Chemo-Enzymatic Approach to Statin Side-Chain Building Blocks

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Abstract: A versatile statin side-chain building block is obtained by an enzymatic desymmetrisation of the symmetrical glutaric acid diethyl ester. The monoacid is produced in almost quantitative yield in the desired high optical purity. The monoacid is easily converted to the corresponding acid chloride, which is a key compound to be elaborated to some statin side-chain derivatives. The optically active C-5 chain is subsequently elongated by two carbon atoms and *syn*-reduced to the final diol fragment.

Keywords: atorvastatin; aza-Wittig-reaction; enzymatic desymmetrisation; statin side-chain

The statins are a class a pharmaceuticals derived from the natural mevilonolacton. The pharmaceutical congeners are potent HMG-CoA-reductase inhibitors, which means that they are very active in lowering blood lipid levels.[1,2] The commercial drugs are generally composed of a large lipophilic residue and a C-7 carboxylic acid chain bearing a *syn*-1,3-diol pattern. The synthesis of this diol chain is approached in different, albeit still quite unsatisfactory, ways by a number of pharmaceutical companies.[3]

One way to introduce unequivocally a chiral centre into a molecule is to start from the chiral pool; so malic acid is used eventually to synthesise the statin-chain in a reliable but lengthy process.[4] A second option is to resolve a racemic precursor and continue the synthesis with the required enantiomer.[5] The resolution can be carried out chemically or enzymatically; but either way, the product yields approach a maximum of 50% only, because the unwanted enantiomer has to be discarded. A more advanced, but less general way is the use of intact cells, which produce and secrete the final building block into the culture broth; but volume yields are often very low and isolation protocols are tedious.[6]

A more efficient strategy commences with the desymmetrisation of a cheap prochiral precursor; this way the theoretical 100% yield can be reached.[7] So we embarked on an enzymatic desymmerisation route, starting with diethyl glutaric acid diethyl ester[8] (see Scheme 1).

Firstly, three parameters had to be screened in order to obtain a satisfactory desymmetrisation of **1** with respect to ee and yield: the ester functionalities, the central hydroxy group or its protected version and the biocatalyst.[9] Finally, diester **1** is first acylated with methoxyacetic acid in almost quantitative yield and subsequently desymmetrised to give acid **2** in 94% yield and optical purities of up to 98% ee. The methoxyacetic acid derivative **2** yields the highest ee values *via* desymmetrisation with the biocatalyst α-chymotrypsin. The methoxyacetic acid ester is also the easiest to be removed at a later stage in the synthesis. The ethyl esters show a significantly fast conversion with α-chymotrypsin and the resulting monoethyl ester **2** is sufficiently soluble in organic solvents for a subsequent extractive work-up (see Experimental Section). The acid **2** is then reacted with oxalyl chloride in the presence of a catalytic amount of DMF to form acid chloride **3** in quantitative yield. This is our key intermediate.

We probed a Friedel–Crafts like chain elongation on this compound. This kind of reaction is not known for such highly oxygenated straight-chain acid chlorides. Surprisingly, we succeeded to introduce two carbon atoms with concomitant functionalisation when the acid chloride **3** is treated with gaseous ethylene in the presence of aluminium trichloride (see Scheme 2). The resulting chloride **9** is deacylated with pig liver esterase (PLE). The enzyme selectively cleaves off the internal methoxyacetyl group leaving the ethyl ester untouched. Chemical deprotection protocols lead to significant decomposition of **9**. The resulting chloride **10** can either be *syn*-reduced to diol **11**, protected and substituted with azide to give **12**. Alternatively, the chloride on **3** can be replaced by the azide. *syn*-Reduction and acetonisation are subsequently performed as described for compound **3**. The ee and de of the final acetonide **12** are identical in either way (see Scheme 2).

Acid chloride **3** can also be treated with gaseous vinyl chloride in the presence of aluminium trichloride giving the dichloro congener of **9**. This chloride may be subsequently processed to iodide **5**, respectively.

The synthesis of atorvastatin, e.g., is completed by reacting the azide **12** directly with diketone **13** (see

