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Application of Chemoselective Pancreatin Powder-Catalyzed Deacetylation Reaction in the Synthesis of Key Statin Side Chain Intermediate (4*R*,6*S*)-4-(*tert*-Butyldimethylsilyloxy)-6-(hydroxymethyl)tetrahydropyran-2-one

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ABSTRACT: A chemoselective biocatalytic procedure for the synthesis of (4*R*,6*S*)-4-(*tert*-butyldimethylsilyloxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-one, a key lactonized statin side chain intermediate, from its acetate precursor is described. The presented method is based on the pancreatin powder-catalyzed cleavage of the acetyl group in ((2*S*,4*R*)-4-(*tert*-butyldimethylsilyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl)methyl acetate. The reaction was conducted in aqueous medium. The overall process is performed in a convenient way and economical manner suitable for industrial use.

INTRODUCTION

Statins are one of the most valuable therapeutic groups of compounds with annual revenues over US $30 billion per year over the past few years. Initially, statins have been obtained as fungal metabolites.[1] However, the quest for increased efficiency has drawn the development of these drugs towards structural refinement of natural compounds with structural modifications that finally resulted in the fully synthetically built derivatives. These are frequently addressed as superstatins.[2] Superstatins are build from a heterocyclic core attached to a chiral 3,5-dihydroxy-6-heptenoic (-heptanoic for atorvastatin) acid side chain (Figure 1), a hydrolyzed form of β-hydroxy-δ-valerolactone. The activity of superstatins essentially depends on the side chain, which remained due to this reason, an unmodified structural element from the origin.[3] Because of the importance of the side chain, numerous attempts have been made for its efficient preparation.[2,4,5] Recently, we have employed acetate **1** and alcohol **2** precursors for the first preparation of formyl substituted lactonized form **3** of the 3,5-dihydroxy-6-heptenoic acid side chain (Figure 1) suitable for use in the Wittig reaction.[6,7] The potential of aldehyde **3** application is substantial due to the fact that Wittig reaction is one the most frequently applied approaches for the assembly of heterocyclic and side chain precursors to the final statin molecule.[2] Furthermore, we have demonstrated that lactone **3** can undergo Wittig reaction[7] and can be used for construction of superstatins.[8] Although we have recently developed an improved highly efficient enzymatic synthesis of acetate **1** from simple prochiral substrates,[9] we have still been hampered to exploit its utility from the industrial point of view. This was due to the moderate yielding, toxic, and expensive tin cluster ($[t\text{-}Bu_2SnOH(Cl)]_2$)[10] catalyzed deacetylation reaction of acetate **1** to alcohol **2**,[6a] which ensued next in the transformation sequence towards aldehyde **3**. The acetyl group protection is frequently applied in multistep syntheses in protection−deprotection sequences, which are indispensable in the preparation of complex functionalized organic molecules.[11] This application of the acetyl group is due to its easy introduction into complex molecules and its removal under mild conditions. Nevertheless, removal of the acetyl moiety in complex molecules containing other protected functional groups and ester moieties such as acetate **1** remains a challenge nowadays. Despite the fact that a large choice of different chemical reagents are available for the acetyl deprotection reaction, the enzymatic hydrolysis of the acetyl group combines the high chemoselectivity achieved under mild reaction conditions, which provides an interesting economic alternative to other reagents.[12] Furthermore, a large variety of enzymes capable of acetyl cleavage are commercially available. Moreover, the research and development of biocatalysis for industrial use has gained impetus over the past decade.[13,14] The use of biocatalysis in water as a solvent in industrial processes can provide a superior selectivity for the chemical reaction and reduce the waste streams during the synthesis. In this paper we report a new, chemoselective deacetylation process of acetate **1** to alcohol **2** by the use of enzymatic catalysis in aqueous medium, which proved to be far superior over the chemical reagents which were tested for this transformation in parallel.

RESULTS AND DISCUSSION

To establish a cheaper, less toxic, and environmentally more acceptable reagent than $[t\text{-}Bu_2SnOH(Cl)]_2$ for the deacetylation reaction of acetate **1**,[6a] we first evaluated some literature known reagents that have proved to be efficient and selective in this type of transformation. Since the lactone **1** is a relatively complex substrate, bearing an acid-sensitive TBS moiety as well as a base-sensitive lactone ester functionality and acetyl moiety, a chemoselective reagent operating at mild reaction conditions would be required to preserve the untouched TBS protection as well as the intact lactone moiety. Therefore, 13 different reactions using

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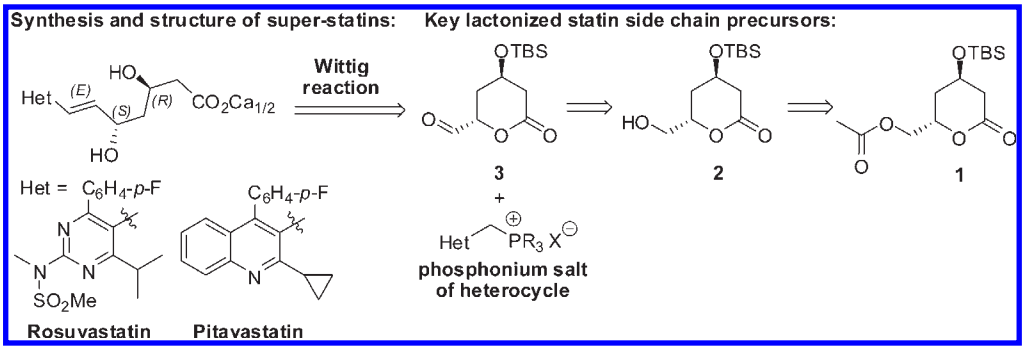
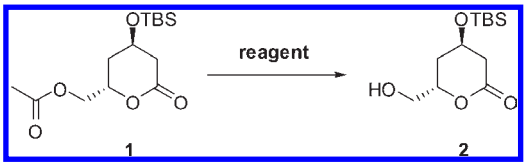


Figure 1. Retrosynthetic analysis of the synthesis of optically pure superstatins containing hept-6-enoic acid residue and structure of their key lactonized side chain precursors.

