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Synthesis of Functionalized δ-Hydroxy-β-keto Esters and Evaluation of Their Anti-inflammatory Properties

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δ-Hydroxy-β-keto esters and δ,β-dihydroxy esters are characteristic structural motifs of statin-type natural products and drug candidates. Here, we describe the synthesis of functionalized δ-hydroxy-β-keto esters in good yields and excellent enantioselectivities using Chan's diene and modified Mukaiyama-aldol reaction conditions. Diastereoselective reduction of δ,β-dihydroxy esters afforded the respective *syn*- and *anti*-diols, and saponification yielded the corresponding acids. All products were evaluated for their anti-inflammatory properties, which uncovered a surprising structure-activity relationship.

Introduction

Main δ-hydroxy-β-keto esters and δ,β-dihydroxy esters are characteristic structural motifs of many natural products and drugs (Figure 1A) with the fungal product lovastatin (**1**), one of the most prominent natural-derived drugs in pharmaceutical history, as prime example.[1,2] Natural and synthetic statins, like the block buster drug atorvastatin (**2**), and its derivatives fluvastatin (**3**) and rosuvastatin (**4**), are currently used for the treatment of dyslipidemia and the prevention of cardiovascular diseases.[3] Statins share common structural features such as a central cyclic core and a lateral chain derived from (3*R*,5*R*)-3,5-dihydroxyheptanoic acid. Due to their structural resemblance with mevalonic acid (**5**), statins act as inhibitors of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, thereby modulating cholesterol and lipid levels *in vivo*.[4] Clinical studies also suggested that statins are also known to have anti-inflammatory and immunomodulatory activity properties by reducing the production of inflammatory markers.[5]

Due to the broad synthetic applicability of δ,β-dihydroxy esters as homochiral synthons for statin-like natural products, we recently investigated the enantioselective synthesis of yet unreported functionalized δ-hydroxy-β-keto esters and their corresponding *syn*- and the *anti*-configured δ,β-dihydroxy esters and acids (Figure 1B). All products were evaluated for their anti-inflammatory properties, which uncovered a surprising structure-activity relationship.

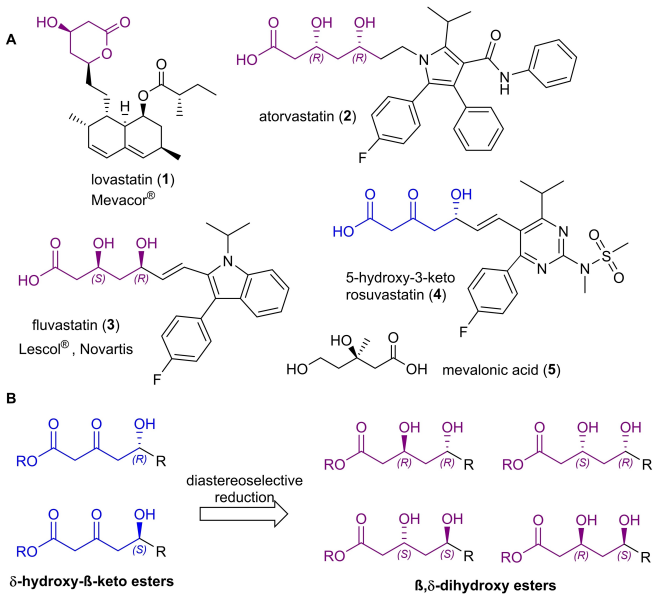

Figure 1. A) Chemical structures of lovastatin (**1**), atorvastatin (**2**), fluvastatin (**3**), rosuvastatin derivative (**4**), and mevalonic acid (**5**). B) Diastereoselective reduction of δ-hydroxy-β-keto esters yields β,δ-dihydroxy esters (structural feature of statins).

Results and Discussion

Synthesis of δ-hydroxy-β-keto esters

To obtain the desired δ-hydroxy-β-keto esters, an asymmetric Mukaiyama aldol reaction protocol was envisaged,[6] which encompasses the Lewis acid-catalyzed reaction of [Si]-trapped enolates (e.g., Chan's diene **6**)[7] with aldehydes (**7a**–**h**).[8] The most frequently used catalyst for this reaction type is the chiral

