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Chemoenzymatic synthesis of statine side chain building blocks and application in the total synthesis of the cholesterol-lowering compound solistatin

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ABSTRACT

The synthesis and enzymatic reduction of several 6-substituted dioxohexanoates are presented. Two-step syntheses of *tert*-butyl 6-bromo-3,5-dioxohexanoate and the corresponding 6-hydroxy compound have been achieved in 89% and 59% yield, respectively. Regio- and enantioselective reduction of these diketones and of the 6-chloro derivative with alcohol dehydrogenase from *Lactobacillus brevis* (LBADH) gave the (5*S*)-5-hydroxy-3-oxo products with enantiomeric excesses of 91%, 98.4%, and > 99.5%, respectively. Chain elongation of the reduction products by one carbon *via* cyanide addition, and by more than one carbon by Julia–Kocienski olefination, gave access to well-established statine side-chain building blocks. Application in the synthesis of the cholesterol-lowering natural compound solistatin is given.

1. Introduction

We have shown previously that the NADPH-dependent alcohol dehydrogenase from *Lactobacillus brevis* (LBADH) or *Lactobacillus kefir* (LKADH) (Weckbecker and Hummel, 2006) accepts 3,5-dioxohexanoates as a substrate for ketone reduction: LBADH reduces 3,5-dioxohexanoates with excellent regioselectivity at position C-5, and the resulting highly enantioenriched 5-hydroxy-3-keto hexanoates are obtained in good yield (Wolberg et al., 2000; Wolberg et al., 2001a). Reduction of *tert*-butyl 6-chloro-3,5-dioxohexanoate by LBADH resulted in the preparation of a synthetically valuable chlorohydrine (Wolberg et al., 2001b; Wolberg et al., 2008). We aimed to broaden the scope of such regioselective enzymatic ketone reduction, and therefore have focused on the synthesis of the corresponding 6-bromo and 6-hydroxy derivatives. The products of such enzymatic transformations are versatile 1,3-diol building blocks, which we demonstrate in the present work by application of these products in the synthesis of the naturally occurring HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitor solistatin (**1**). Application in the synthesis of an advanced building block for synthetic HMG-CoA reductase inhibitors, such as atorvastatin (**2**), is presented as well.

Solistatin (**1**), an aromatic compactin (**3**) analogue, has been isolated from *Penicillium solitum* and its structure elucidated by Sorensen et al. (1999) and Larsen et al. (2002). Naturally, solistatin occurs in its (+)-(3*R*,5*R*) configuration. Compactins are biosynthesized by fungi and arise from polyketidic pathways (Manzoni and Rollini, 2002; Abe et al., 2002). They show *in vitro* HMG-CoA reductase inhibitory effects. These effects are caused by their mevalonic acid-mimicking partial structure (Prugh et al., 1990). Therefore, the correct stereochemistry in this partial structure (often designated as 'statin side chain') (Müller, 2005) is essential for their pharmacological effects (Fig. 1).

2. Materials and methods

2.1. General

All solvents were HPLC grade or analytical grade. When necessary, solvents were dried prior to use. Methanol and dichloromethane were stored over 4 Å molecular sieves. Acetonitrile was distilled from calcium hydride. Air- and/or moisture-sensitive reactions were carried out under an atmosphere of dry nitrogen in oven-dried glassware. Silica gel 60F254 precoated aluminium sheets (Merck) were used for analytical thin layer chromatography. Visualization was accomplished by UV light or by dipping the plate into a solution of *p*-anisaldehyde (1 mL) in acetic acid/conc. sulfuric acid (100 mL, 98:2 v/v) followed by heating.

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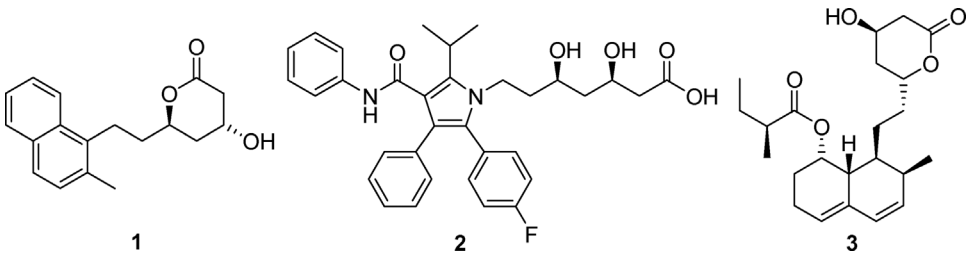

Fig. 1. Structure of solistatin (**1**), atorvastatin (**2**), and compactin (**3**).