Scheme 1. Deacetylation of Acetate 1 with Various Reagents



chemical reagents that have proved to be selective with some other substrates were conducted for the deacetylation of acetate **1** (Scheme 1, Table 1). As shown in Table 1, none of the reagents were able to afford full conversion to alcohol **2**. Moreover, reactions with anhydrous $HBF_4 \cdot OEt_2$ (entry 1, Table 1),[15] guanidinium nitrate/NaOMe couple (entry 2, Table 1),[16] a catalytic amount of acetyl chloride in methanol (entry 3, Table 1),[17] scandium trifluoromethanesulfonate catalyst (entry 4, Table 1),[18] dibutyltin oxide (entry 5, Table 1),[19] *p*-toluenesulfonic acid (entry 6, Table 1),[20] hydrazine hydrate in THF (entry 7, Table 1),[21] and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (entry 8, Table 1)[22] resulted in complete decomposition and no alcohol **2** was detected in the crude reaction mixture although acetate **1** was completely consumed. When reaction was performed with ammonium acetate (entry 9, Table 1)[23] in methanol or iodine in methanol (entry 10, Table 1)[24] the conversion was poor and the majority of acetate **1** was not consumed. Nevertheless, ^{1}H NMR analysis of the crude mixture did not reveal, besides the side products, any presence of alcohol **2**. The next reaction was performed with potassium carbonate in methanol (entry 11, Table 1).[25] In this case acetate **1** was completely consumed and 16% of alcohol **2** was detected in the crude mixture besides side products giving 9% of alcohol **2** after isolation. Similarly, reaction with sodium methoxide in methanol (entry 12, Table 1)[26] afforded 32% of alcohol **2** after complete consumption of acetate **1**, which resulted in a 20% isolated yield of alcohol **2**. Finally, treatment of acetate **1** with cyanide on polymer support (entry 13, Table 1)[27] gave a clean reaction that resulted in partial conversion to alcohol **2** in 60% and the unreacted acetate **1** in 40%. Alcohol **2** was isolated in 51% yield.

Since only the costly cyanide on polymer support provided a clean deacetylation reaction of **1** and desired product was isolated in even lower yield (51%) than with a tin catalyst (66%),[6a] we were encouraged to explore further possibilities for more effective deacetylation of acetate **1**. Therefore, we

Table 1. Deacetylation of Acetate 1 with Chemical Reagents

		product composition[b]		
entry	reagent[a]	acetate **1**	alcohol **2**	yield[c] [%]
1	$HBF_4 \cdot OEt_2$[15]	0	0[e]	0
2	guanidium nitrate, NaOMe[16]	0	0[e]	0
3	AcCl, MeOH[17]	0	0[e]	0
4	$Sc(OTf)_3$, MeOH[18]	0	0[e]	0
5	Bu_2SnO, MeOH[19]	0	0[e]	0
6	*p*-TsOH[20]	0	0[e]	0
7	hydrazine hydrate[21]	0	0[e]	0
8	DBU, MeOH[22]	0	0[e]	0
9	ammonium acetate, MeOH[23]	79[d]	0	0
10	I_2, MeOH[24]	84[d]	0	0
11	K_2CO_3, MeOH[25]	0[d]	16	9
12	NaOMe, MeOH[26]	0[d]	32	20
13	cyanide on polymer support[27]	40	60	51

[a] The experimental procedures were followed, regarding the temperature, reaction time, and stoichiometry, as described in the literature. Reactions were followed by TLC until disappearance of acetate **1** or until no change of reaction progress was detected by NMR in the reaction mixture. [b] Ratio of acetate **1** and alcohol **2** determined by ^{1}H NMR integral of crude product after extractive workup. [c] Isolated yield after column chromatography. [d] Remaining percentages correspond to decomposition products. [e] Only decomposition products were present according to the ^{1}H NMR analysis.

turned our attention to the biocatalytic approach where appropriate enzymes would be used as highly chemoselective catalysts for deacetylation of acetate **1**. For the proof of our concept we tested seven different enzymes in deacetylation of acetate **1** (Scheme 1, Table 2) in the mixture of buffer/dioxane due to the poor solubility of acetate **1** in water. The first experiment was performed with lipase from *rhizopeus niveus*[28] (entry 1, Table 2). To our surprise reaction did not take place and only the starting acetate **1** was present in the reaction mixture as evidenced by ^{1}H NMR of the crude mixture after extractive workup and evaporation of the solvent. When the reaction was performed with lipase from *Candida rugosa*[29] (entry 2, Table 2), only acetate **1** and alcohol **2** were observed by TLC in the reaction mixture without any side products. Nevertheless, conversion was not complete and a mixture of alcohol **2** and acetate **1** in a ratio of 70:30 was obtained as evidenced by ^{1}H NMR, which gave after isolation a 41% yield of alcohol **2**. A similar mixture (ratio of 70:30) of

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Table 2. Deacetylation of Acetate 1 with Enzymes

entry	reagent (enzyme)[a]	dioxane (% v/v)	enzyme load, equiv (w/w)	product ratio **1**/**2**[b]	yield[c] [%]
1	lipase from *rhizopeus niveus*[28]	8	1.5	100/0	0
2	lipase from *Candida rugosa*[29]	20	0.7	30/70	41
3	lipase from wheat germ[28]	17	3	30/70	54
4	lipase from hog pancreas[28]	17	3	34/66	36
5	lipase from *Aspergillus niger*[28]	17	3	3/97	49
6	lipase *thermocymes lanuginose*[30]	17	3	0/100	51
7	pancreatin powder[28]	17	3	0/100	77

[a] The reactions were done at 34−37 °C with a single batch addition at the beginning, phosphate buffer pH 7.0. Reactions were followed by TLC until disappearance of acetate **1** or until no change of reaction progress was detected by NMR. [b] Ratio between acetate **1** and alcohol **2** determined by ^{1}H NMR integral of crude product after extractive workup and evaporation of the solvent. [c] Yield of alcohol **2** after column chromatography.