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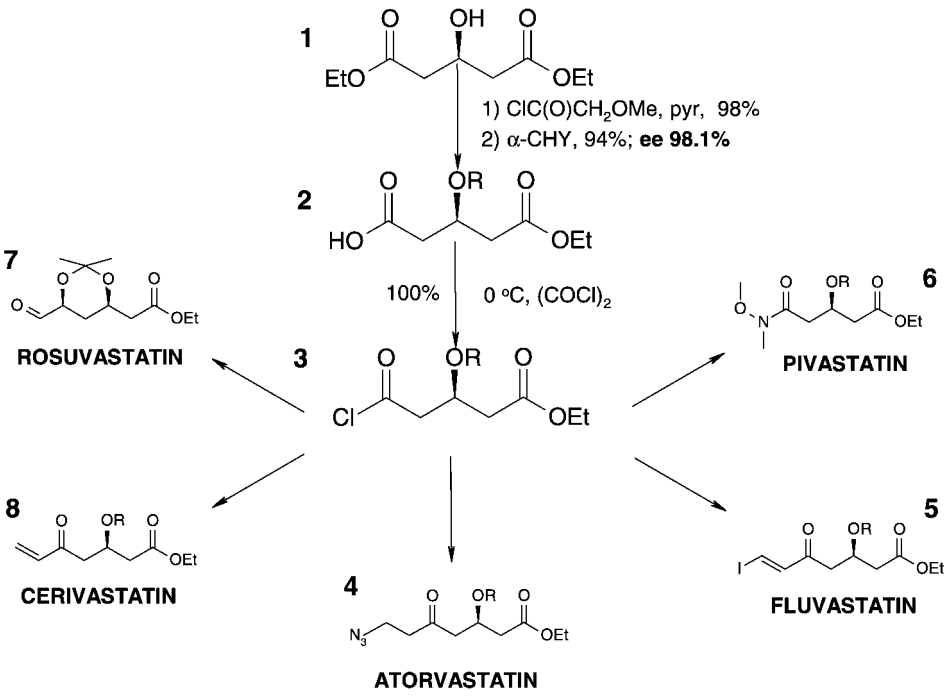
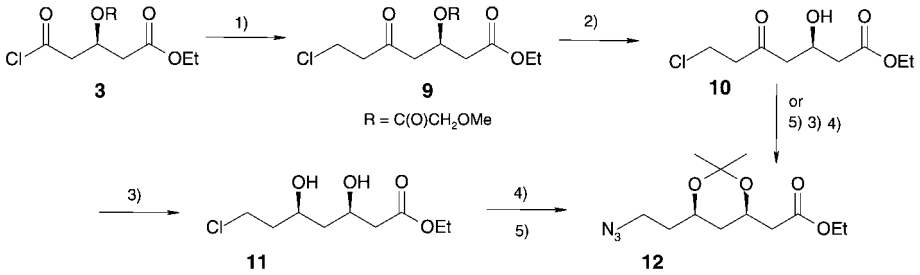


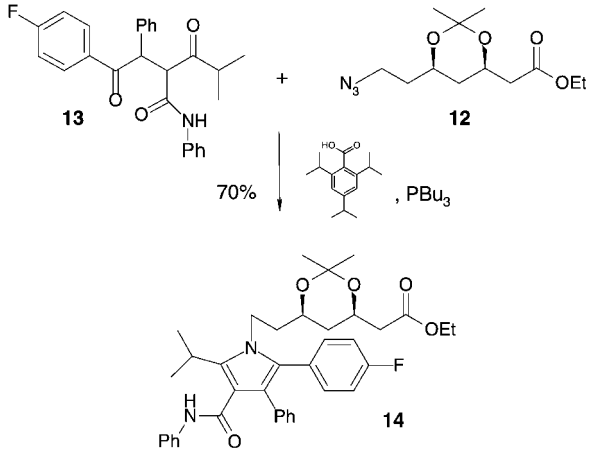
Scheme 1. Enzymatic desymmetrisation and further building blocks.



Scheme 2. Chain elongation and functionalisation. *Reaction conditions:* 1) $(CH_2Cl)_2$, 0 °C, ethylene, $AlCl_3$, 89%; 2) PLE, pH = 7, 76%; 3) BEt_3, $NaBH_4$, −78 °C, 91%; 4) dimethoxypropane, H^+, 98%; 5) NaN_3, DMF, 94%, ee 98.1%, de 96.8%.

Scheme 3). This so called 'aza'-Wittig reaction, which proceeds *via* an imine intermediate is promoted by a phosphine reagent and a weak, sterically hindered acid. This way the sensitive, pentasubstituted pyrrole ring system **14** is obtained directly under mild conditions in good yield after chromatographic purification, as described in the Experimental Section.[10]

The overall sequence towards atorvastatin excels by its straightness and versatility. Two biocatalysts are integrated in the synthetic scheme. One biocatalyst is applied to set the desired asymmetric centre and the second one to remove a transient auxiliary selectively. In addition, the azide in the side-chain building block allows the direct merger with the 1,4- diketone to a pentasubstituted pyrrole ring system.



Scheme 3. Ring closure.