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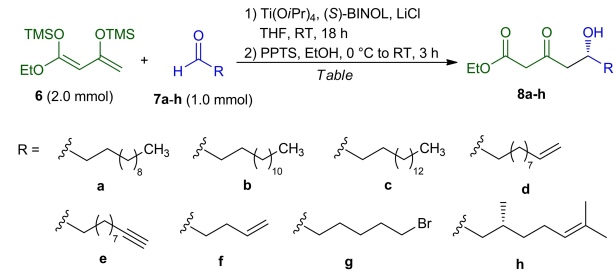
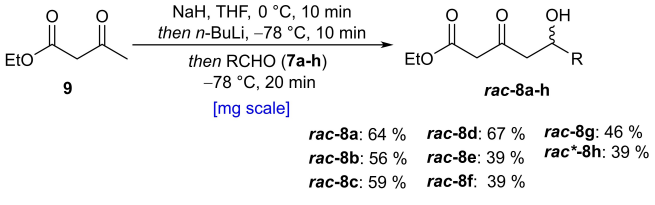
Lewis acid complex prepared *in situ* from a 1:1 mixture of $Ti(OiPr)_4$ and (*S*)- or (*R*)-BINOL in the presence of molecular sieves (4 Å) or LiCl.[9,10] While the roles of the additives are not completely understood, trace amounts of residual water in the molecular sieves were reported to be essential for the formation of the active catalyst.[11] Thus, in the first step, eight different aliphatic alcohols, including linear, branched, unsaturated and halogenated derivatives, were oxidized using Swern oxidation conditions yielding aldehydes (**7a–h**) in 68–92% yield. Chan's diene **6**, synthesized from ethyl acetoacetate in two steps,[7] was then reacted with aldehydes **7a–h** in the presence of an *in situ* generated Ti-BINOL (6 mol%) complex as catalyst and LiCl as additive (12 mol%).[12] As upon nucleophilic addition the silyl protection group is transferred to the newly formed hydroxyl group, the obtained products were deprotected (one-pot) yielding the desired δ-hydroxy-β-keto esters (**8a–h**) in good yields and excellent enantioselectivities (Table 1).[13] However, sterically more hindered aldehyde **7h** could not be converted to the desired product and only starting material remained. Intriguingly, the use of (*R*)-BINOL instead of (*S*)-BINOL as a chiral ligand afforded the aldol product **8h** in only 10% yield and high stereoselectivity (*dr* = 95:5). Neither increased catalyst loading nor change in additives and temperature resulted in higher yields; instead, the unreacted aldehyde was reisolated in all cases. The enantioselectivity of obtained δ-hydroxy-β-keto esters (**8a–h**) was determined by chiral HPLC separation and assignment of the stereocenter was determined using Mosher's method after the removal of TMS protecting group.

For comparison, racemic δ-hydroxy-β-keto esters (***rac*-8a–h**) were prepared by reacting aldehydes **7a–h** with ethyl acetoacetate (**9**) in a non-stereoselective aldol addition yielding the desired products in 39–67% yield (Figure 2).

Table 1. Ti-BINOL catalyzed asymmetric aldol reaction of Chan's diene (**6**) and aldehydes **7a–h**.



Entry	Aldehyde	Yield [%]	*er* (*dr*) [%]
1	**7a**	53	99:1[14]
2	**7b**	79	99:1[14]
3	**7c**	72	99:1
4	**7d**	73	99:1
5	**7e**	74	99:1
6	**7f**	60	99:1
7	**7g**	48	99:1
8	**7h**	0	-



***rac*-8a**: 64 % ***rac*-8d**: 67 % ***rac*-8g**: 46 %
***rac*-8b**: 56 % ***rac*-8e**: 39 % ***rac**-8h**: 39 %
***rac*-8c**: 59 % ***rac*-8f**: 39 %

Figure 2. Reaction of functionalized aldehydes (**7a–h**) with ethyl acetoacetate (**9**) yielding racemic δ-hydroxy-β-keto esters (***rac*-8a–h**) (* *dr* 1:1).[14]

Synthesis of β,δ-dihydroxy esters

Syn-configurated 1,3-diols (***syn*-10**) were obtained from δ-hydroxy-β-keto esters (**8a,d,f,g**) after treatment with sodium borohydride in the presence of the chelating agent diethyl methoxyborane (Narasaka-Prasad reduction), affording almost exclusively the corresponding *syn*-diols in 77–96% yield (Figure 3).[15,16] The intermediate six-membered methoxyborane chelate complex enforces the substituent R^2 in pseudo-equatorial position,[17] and allows the formation of a chair-like transition state leading to 1,3-*syn*-diols as major products. In analogy, the respective 1,3-*anti*-diols (***anti*-10a–g**) were obtained in good diastereoselectivity and moderate to excellent yields (50–97%) when $Me_4NHB(OAc)_3$ was used as reducing agent (Evans-Saksena reduction) instead (Figure 3).[18] Overall, eleven β,δ-dihydroxy esters were obtained in good yields and stereoselectivity, from which eight were yet undescribed.