Table 3. Optimization of Pancreatin Powder Load

entry[a]	load of pancreatin powder, equiv (w/w)[b]	**1**/**2**[c]
1	0.1	90/10
2	0.5	43/57
3	1	50/50
4	1.3	24/76
5	1.6	25/75
6	2	0/100
7	3	0/100

[a] Performed at 34−37 °C, phosphate buffer pH 7.2 and 17% (v/v) dioxane, 7 h. [b] In equivalents (w/w) compared to acetate **1**. Pancreatin powder added in a single batch addition at the beginning of the reaction. [c] Ratio between **1** and **2** determined by ^{1}H NMR of isolated crude product after extractive workup and evaporation of the solvent.

alcohol **2** to acetate **1** was obtained when lipase from wheat germ[28] (entry 3, Table 2) was used though it afforded a better yield with 54% of alcohol **2**. A lipase from hog pancreas[28] (entry 4, Table 2) provided slightly lower conversion with a 66:34 ratio of alcohol **2** to acetate **1**, giving only 36% of alcohol **2** after isolation. Interestingly, lipase from *Aspergillus niger*[28] (entry 5, Table 2) gave a significantly higher conversion than lipases used in previous experiments. Indeed, a 97:3 ratio of alcohol **2** to acetate **1** was obtained in a crude product, though alcohol **2** was isolated in only 49% yield. In the same way, when lipase *thermocymes lanuginose*[30] (entry 6, Table 2) was used, a full conversion to alcohol was observed, with a similarly mediocre 51% isolated yield of alcohol **2**. Finally, we also tested *Pancreatin powder*[28] (entry 7, Table 2). Similarly, as in the case of the previous reaction, the conversion was complete and only alcohol **2** was detected in the crude mixture after extractive workup and evaporation of the solvent. To our delight, alcohol **2** was obtained in good 77% yield after isolation. Although high conversions to alcohol **2** were achieved in entries 5−7, the isolated yield was rather low in entries 5 and 6. This could be due to the lower selectivity of lipase from *Aspergillus niger* and lipase *thermocymes lanuginose* to lactone ester moiety, which probably resulted in lactone ring-opening and subsequent loss of product in extractive workup.

Having identified pancreatin powder as the most suitable catalyst for our transformation, we turned our attention to further optimization of the process with pancreatin powder (from here on referred to as "catalyst") regarding the industrial applicability (removal of chromatographic purification and minimization of organic cosolvent use) and catalyst load. The main challenge of the further optimization was to decrease the high initial 3 equiv (w/w) load[28] of catalyst and to isolate alcohol **2** crystals without performing the chromatographic purification. First, we investigated the minimum required load of the catalyst with the initial batch addition for complete conversion of acetate **1** to alcohol **2**. In the first attempt 0.1 equiv (w/w) of catalyst was used (entry 1, Table 3) in the reaction. However, only 10% conversion was observed. When the load of catalyst was increased to 0.5 equiv (w/w) (entry 2, Table 3) a 43/57 mixture of acetate **1** to alcohol **2** was obtained. Similarly, 1 equiv (w/w) of catalyst (entry 3, Table 3) gave a 1:1 mixture of **1** and **2**. Next, 1.3 and 1.6 equiv (w/w) of catalyst gave a 1/3 ratio of **1** to **2** (entries 4 and 5, Table 3). Application of 2 equiv (w/w) of catalyst (entry 6, Table 3) resulted in complete conversion of acetate **1** to alcohol **2**. The same result was observed when 3 equiv (w/w) of catalyst were applied, which indicates that 1 equiv (w/w) excess of catalyst compared to entry 6 did not result in a side reaction with the lactone moiety (entry 7, Table 3).

We then decided to investigate different possibilities on how to enable sufficient contact of substrate **1** with the catalyst in order to decrease the load of catalyst and improve the yield. A very appealing approach would be a slow stepwise addition of acetate **1** to the catalyst solution compared to initial single batch addition as described previously. This way the load of catalyst would be relatively high at all times compared to the substrate even if the absolute load would be lower, which would lead to the intrinsic decrease of catalyst load. To test this approach we performed this type of experiment and monitored it with an in-line IR probe. The reaction was performed with an excess of acetate **1**, which was added stepwise over 8 h to the solution of 0.2 equiv (w/w) of catalyst in phosphate buffer (pH 7.0)/dioxane mixture (4/1). According to the data obtained by in-line IR (Figure 2), the conversion from acetate **1** to alcohol **2** was very fast initially and all the acetate **1** entering the reaction was consumed (flat red curve in Figure 2) and converted to alcohol **2**. A steep curve for alcohol **2** concentration rise was observed (green curve in Figure 2) in the period of the first 2.5 h when the maximum of concentration of alcohol **2** was reached. Surprisingly, in-line FTIR data revealed that after ca. 3.75 h the concentration level of the alcohol **2** started to decrease gradually until 8 h of reaction. At the same time accumulation of acetate **1**, which was still added stepwise in the reaction mixture, was observed. Indeed, a rapid increase of the concentration of acetate **1** was observed, which correlated with its addition as evidenced by a steep curve for acetate **1** from 3.75 to 8 h (red curve in Figure 2). This suggests that the catalyst started to react preferably with alcohol **2**, when its concentration reached the appropriate level compared to acetate **1** after prolonged exposure in the phosphate buffer/dioxane mixture. This reaction results in degradation of

