3)_6]$, H_2O, CH_3CN; (b) *C. antarctica* lipase, vinyl acetate, 30 °C; (c) Ac_2O, pyridine (three steps, 42%).

tain acyl migration. The *p*-methoxyphenyl blocking on C-1 was necessary, since the treatment of **2bp** with *C. antarctica* lipase gave highly polar by-products due to the hydrolysis of the acetyl groups on both positions C-1 and C-4.

An alternative chemo-enzymatic route to liberate C-4 hydroxyl group of D-lyxose **2a** was attempted. It was a combination of *C. antarctica* lipase-catalyzed selective acylation of anomeric hydroxyl group [13] and the subsequent acetonide formation between *cis*-oriented hydroxyl groups on C-2 and C-3. However, probably due to some acyl migration in the acid-catalyzed acetonide formation, the attempts only resulted in a complex mixture.

The next work was the inversion of the configuration of C-4 in **2c**. It was revealed that an oxidation-reduction pathway was successful. First, oxidation with IBX in AcOEt [14] provided the proper ketone **3** in 99% yield. Through the screening of reductants providing an equatorial attack of hydride, $NaBH(OAc)_3$ in CH_3CN–AcOH [15] showed the highest stereoselectivity to give **1b** with the desired axial hydroxyl group, accompanied with only small amount of **2d** (91:9) as an inseparable mixture.

The introduction of the leaving group such as chloromethylsulfonate [16,17] at C-4 of **2d**, and the subsequent nucleophilic attack with potassium or cesium salts were also attempted. The steric hindrance of axially oriented acetoxy group on C-2 showed an adverse effect, and the reaction only resulted in the undesired elimination product.

Final task was the isolation of desired isomer from the mixture, as well as the fixation to **1af**. *p*-Methoxyphenyl group was first deprotected with CAN [$(NH_4)_2[Ce(NO_3)_6]$] [18] to give **1c**, but which was still contaminated with **2e**. And unfortunately, a small amount of other impurities appeared, probably caused by acyl migration under reaction conditions. The above mixture, however, was treated with *C. antarctica* lipase in vinyl acetate, to give an anomeric mixture of **1d** as triacetates. Anomeric hydroxyl group was further acetylated in a conventional manner to give **1af** as a 1:6 mixture of α- and β-anomers, in 42% yield, through three steps from **1b** as shown in Scheme 3. It was noteworthy that the desired compound **1af**, was obtained in pure state, free from **1ap** [19] or **2bf** originated from the contaminant.

We explain the reason for the formation of **1af** as follows. First, in view of the destination of the contaminant, the acylation of C-4 hydroxyl group in **2ep** might be fast, which was experienced in the case of **2d** from **2c** in Scheme 2. Furthermore, even if **2ef** is major as shown in Scheme 3, the *cis*-oriented acetoxy group on C-3 would exhibit steric hindrance [20] over the primary alcohol to some extent. The very slow reaction of **1cp** is agreeable, due to the steric hindrance essentially in axial secondary alcohol, as well as the low nucleophilicity of anomeric hydroxyl group. In

contrast, primary hydroxyl group in **1c*f*** was acylated very fast, in good accordance with the previous example for similar substrates [20].

4. Conclusion

In this way, a new chemo-enzymatic way to tetra-*O*-acetyl-L-ribofuranose from D-lyxose was established. Two regioselective lipase-catalyzed reactions were demonstrated as the key step; first, for the purpose of inversion of the stereochemistry on the specific secondary alcohol in the starting material, and second, the isolation of desired stereoisomers under the final convergence to ribofuranose, from a complex stereo- and structural isomeric mixture of pentoses.

Acknowledgements

The authors thank Professor Vicente Gotor and Dr. Jesús Joglar, of Department of Chemistry, University of Oviedo and Department of Biological Chemistry and Molecular Modeling, Spanish National Research Council (CSIC), for their kindly providing valuable NMR spectra. We are indebted to Amano Enzyme Inc. for lipase PS-IM. Professor Kazuo Umezawa's encouragement on this study was acknowledged with thanks. J. C. thanks Post-Doctoral fellowship from the Japanese Society for the Promotion of Science (No. P07435). This work was supported both by a Grant-in-Aid for Scientific Research (No. 18580106) and "High-Tech Research Center" Project for Private Universities: matching fund subsidy 2006–2011 from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and acknowledged with thanks.

References

[1] P. Wang, J.H. Hong, J.S. Cooperwood, C.K. Chu, Antiviral Res. 40 (1998) 19.
[2] C. Mathé, G. Gossselin, Antiviral Res. 71 (2006) 276.
[3] G.G. Sivets, T.V. Klennitskaya, E.V. Zhernosek, I.A. Mikhailopulo, Synthesis (2002) 253.
[4] M.E. Jung, Y. Xu, Tetrahedron Lett. 38 (1997) 4199.
[5] E. Moyroud, P. Strazewski, Tetrahedron 55 (1999) 1277.
[6] Z.-D. Shi, B.-H. Yang, Y.-L. Wu, Tetrahedron Lett. 42 (2001) 7651.
[7] Z.-D. Shi, B.-H. Yang, Y.-L. Wu, Tetrahedron 58 (2002) 3287.
[8] G. Agnihotri, P.K. Mandal, A.K. Misra, Tetrahedron 63 (2007) 7240.
[9] M. Mastihubová, P. Biely, Carbohydr. Res. 339 (2004) 1353.
[10] G.M. Watt, S.L. Flitsch, S. Fey, L. Elling, U. Kragl, Tetrahedron: Asymmetry 11 (2000) 621.
[11] Y. Igarashi, S. Otsutomo, M. Harada, S. Nakano, Tetrahedron: Asymmetry 8 (1997) 2833.
[12] T. Miyazawa, M. Hamada, R. Morimoto, T. Murashima, T. Yamada, Tetrahedron Lett. 48 (2007) 8334.
[13] R. Pulido, F.L. Ortiz, V. Gotor, J. Chem. Soc., Perkin I (1992) 2891.
[14] M. Ocejo, J.L. Vicario, D. Badia, L. Carrillo, E. Reyes, Synlett (2005) 2110.
[15] D.A. Evans, K.T. Chapman, E.M. Carreira, J. Am. Chem. Soc. 110 (1988) 3560.
[16] T. Shimizu, S. Hiranuma, T. Nakata, Tetrahedron Lett. 37 (1996) 6145.
[17] N. Ito, Y. Tokuda, S. Ohba, T. Sugai, Bull. Chem. Soc. Jpn. 77 (2004) 1181.
[18] T. Fukuyama, A.A. Laird, L.M. Hotchkiss, Tetrahedron Lett. 26 (1985) 6291.
[19] R.U. Lemieux, J.D. Stevens, Can. J. Chem. 43 (1965) 2059.
[20] A.K. Prasad, S. Roy, R. Kumar, N. Kalra, J. Wengel, C.E. Olsen, A.L. Cholli, L.A. Samuelson, J. Kumar, A.C. Watterson, R.A. Gross, V.S. Parmar, Tetrahedron 59 (2003) 1333.