Synthesis of carboxylic acids

Finally, eight β,δ-dihydroxy esters (***syn*-10a,d,f,g** and ***anti*-10a,d,f,g**) were saponified using LiOH in a mixture of methanol and water to obtain the respective carboxylic acids (Figure 4).

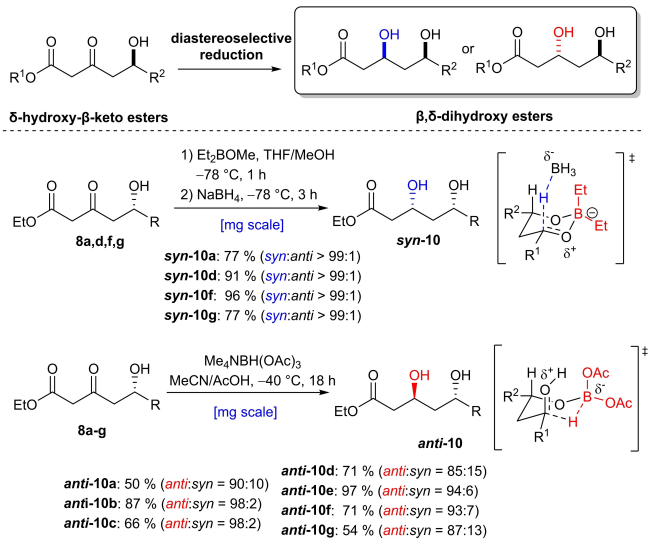


Figure 3. Diastereoselective Narasaka-Prasad and Evans-Saksena reduction of δ-hydroxy-β-keto esters **8a–g** yielding *syn*- and *anti*-β,δ-dihydroxy esters. Diastereomeric ratios were determined based on comparative ^{1}H-NMR analysis and ^{1}H-signal integration after reactions work-up of product mixture.

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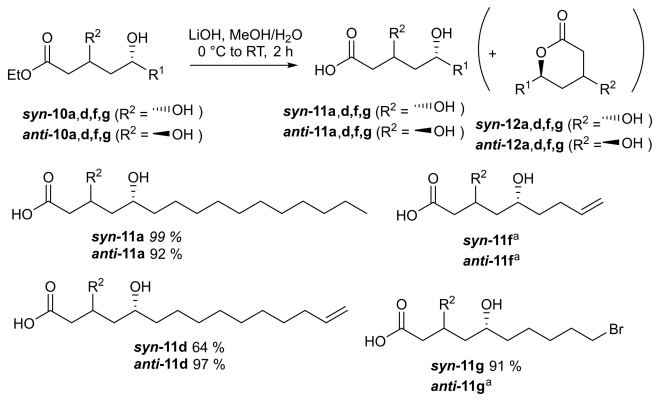

Figure 4. Saponification of β,δ-dihydroxy esters. [a] Inseparable mixture of free acid and δ-lactone.

To avoid the formation of the undesired δ-lactones (***syn***- and ***anti*-12a,d,f,g**) control of the pH of the reaction and work-up was required. Due to the preferential formation of a six-membered lactone, compounds ***syn***- and ***anti*-11f** and ***anti*-11g** were only isolated as a mixture of acid and lactone. The ratio of lactone formation was determined by ^{1}H NMR through the appearance of characteristic ^{1}H signals of H-3 and H-5. Similarly, ^{13}C NMR analysis revealed newly appearing signals for C-1, C-3 and C-5 that related to the presence of the corresponding lactone (Supporting Information, Figure S153). Overall, five previously undescribed 3,5-dihydroxy carboxylic acids (***syn***- and ***anti*-11a**, ***syn***- and ***anti*-11d** and ***syn*-11g**) were obtained in very good yields.

NMR analysis

To assist analyses efforts of natural products with similar structural features in future, we thoroughly investigated the NMR shift patterns of *syn*- and *anti*-diols. As depicted in Table 2, comparison of the ^{1}H NMR spectra uncovered significant differences in chemical shifts of *syn*- and *anti*-diols for proton H-3 as well as the hydroxy protons. However, only minor differences were observed for the chemical shift of H-5. Comparison of corresponding ^{13}C NMR spectra revealed significant chemical shift differences for hydroxylated carbons. While signals of C-3 and C-5 for *syn*-diols appeared at 69 and 72 ppm (Table 3), corresponding *anti*-configurated diols exhibited signals at 66 and 69 ppm.