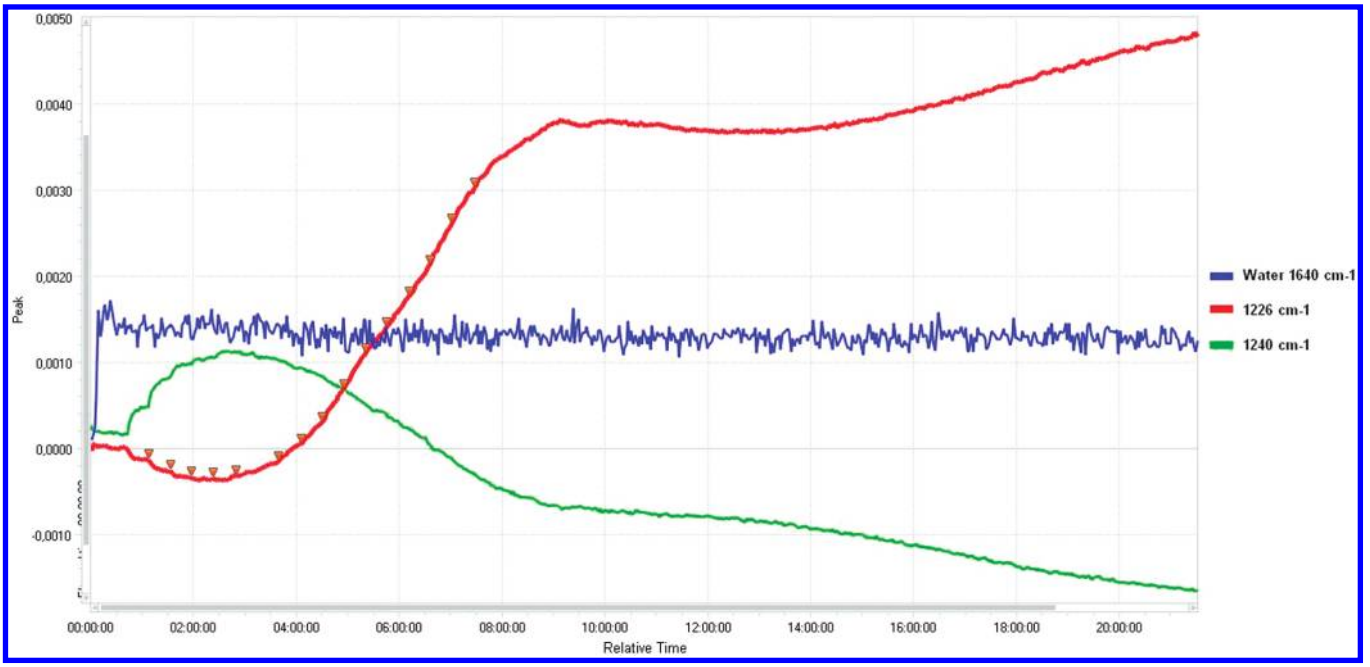

Figure 2. Monitoring of acetate **1** to alcohol **2** conversion by in-line IR. The blue curve represents water. The red curve represents the reaction profile for acetate **1** (points on the curve represent the time points when substrate was dosed to the reaction mixture). The green curve represents the reaction profile for alcohol **2**.

Table 4. Alcohol 2 Synthesis in Aqueous Medium

entry[a]	C [mol/L]	catalyst, equiv (w/w)	mode of addition	1/2[c]
1	0.11	2[b]	2	2/98
2	0.11	2	0.50 + 5 × 0.30	2/98
3	0.11	1	0.25 + 5 × 0.15	2/98
4	0.11	0.7	0.20 + 5 × 0.10	5/95
5	0.11	0.5	0.15 + 5 × 0.07	2/98
6	0.11	0.4	0.10 + 5 × 0.06	10/90
7	0.2	0.5	0.15 + 5 × 0.07	1/99
8	0.3	0.5	0.15 + 5 × 0.07	50/50
9	0.5	0.7	0.20 + 5 × 0.10	1/99

[a] Reactions performed with pancreatin powder catalyst at 34−37 °C. Reaction time was 24 h. pH controlled at 4.8−5.0 (PBS buffer 5.2 + addition of $NaHCO_3$ 1.0 M). [b] Single batch pancreatin powder addition. [c] Ratio between **1** and **2** by ^1H NMR analysis after extractive workup and evaporation of the solvent.

alcohol **2** (lactone ring-opening). Consequently, acetate **1** remained unreacted in the mixture and its concentration started to rise rapidly during the stepwise addition. Furthermore, even though the reaction of catalyst with alcohol **2** did not proceed significantly after 8 h, the catalyst did not start to react with acetate **1** and its concentration remained unchanged (a slight rise is observed due to the solvent evaporation over 20 h).

This could suggest that the catalyst was inhibited by the product **2** or side products of the undesired consecutive or/and parallel reactions of lactone moiety in **1** or **2** with catalyst, which could lead to ring-opened products. All these observations support our initial hypothesis concerning the undesired consecutive reaction of catalyst with the lactone ring moiety.

To circumvent this parasitic consecutive reaction we were stimulated to further modify the process. Our first objective was to ensure that alcohol **2**, after being formed, was not placed into contact with catalyst; this should result in a clean reaction with acetate **1**. To ensure these conditions we decided not to use the cosolvent, which was primarily used to facilitate the solubilization of water poorly miscible acetate **1**. The omission of cosolvent should also result in precipitation of the newly formed alcohol **2** and prevent its further reaction with the catalyst. When the reaction was performed without cosolvent with initially 2 equiv (w/w) of catalyst the conversion was almost complete (entry 1, Table 4). As the progress of the reaction is limited by the poor solubility of acetate **1**, which is dispersed in the water phase, we concluded that a high 2 equiv (w/w) initial load of catalyst is not required. Moreover, since the catalyst containing water phase in this biphasic system is saturated with acetate **1** at low concentration, the progress of the reaction would be dependent upon the consumption of acetate **1** in the water phase and not the load of catalyst. The consumption of **1** by the reaction in the water phase, followed by removal of alcohol **2** product from the mixture by precipitation, would facilitate additional mass transport of acetate **1** from the bulk mass of **1** to the water phase and subsequent deacetylation already at relatively low catalyst loads. This fact stimulated us to further modify the catalyst dosing. This could consist of the stepwise addition of the catalyst into the mixture of acetate **1** and phosphate buffer. This type of feed could provide a maximum initial substrate concentration and linearly variable catalyst concentration, which would gradually increase through the reaction progress and compensate low final substrate concentration in bulk mass and low concentration gradient for the mass transfer to the water phase. This feed type could maximize the rate of conversion of acetate **1** to alcohol **2**, compared to the previous feed approach, because the catalyst would always be in the presence of a maximum possible concentration of acetyl moiety in **1** and would react with it in preference compared to the lactone ring moiety. To test our idea of stepwise catalyst addition, the load was changed to the initial 0.5 equiv (w/w) addition of