Flash chromatography was performed on silica gel 60 (40 ± 63 µm), Merck. NMR spectra were recorded with a Bruker DRX-400 spectrometer at 20 °C. ^{1}H NMR: 400.13 MHz, residual undeuterated solvent as internal standard ($CHCl_3$: δ = 7.27). ^{13}C NMR: 100.62 MHz, deuterated solvent as internal standard ($CDCl_3$: δ = 77.2), broadband proton decoupling. GC–MS analysis was carried out with an Agilent Technologies 6890N GC system (HP-5MS column) coupled to a HP5973 mass selective detector. Mass peaks with relative intensity < 10% were omitted unless they correspond to $[M]^+$ or some other informative fragment. GC on chiral stationary phase was performed with a Hewlett-Packard 6890 GC system (Cyclodex-β column) equipped with a flame ionization detector. HPLC on chiral stationary phase was performed with a Hewlett-Packard HP1100 system (UV-DAD, Chiracel OB column). Optical rotations were determined with a Jasco P2010 polarimeter (1 dm cell). Commercially available reagents were used as delivered unless otherwise stated.

2.2. tert-Butyl 6-bromo-3,5-dioxohexanoate (6b)

4.7 mL Diisopropylamine (36 mmol) were dissolved in 125 mL anhydrous THF, and cooled to 0 °C. 12.2 mL (2.7 M in hexanes; 33 mmol) *n*-Butyllithium were added dropwise, and stirred for 15 min at 0 °C. The mixture was cooled to −45 °C and 2.5 mL (15 mmol) *tert*-butyl acetoacetate (**4**) were added dropwise, followed by stirring for 30 min at −45 °C. After cooling to −78 °C 3.0 mL (15 mmol) methyl bromoacetate (**5b**) were added dropwise, and stirred for 30 min (T_{max} = −65 °C). The mixture was poured onto 150 mL of a vigorously stirred 1:1 mixture of ethyl acetate and 2 M HCl. The organic layer was isolated, and the aqueous layer extracted twice with 50 mL ethyl acetate. The pooled organic phases were washed with brine, and dried over $MgSO_4$. The solvent and unreacted educt were removed *in vacuo*. 3.6 g (89%) of a pale yellow oil were obtained. The crude product was used for further synthesis without purification. An analytical sample was purified by column chromatography (Si-60, cyclohexane/ethyl acetate 70:30).

^{1}H NMR (400 MHz, $CDCl_3$): enol form (ef)/keto form (kf) = 87: 13; δ = 1.49 (s, $9H_{kf+ef}$ $(CH_3)_3$), 3.32 (s, $2H_{ef}$, H-2), 3.50 (s, $2H_{kf}$, H-2), 3.89 (s, $2H_{ef}$, H-6), 3.99 (s, $2H_{kf}$, H-4), 4.04 (s, $2H_{kf}$, H-6), 5.92 (s, $1H_{ef}$, H-4), 14.63 (br s, $1H_{ef}$, OH).

^{13}C NMR (100 MHz, $CDCl_3$): enol form; δ = 28.0 (*tert*-butyl), 46.0 (C-2), 51.3 (C-6), 82.3 (Cq), 99.5 (C-4), 166.3 (C-1), 185.0 (C-5), 188.0 (C-3).

MS (EI), *m/z* (%): 278 (6) $[M]^+$, 205 (38) [M-*tert*-butanol]$^+$, 177 (13), 147 (9), 119 (11), 57 (100) [*tert*-butyl]$^+$.