Bioactivity studies

Leukotrienes (LTs) are lipid mediators involved in the regulation of inflammatory processes and allergic reactions.[4] LTs are produced from arachidonic acid (AA) via the action of the enzyme 5-lipoxygenase (5-LOX).[19] As some statin drugs are known to modulate 5-LOX activity,[20] the influence of all synthesized compounds (**8**–**11**) on the formation of 5-LOX products in polymorphonuclear leukocytes (PMNL) was tested.

As depicted in Figure 5A, saturated long-chain fatty acid esters ***syn*-10a**, ***anti*-10a** and **8a** led to a strong inhibition of the formation of 5-LOX products in PMNL after exposure to 2.5 μM Ca^{2+}- ionophore A23187 and 20 μM AA. Overall, esters with saturated alkyl chains appeared to be more active compared to halogenated and unsaturated derivatives and acids in general. Furthermore, a certain chain length seemed to be crucial for inhibiting 5-LOX product formation, since the saturated long-chain fatty acid esters **8b** and **c** as well as ***anti*-10b** and ***anti*-10c** only showed impaired activity. The three most potent compounds (***syn*-10a**, ***anti*-10a** and **8a**) showed IC_{50} values for inhibition of 5-LOX product formation in the range of 10 to 30 μM in PMNL (Figure S3A). We also tested if the most active compounds are inhibitors of the isolated 5-LOX enzyme; however, no direct inhibitory activity was observed (Figure S3B). Instead, cell viability assay revealed that the most potent inhibitors caused a marked disruption of membrane integrity with an intriguing structure-activity dependence in PMNL (Figure 5).

Conclusion

In summary, we have achieved the synthesis of yet non-reported chiral aliphatic δ-hydroxy-β-keto esters from aliphatic aldehydes and Chan's diene via optimized asymmetric Mukaiya-

Table 2. Significant chemical shifts in ^{1}H NMR (300 MHz, $CDCl_3$) of dihydroxy esters ***syn*-10a,d,f,g** and ***anti*-10a,d,f,g**.

	Cpd	$\delta_{H\text{-}3}$	$\delta_{H\text{-}5}$	$\delta_{OH\text{-}3}$	$\delta_{OH\text{-}5}$
syn	**10a**	4.27	3.87	3.78	3.20
	10d	4.27	3.87	3.77	3.21
	10f	4.27	3.90	3.79	3.34
	10g[a]	4.27	3.87	3.76	3.36
anti	**10a**	4.36	3.91	3.44	2.40
	10d	4.36	3.91	3.44	2.39
	10f	4.35	3.95	3.48	2.56
	10g[a]	4.35	3.92	3.45	2.51

[a] Measured at 500 MHz in $CDCl_3$.

Table 3. Significant chemical shifts in ^{13}C NMR (75 MHz, $CDCl_3$) of dihydroxy esters ***syn*-10a,d,f,g** and ***anti*-10a,d,f,h**.

	Cpd	$\delta_{C\text{-}1}$	$\delta_{C\text{-}2}$	$\delta_{C\text{-}3}$	$\delta_{C\text{-}4}$	$\delta_{C\text{-}5}$	$\delta_{C\text{-}6}$	$\delta_{C\text{-}7}$
syn	**10a**	172.7	41.9	69.3	42.5	72.4	38.0	25.5
	10d	172.7	41.9	69.3	42.4	72.4	38.0	25.5
	10f	172.7	41.8	69.2	42.4	71.7	37.0	29.8
	10g[a]	172.6	41.8	69.3	42.4	72.1	37.7	24.6
anti	**10a**	173.1	41.4	65.9	42.1	69.1	37.7	25.9
	10d	173.1	41.4	65.9	42.1	69.1	37.7	25.9
	10f	173.1	41.4	65.8	42.1	68.5	36.6	30.3
	10g[a]	173.1	41.3	65.8	42.2	68.8	37.4	25.1

[a] Measured at 125 MHz in $CDCl_3$.