Table 5. Influence of pH on the Reaction[a]

entry	pH reaction	yield [%]
1	<3.8	50
2	4.8 ± 0.5	73
3	6.5 ± 0.5	66
4	5.0 ± 0.5	83

[a] Load of pancreatin powder was 0.7 equiv (w/w) compared to acetate **1**. Reactions were done at 34−37 °C. Reaction time was 24 h. pH was controlled by addition of 1.0 M $NaHCO_3$. Starting PBS buffer was at pH 4.0, 5.2, or 7.0 depending on the desired pH range.

catalyst to the dispersion of **1** (0.11 M) in phosphate buffer followed by five subsequent stepwise additions of 0.3 equiv (w/w) after each hour of reaction until the total amount of 2.0 equiv (w/w) of catalyst, which resulted in practically complete conversion to **2** in 24 h (entry 2, Table 4). Next, the load of catalyst was decreased to the initial addition of 0.25 equiv (w/w) of catalyst followed by five subsequent stepwise additions of 0.15 equiv (w/w) after each hour of reaction until the total amount of 1.0 equiv (entry 3, Table 4). In this case also a 2/98 ratio of **1** to **2** was obtained. Further lowering of the catalyst load to 0.7 equiv (w/w) (initial 0.2 equiv followed by 5 times 0.1 equiv) provided a 5/95 ratio of **1** to **2** (entry 4, Table 4). Moreover, when the total catalyst load of 0.5 equiv (w/w) (initial 0.15 equiv followed by 5 times 0.07 equiv) was applied (entry 5, Table 4) almost complete conversion of acetate **1** to alcohol **2** was also achieved in 24 h. Markedly, when an even lower catalyst load of 0.4 equiv (w/w) was applied (initial 0.1 equiv followed by 5 times 0.06 equiv) the reaction was not complete and a 10/90 ratio of **1** to **2** was obtained (entry 6, Table 4). This experiment suggested that we determined the borderline catalyst load required for the completion of the reaction with 0.5 equiv (w/w) load at 0.11 M substrate concentration. Next, we have decided to explore the reaction performance at higher substrate concentrations in order to increase the through-put. When a 0.2 M concentration of substrate was used and a total catalyst load of 0.5 equiv (w/w) (initial 0.15 equiv followed by 5 times 0.07 equiv) was applied, practically full conversion to alcohol **2** was achieved (entry 7, Table 4). Increasing the substrate **1** concentration to 0.3 M with the same total catalyst load and mode of addition resulted in only 50% conversion (entry 8, Table 4). With a higher total catalyst load of 0.7 equiv (w/w) (initial 0.20 equiv followed by 5 times 0.1 equiv) we were able to achieve full conversions at substrate **1** concentrations up to 0.5 M (entry 9, Table 4). However, as the catalyst is a key cost driver of the transformation, our objective was to elaborate the optimal process at 0.2 M substrate concentration and not to pursue the development at higher catalyst loads. Importantly, by applying stepwise catalyst feed to substrate we succeeded in lowering the catalyst load from 2 equiv (w/w) in a single batch addition (entry 1, Table 4) to 0.5 equiv (w/w) in a stepwise addition (entry 7, Table 4) and increase the initial substrate **1** concentration from 0.11 to 0.2 M, which enables the increased through-put of the process.

Another important parameter of the reaction to be investigated was pH. Since the lactone moiety is sensitive to basic conditions and the TBS protection to acidic medium (pH < 2),[11] we have investigated several possibilities of pH control in different pH ranges in order to facilitate the optimum reaction conditions. Importantly, deprotection reaction releases 1 mol of acetic acid per mole of acetate **1**, which could rapidly lead to significant pH decrease and deterioration of the substrate or product. We considered first to control the pH by regulated addition of 1.0 M NaOH solution or 1.0 M $NaHCO_3$ solution to the reaction mixture consisting of phosphate buffer at pH 7. The 1.0 M $NaHCO_3$ solution proved to be better choice due to the higher yields of recovered alcohol **2**, which could be ascribed to the opening of the lactone ring by a strong base such as NaOH and consequently loss of yield. Initially, a screening had been done with pH maintained through reaction progress at values of 3.8, 4.8, and 6.5. It was noticed that when pH <4 was maintained through reaction progress with 1.0 M $NaHCO_3$ solution (entry 1, Table 5) the reaction yield decreased to 50%. When the pH was maintained through the reaction at 4.8 ± 0.5 (entry 2, Table 5), the yield of **2** increased to 73%. However, when pH was maintained at 6.5 ± 0.5 (entry 3, Table 5), the isolated yield of alcohol **2** lowered to 66%. These results indicated that favorable results would be obtained with application of phosphate buffer with a pH of approximately 5 followed by pH correction in the range of 5 ± 0.5 by continuous addition of 1.0 M $NaHCO_3$ solution to the reaction mixture. Indeed, when a PBS buffer with pH of 5.2 was used and pH 5.0 ± 0.5 was maintained through the reaction progress a maximum yield of 83% (entry 4, Table 5) was achieved.