2.3. syn/anti tert-Butyl 6-bromo-3,5-dihydroxyhexanoate (10b) from 6b

1.34 g (4.8 mmol) Diketone **6b** were dissolved in 25 mL anhydrous ethanol. 190 mg (5 mmol) $NaBH_4$ were added. After TLC indicated completion of the reaction (2 h), 2 mL 1 M HCl were added. The mixture was concentrated *in vacuo*. The residue was dissolved in 50 mL ethyl acetate and water (1:1). The aqueous phase was extracted twice with 25 mL ethyl acetate. The pooled organic phases were washed with brine, and dried over $MgSO_4$. The solvent was removed *in vacuo*, and the crude product purified by column chromatography (SI-60, cyclohexane/ethyl acetate 1:1). 0.96 g (68%) of a colourless liquid were obtained.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.49 (s, 9H, $(CH_3)_3$), 1.55–1.83 (m, 2H, H-4), 2.45 (d, *J* = 6.7 Hz, 2H, H-2), 3.01 (br s, 1H, OH_{anti}), 3.51 (m, 2H, H-6), 3.59 (br s, 1H, OH_{anti}), 3.73 (br s, 1H, OH_{syn}), 3.83 (br s, 1H, OH_{syn}), 4.13 (m, 1H, H-5), 4.31 (m, 1H, H-3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.0 (*tert*-butyl), 38.4, 38.9, 39.9, 40.2, 42.0, 42.2 ($CH_{2syn/anti}$; C-2, C-4, C-6), 65.3, 68.1 (C-$5_{syn/anti}$), 68.4, 71.0 (C-$3_{syn/anti}$), 81.7 (Cq), 172.0, 172.3 (C-$1_{syn/anti}$).

2.4. syn/anti tert-Butyl 6-bromo-3,5-(isopropylidendioxy)-hexanoate (11b)

1.3 g (4.8 mmol) Dihydroxyester **10b**, 5.0 g (48 mmol) 2, 2-dimethoxypropane, and 57 mg (0.3 mmol) *p*-toluenesulfonic acid were dissolved in 15 mL acetone, and the mixture was stirred for 2 h at room temperature. The mixture was neutralized with triethylamine, and the solvent removed *in vacuo*. The residue was dissolved in 20 mL ethyl acetate, washed with brine, and dried over $MgSO_4$. The solvent was removed *in vacuo*, and the crude product purified by column chromatography (SI-60, cyclohexane/ethyl acetate 9:1). 0.45 g (29%) of an off-white liquid were obtained.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.25 (m, 1H, H-4), 1.37–1.44 (m, 6H, 2 CH_3 acetonide), 1.48 (s, 9H, $(CH_3)_3$), 1.74–1.87 (m, 1H, H-4), 2.32–2.50 (m, 2H, H-2), 3.25–3.43 (m, 2H, H-6), 4.06 (m, 1H, H-5), 4.28 (m, 1H, H-3).

syn-**11b** ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.7, 29.8 (2 CH_3 acetonide), 28.0 (*tert*-butyl), 34.8, 35.2, 42.5 (CH_2, C-2, C-4, C-6), 66.0, 68.9 (C-3, C-5), 80.7 (Cq *tert*-butyl), 99.3 (Cq acetonide), 170.0 (C-1).

anti-**11b** ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.5, 24.6 (2 CH_3 acetonide), 28.0 (*tert*-butyl), 35.1, 36.3, 42.0 (CH_2, C-2, C-4, C-6), 63.8, 66.6 (C-3, C-5), 80.7 (Cq *tert*-butyl), 101.1 (Cq acetonide), 169.9 (C-1).

MS (EI), *m/z* (%): 307 (46) $[M-CH_3]^+$, 251 (100) $[M-$*tert*-butanol$]^+$, 209 (33), 191 (100), 149 (54), 129 (82), 111 (40), 57 (100) [*tert*-butyl]$^+$.

2.5. tert-Butyl (S)-6-bromo-5-hydroxy-3-oxohexanoate (9b)

5 mg (0.25 mmol) $MgCl_2$ and 20 mg (21.6 µmol) $NADP^+$ were dissolved in 50 mL citric acid/disodiumhydrogen phosphate buffer (375 mM, pH 5.5). 1.5 mL (20 mmol) 2-Propanol and 200 U *rec*-LBADH were added. The mixture was stirred at 100 rpm at room temperature for 10 min using Fish-Clip®. 220 mg (0.8 mmol) Diketo ester **6b** in 50 mL MTBE were added. Stirring was accelerated to 700 rpm, and maintained for 24 h at room temperature. Another 200 U *rec*-LBADH were added, and the reaction mixture stirred for 24 h.The reaction mixture was filtered, saturated with NaCl, and the aqueous phase was extracted twice with 50 mL ethyl acetate. The organic phases were pooled, washed with brine, and dried over $MgSO_4$. The solvent was removed *in vacuo* giving 250 mg of a raw product. ^{1}H NMR analysis showed a content of 62% **9b** and 24% furanone **7**. Hexanoate **9b** was purified twice by column chromatography over silica (cyclohexane/ethyl acetate 4:1). 29 mg (12%) of **9b** were obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.49 (s, 9H, (*tert*-butyl)), 2.70 (m, 1H, H-4), 2.89 (m, 1H, H-4), 3.02 (br s, 1H, OH), 3.43 (s, 1H), 3.70