Experimental Section

Desymmetrisation of 3-Acetoxymethoxy Derivative of Diethyl Glutarate (1) with the Biocatalyst α-Chymotrypsin

Diester **1** (1.086 kg, 5.32 mol) is dissolved in 250 mL dichloromethane at 0 °C. To this mixture are successively added 411 mL (5.85 mol) of pyridine and 635 mL (5.85 mol) of methoxyacetyl chloride. This mixture is stirred at room temperature overnight until TLC shows consumption of the diester **1**. The mixture is diluted with ethyl acetate and successively washed with 2 L of 1 N hydrogen chloride, saturated sodium hydrogen carbonate and brine. The organic phase is dried over magnesium sulphate and finally evaporated. The residue is distilled at 120 – 122 °C/ 0.08 mbar to give a colourless liquid, which is desymmetrised in the next step; yield: 1.426 kg (97%).

The triester of the previous step (400.5 g, 1.45 mol) is suspended in 1.1 L of deionised water and 310 mL 0.1 M KH_2PO_4 buffer (pH = 7). α-Chymotrypsin (BioFac) is added in 3 portions (overall 2.8 g) and the pH of the emulsion kept at 7.8 with a 0.5 N sodium hydroxide solution (pH-stat.). When the theoretical amount of the base is consumed (*ca.* 24 – 30 h) about 10 g Celite are added and the mixture is stirred for 0.5 h. The mixture is filtered and the aqueous phase thoroughly extracted with ethyl acetate or ether, or mixtures of both solvents. After acidification of the aqueous phase with concentrated hydrogen chloride to pH = 1 – 1.5 and filtration over Celite the aqueous phase is again thoroughly extracted with ethyl acetate. The organic phase is dried over $MgSO_4$ and evaporated to give acid **2** as a slightly yellow oil; yield: 338.2 g (94%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.24 (t, 3H), 2.74 (d, 2H), 2.75 (d, 2H), 3.42 (s, 3H), 3.99 (s, 2H), 4.14 (q, 2H), 5.59 (quint., 1H), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 13.9, 37.7, 38.1, 59.0, 60.8, 67.0, 69.3, 169.2, 169.6, 174.2; ee (HPLC, chiracel OD; hexane-ethanol, 9:1): 98.2% from the corresponding benzylamide derivative of the acid **2**.

Aza-Wittig Reaction of Azide 12 with Diketone 13 to give Highly Functionalised Pyrrole 14

In an argon atmosphere 1.00 g (3.73 mmol) of azide **12** is stirred together with 0.92 mL (3.73 mmol) of tributylphosphine in 3 mL of dry toluene at room temperature until gas evolution ceases. Hereafter 1.20 g (2.87 mmol) of diketone **13** and 0.61 g (3.73 mmol) of 2,4,6-triisopropylbenzoic acid are added and the mixture is warmed up to 60 °C and stirred until the starting diketone is consumed (TLC). It proved advantages to add molecular sieves 3 Å (*ca.* 300 mg) from the start to remove traces of moisture of the reaction mixture. The reaction mixture is finally cooled down, diluted with toluene and successively extracted with 1 N sodium hydroxide, 1 N hydrogen chloride, and brine. The organic phase is dried over $MgSO_4$ and evaporated. The residue is chromatographed over silica gel (eluent: dicholomethane-ethyl acetate, 30:0.5 to 3:2) to afford the syrupy pyrrole **14**; yield: 1.26 g (70%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06 (q, 1H, J = 12.5 Hz), 1.2 (t, 3H, J = 7.5 Hz), 1.31 (s, 3H), 1.35 – 1.40 (m, 1H), 1.37 (s, 3H), 1.54 (d, 6H, J = 7.3 Hz), 1.63 – 1.74 (m, 2H), 2.31 (dd, 1H, J = 6.2 Hz, 15.3 Hz), 2.49 (dd, 1H, J = 7.0 Hz, 15.3 Hz), 3.58 (sept., 1H, J = 7.3 Hz), 3.65 – 3.75 (m, 1H), 3.78 – 3.89 (m, 1H), 4.02 – 4.26 (m, 4H), 6.87 – 7.20 (m, 15H); ^{13}C NMR (75.4 MHz, $CDCl_3$): = 14.57, 20.05, 21.95, 22.12, 26.47, 30.27, 36.33, 38.40, 41.61, 60.67, 65.95, 66.69, 99.00, 115.54 (d, $J_{C,F}$ = 21.3 Hz), 115.57, 119.77, 122.01, 123.67, 126.73, 128.51, 128.83, 128.97, 130.67, 133.55 (d, $J_{C,F}$ = 8.1 Hz), 134.99, 138.61, 141.64, 162.40 (d, $J_{C,F}$ = 247.3 Hz), 164.94, 170.90; HPLC (chiracel OJ): ee 98.6%, de 98.6%. α_D: + 8.1 (*c* 2, $CHCl_3$).

The product is further processed to the atorvastatin calcium salt according to WO89/07598. The physical data of the salt are in good agreement to the given values.

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