To determine the optimum reaction time and avoid the prolonged exposure of fragile lactone moiety to pancreatin powder and phosphate buffer we monitored the reaction progress by PAT with an in-line IR probe and a pH probe (Figure 3). The reaction was performed at 35 °C in PBS and the pH was maintained between the initial 5.6 and final 5.1. The pancreatin powder was added initially (0.2 equiv, w/w) and then stepwise each hour 0.1 equiv (w/w) until a total amount of 0.7 equiv (w/w) was reached. The reaction was performed over 21 h. The reaction progress was monitored by acetate **1** consumption via IR analysis (blue curve, Figure 3b) and pH drop due to the released acetic acid (green curve, Figure 3b). Complete conversion of acetate **1** could be observed when constant pH would be reached indicating no change of acetic acid concentration. Indeed, the in-line IR curve for acetate **1** concentration change indicated that acetate **1** was consumed rapidly in the first few hours (steep slope in the blue curve, Figure 3b) and reached steady-state after ca. 9 h. Similarly, the acetic acid concentration rise was rapid in the first few hours (steep slope in the green curve, Figure 3b) measured by drop of pH with pH probe and reached a steady-state also at approximately 9 h. Both curves show good agreement between two different approaches of reaction monitoring. According to the results obtained by the in-line FTIR probe and pH probe all acetate **1** was consumed already in ca. 9 h of reaction (Figure 3). After isolation by extractive workup and crystallization from hexane only a 66% yield of alcohol **2** was obtained. This result indicated that prolonged exposure of product to catalyst and phosphate buffer leads to degradation of the lactone ring moiety, which results in product loss through chain opened ring carboxylate side product. Therefore, the reaction should be stopped after approximately 9−10 h, followed by the downstream isolation process in order to achieve the best results.

With an established chemical process in hand we turned our attention to a downstream process in order to provide alcohol **2** in pharmaceutically acceptable quality without residual proteins with simple and scalable downstream operations. An additional aspect of this development was the elimination of concentration to dryness under reduced pressure after extractive workup, which followed recrystallization from hexane. Furthermore, investigations of solubility of alcohol **2** by construction of solubility curves

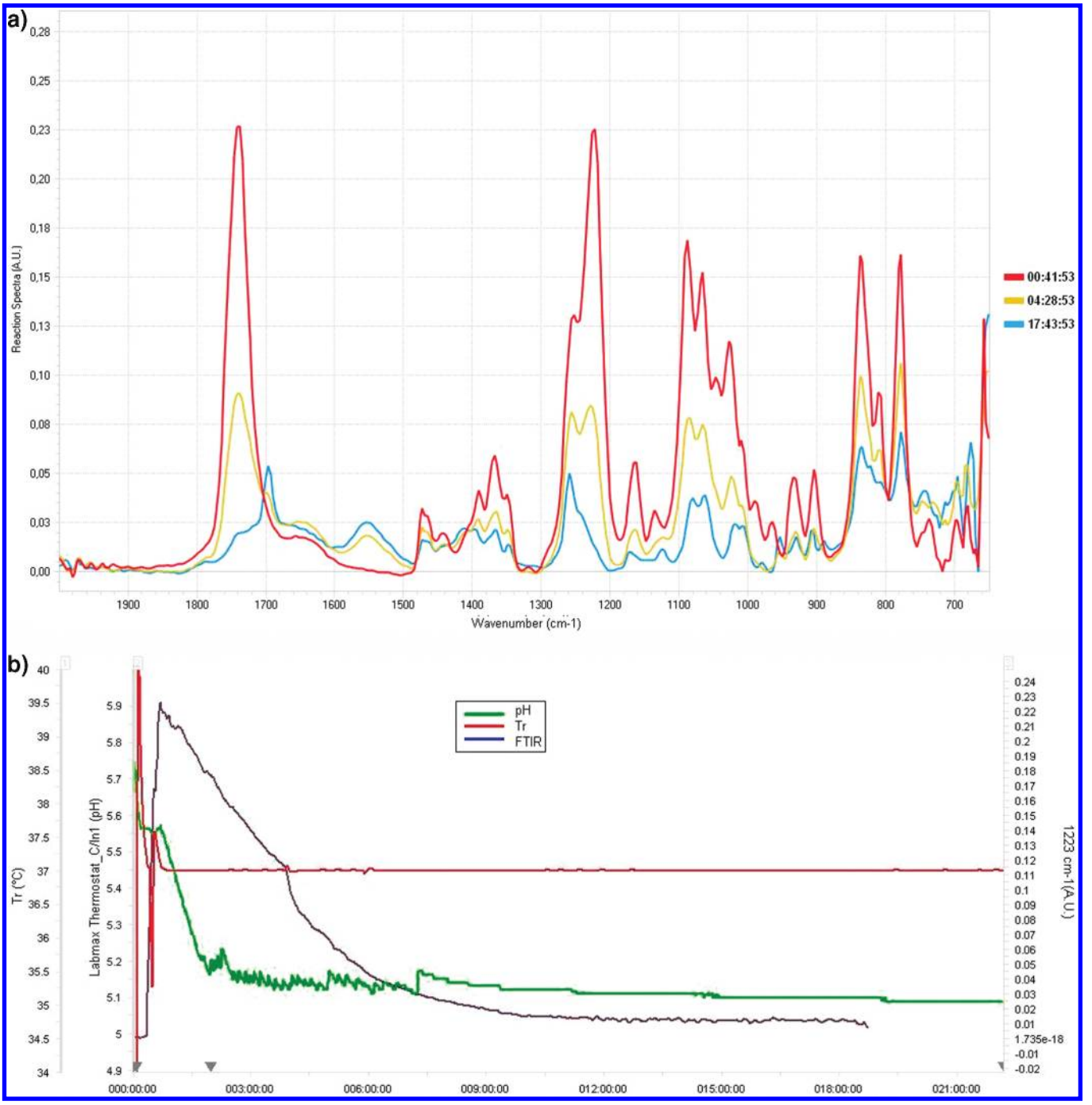

Figure 3. (a) Accumulated in-line FTIR spectra at 42 min, 4h 29 min, and 17h 44 min. (b) The blue curve represents the acetate **1** solubilization and consumption monitored by in-line FTIR at 1223 cm^{-1}. The red curve is the reaction temperature profile. The green curve is the pH of the reaction. Small pH leaps correspond to pH correction with 1.0 M $NaHCO_3$ solution. Formation of alcohol **2** is not observed due to the insolubility of alcohol **2** in aqueous media.

in hexane, methylcyclohexane, and heptane demonstrated that the best solvent for alcohol **2** crystallization is methylcyclohexane, which provides maximum solubility at the boiling point temperature and lowest solubility of alcohol **2** at temperatures close 0 °C. These properties of methylcyclohexane would allow minimum consumption of solvent for solubilization of alcohol **2** and maximum precipitation at low temperatures. Therefore, when the reaction was complete, Celite was added to the mixture and filtration was performed to remove the enzyme residues. The filtrate was then extracted with EtOAc and the organic phase was concentrated to provide the oily residue, which still contained ca. 5−10% of EtOAc. Then 3 times volume of methylcyclohexane or heptane was added (both solvents have a higher boiling point than EtOAc). Afterwards, the solvents were evaporated under reduced pressure to about 40% of the volume, which removed all the EtOAc and precipitated alcohol **2** from the remaining pure alkane solvent providing the suspension of alcohol **2**. The suspension was warmed at 80 °C until all the precipitated alcohol

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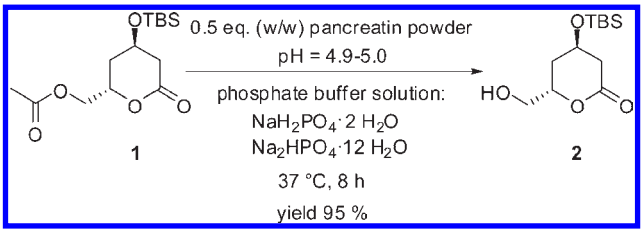
Scheme 2. Final Procedure for Deacetylation of ((2*S*,4*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl)methyl Acetate 1 by Using Pancreatin Powder Lipase



2 was completely dissolved. The solution was then cooled to room temperature and stirred for 30 min and further cooled to 0 °C. The precipitated alcohol **2** was collected by filtration at 2 to 4 °C. Alcohol **2** was recovered as white crystals with 82% yield after drying.

With all the reaction and downstream parameters in hand we decided to apply the knowledge acquired on lab scale experiments to perform the scale up from lab scale to bench scale. When then reaction was performed with the initial 120 g of acetate **1** (0.2 M) in PBS (pH 5.20) at 37 °C with stepwise addition of pancreatin powder (0.5 equiv w/w) in 8 h and pH was maintained at 4.9−5.0 with addition of 1.0 M $NaHCO_3$ solution, alcohol **2** was isolated in excellent 95% yield (Scheme 2).

■ CONCLUSION

In conclusion, we have developed a high-yielding and practical biocatalytic deprotection of acetyl moiety in ((2*S*,4*R*)-4-(*tert*-butyldimethylsilyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl)methyl acetate to provide a key lactonized statin side chain precursor (4*R*,6*S*)-4-(*tert*-butyldimethylsilyloxy)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-2-one. Enzymatic deprotection proved to be far superior compared to the chemical reagents that were tested in parallel for comparison and previously reported homogeneous organotin catalyzed deacetylation.[6a,31] From an economical point of view pancreatin powder was selected for further reaction optimization.[32] Cosolvent utilization has been investigated with in-line FTIR, which revealed an unfavorable effect on the conversion. Furthermore, the pancreatin powder load was significantly decreased from the initial 3 equiv (w/w) to 0.5 equiv (w/w) by application of stepwise pancreatin powder addition through the reaction progress. Additionally, the optimal pH range of the reaction has been determined. The final optimized procedure proceeds in aqueous medium and provides (4*R*,6*S*)-4-(*tert*-butyldimethylsilyloxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-one in up to 95% yield, which enables economical utilization of the respective transformation.

■ EXPERIMENTAL SECTION

General Considerations. Reagents and solvents were used as purchased. **NMR spectra** were recorded at 302 K on a Varian VNMRS 400 spectrometer operating at 400 (^{1}H) and 100 MHz (^{13}C), respectively, using deuterated chloroform ($CDCl_3$) as solvent. Proton and carbon spectra were referenced to TMS as internal standard or residual solvent signals. The assay is given as the NMR percent for crude product based on the integral compared to the internal standard 1,3,5-trimethoxybenzene. **Infrared spectra** were recorded on a Thermo Nicolet Nexus spectrometer, using samples in potassium bromide disks or sodium chloride plates. PAT monitored reactions were performed in a Mettler Toledo LabMax automated lab reactor system equipped with a 80 and 600 mL glass vessels, pH probe, and powered by a Huber Unistat 815-W thermostat. In situ reaction analysis was performed with a Mettler Toledo ReactIR iC10 system equipped with flexible silver halide (AgX) FiberConduit probe. Data sampling for compound **1** was performed for the peak at 1223 cm^{-1} and for compound **2** for the peak at 1703 cm^{-1}. **Solubility curves** for alcohol **2** in various solvents were constructed with an Avantium Crystal16 apparatus. **Melting points** were determined on a Kofler block or on a Mettler Toledo DSC apparatus 822^e. **TLC** was performed on silica gel 60 F_{254} plates (Merck). For TLC analyses hexane and ethyl acetate at different proportions were used as the mobile phase and revealed with use of UV light. Acetate **1** R_f (hexane/Et_2O = 2:1) = 0.53, alcohol **2** R_f (hexane/Et_2O = 2:1) = 0.21. **Residual proteins** in the product were determined by an EZQ Protein Quantitation Kit (R33200), Molecular Probes, Invitrogen. **Materials**: ((2*S*,4*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl)methyl acetate (**1**) was obtained according to the procedures described in the literature.[6a,9] Celite (Celite 535; Merck), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$; 99+%; Kemika), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$; 98+%; Merck), fluoroboric acid diethyl ether complex ($HBF_4 \cdot OEt_2$ 51% to 57% in diethyl ether; Fluka), sodium methoxide solution (NaOMe 0.5 M in methanol; Fluka), methanol (MeOH; 99+%; Acros Organics), iodine (I_2; 99.8+%; Sigma Aldrich), guanidine nitrate (98%; Aldrich), acetyl chloride (AcCl; 98%, Sigma Aldrich), cyanide on polymer support (CN on polymer support; 3 mmol CN/g resin; Fluka), dibutyltin oxide (Bu_2SnO; 98%, Aldrich), potassium carbonate (K_2CO_3; 99+%; Riedel de Haen), 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU; 98%; Acros Organics), ammonium acetate (98%; Merck), *p*-toluenesulfonic acid (*p*-TsOH; 12% in acetic acid; Acros Organics), hydrazine hydrate solution (78% to 82%; Riedel de Haen), and scandium triflate ($Sc(OTf)_3$; 99%; Aldrich) were used. Commercial lipases were used: pancreatin powder (lipase (55 U/mg), amylase (49 U/mg) and protease (3.7 U/mg; Sandoz), lipase from thermocymes lanuginosa (29.7 U/mg; Fluka), lipase from rhizopeus niveus (4.0 U/mg; Fluka), lipase from *Candida rugosa* (1560 U/mg; Sigma), lipase from wheat germ (0.10 U/mg; Fluka), lipase form *Aspergillus niger* (0.187 U/mg; Fluka), and lipase from hog pancreas (27.4 U/mg; Fluka).

Synthesis of (4*R*,6*S*)-4-(*tert*-Butyldimethylsilyloxy)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-2-one (2) via Enzymatic Deacetylation of ((2*S*,4*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl)methyl Acetate (1) with Pancreatin Lipase. *a. Initial Unoptimized Procedure.* ((2*S*,4*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl)methyl acetate (**1**)[6a,9] (300 mg; 0.99 mmol) was diluted in a phosphate buffer solution with pH 7 (20 mL). Then the pancreatin lipase (0.90 g; 3 equiv, w/w, Sandoz) was added followed by dioxane (4 mL). The PBS/dioxane volume ratio was 5/1. The pH was monitored and regulated by addition of NaOH solution (1.0 M, 0.2 mL) to maintain the pH between 6.5 and 7.1. The reaction mixture was stirred at 34 °C for 2 h. Then, the reaction mixture was washed with water (15 mL) and filtered through Celite. Traces of acetate **1** were observed according the TLC. After extraction into EtOAc followed by solvent evaporation and chromatographic purification 0.20 g (77%) of (4*R*,6*S*)-4-(*tert*-butyldimethylsilyloxy)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-2-one (**2**) was obtained.

b. Final Optimized Procedure. ((2*S*,4*R*)-4-(*tert*-Butyldimethylsilyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl)methyl acetate (**1**)[6a,9] (120.0 g, 0.397 mol) was added to phosphate buffer

solution (mixture of $NaH_2PO_4 \cdot 2H_2O$ 100 mM and $Na_2HPO_4 \cdot 12H_2O$ 100 mM) with pH 5.2 (2.0 L). The obtained mixture was warmed to 37 °C. Then, pancreatin powder (60 g; 0.5 equiv w/w, Sandoz) was added stepwise to the buffer mixture containing the substrate **1** (at t_0 0.15 equiv w/w (18.0 g) was charged and afterwards each hour 0.07 equiv w/w (5 × 8.4 g) was added up to a total amount of 0.5 equiv w/w). In parallel, the pH of the reaction mixture was monitored and regulated by simultaneous addition of 1.0 M $NaHCO_3$ water solution to maintain a pH at 4.9. The reaction mixture was stirred for 8 h at 37 °C after the first addition of pancreatin powder. The conversion of the reaction was almost quantitative (<2% of acetate **1** remaining) as indicated by in-line FTIR and stabilization of pH. Then, Celite (120 g) was added to the crude mixture. The solution was filtered through the Celite. A pale yellow liquid was recovered. The remaining solid on the filter was washed with EtOAc (4 × 0.5 L). TLC indicated that the last wash contained no product **2**. The last portion of EtOAc washed was thus used to extract the water solution obtained after filtration through Celite. The water solution was washed with an additional portion of EtOAc (0.5 L). The combined organic phases (ca. 2.5 L) were dried with $MgSO_4$ and partially concentrated under reduced pressure at 40 °C to obtain a concentrate of the product **2** in ca. 100 mL of EtOAc. Then, methylcyclohexane (450 mL) was added. The solution was concentrated under reduced pressure to ca. 2/3 of the initial volume in order to remove EtOAc. The white precipitate of alcohol **2** appeared in the solution. The solution was warmed to 80 °C to dissolve all the solid. Afterwards, the mixture was cooled to ambient temperature (after 10 min at ambient temperature a white precipitate was observed) and stirred at ambient temperature for 30 min and then cooled to 0 °C and stirred for 30 min at 0 °C. The product was recovered by filtration. The obtained white solid was dried under reduced pressure at 40 °C until a constant weight to give 97.6 g (95%) of (4*R*,6*S*)-4-(*tert*-butyldimethylsilyloxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-one (**2**) as white crystals with physical and spectroscopic properties in accordance with those reported in the literature and purity above 99.5%.[6a] ^{1}H NMR analysis of compound **2** showed that no diastereoisomers are present even with high scan accumulation at the detection limit, which indicates that the diastereoisomeric level in **2** is significantly below 1%. Enantiomeric purity was determined to be >99.8% ee.[9] Residual proteins in product **2** were determined on the level up to 130 ppm for all produced batches. In some batches these levels were below 30 ppm. The suggested allowable limit for residual proteins in APIs is set at 1−100 or below 50 ppm.[33] The level of residual proteins in API prepared from alcohol **2** acquired from the presented process was below the detection limit of the applied method. This indicates that four synthetic steps,[7,8] leading from intermediate **2** to API, have significant depletion capacity for removal of an already low level of residual proteins in alcohol **2**. EtOAc used for extraction was recycled by distillation at reduced pressure and 40 °C (recovery 2.3 L, 92%). Recycled EtOAc had the same GC purity of 99.92 area % as the originally used EtOAc.

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