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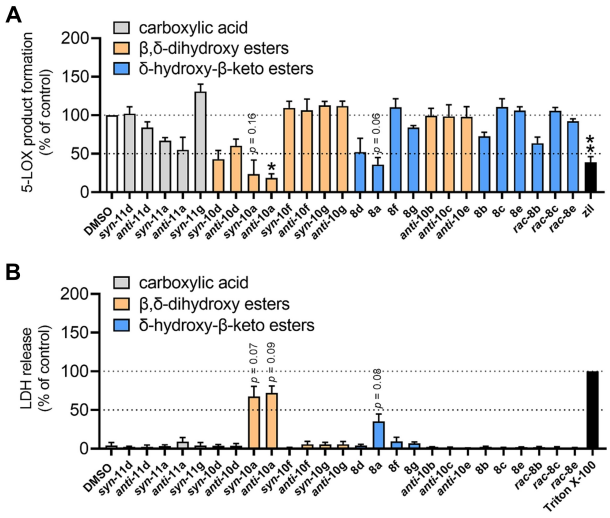

Figure 5. A) Inhibition of 5-LOX product formation. PMNL were preincubated with test substances at 30 μM, zileuton (zil, 3 μM) as reference 5-LOX inhibitor or DMSO as control (0.1%) for 15 min at 37 °C and stimulated with 2.5 μM Ca^{2+}-ionophore A23187 plus 20 μM AA for 10 min. 5-LOX products were analyzed by HPLC. B) Lactate dehydrogenase (LDH) release from PMNL was measured upon stimulation with 30 μM test substances, 1% Triton X-100 as positive control or DMSO (vehicle control) for 60 min at 37 °C. Statistical analysis was performed by using Student's ratio paired t-test vs. control; * $p < 0.05$; ** $p < 0.01$, $n = 3$.

ma aldol conditions. The use of a Ti-BINOL catalyst with LiCl as additive proved to be a reliable method to achieve satisfying yields and excellent enantioselectivities with unbranched aldehydes. Obtained δ-hydroxy-β-keto esters were diastereoselectively reduced leading to eight previously undescribed 3,5-*syn*- and *anti*-dihydroxy esters in good yields and diastereoselectivities and subsequent saponification furnished the analogous dihydroxylated carboxylic acids. In bioactivity studies, the synthesized compounds inhibited the production of pro-inflammatory lipid mediators in neutrophils by reducing 5-LOX activity via yet unknown mechanisms in a structure-activity dependence. Future studies are directed towards understanding their mode of action.

Experimental Section

The datasets supporting this article have been uploaded as part of the ESI and contains details for chemical procedures, 1D and 2D NMR of described compounds, as well as HRMS data and bioassay data.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: diastereoselective reduction · dihydroxy esters · keto esters · Mukaiyama aldol reaction · statins

[1] A. P. Lea, D. McTavish, *Drugs* **1997**, *53*, 828–847.
[2] B. Debnath, L. Q. Al-Mawsawi, N. Neamati, *Drugs News Perspect.* **2010**, *23*, 670–684.
[3] T. Hirota, Y. Fujita, I. Ieiri, *Expert Opin. Drug Metab. Toxicol.* **2020**, *9*, 809–822.
[4] I. Pinal-Fernandez, M. Casal-Dominguez, A. L. Mammen, *Med. Clin.* (Barc) **2018**, *150*, 398–402.
[5] a) B. Kwak, F. Mulhaupt, S. Myit, F. Mach, *Nat. Med.* **2000**, *6*, 1399–1402; b) L. M. Blanco-Colio, J. Tuñón, J. L. Martín-Ventura, J. Egido, *Kidney Int.* **2003**, *63*, 12–23; c) M. K. Jain, P. M. Ridker, *Nat. Rev. Drug Discovery* **2005**, *4*, 977–987; d) R. Zeiser, *Immunology* **2018**, *154*, 69–75.
[6] T. Mukaiyama, K. Narasaka, K. Banno, *Chem. Lett.* **1973**, *2*, 1011–1014.
[7] A. Soriente, M. De Rosa, R. Villano, A. Scettri, *Tetrahedron: Asymmetry* **2000**, *11*, 2255–2258.
[8] Y. Yamashita, T. Yasukawa, W. J. Yoo, T. Kitanosono, S. Kobayashi, *Chem. Soc. Rev.* **2018**, *47*, 4388–4480.
[9] A. Soriente, M. De Rosa, R. Villano, A. Scettri, *Curr. Org. Chem.* **2004**, *8*, 993–1008.
[10] D. J. Ramon, M. Yus, *Chem. Rev.* **2006**, *106*, 2126–2208.
[11] M. Terada, Y. Matsumoto, Y. Nakamura, K. Mikami, *Chem. Commun.* **1997**, 281–282.
[12] G. E. Keck, D. Krishnamurthy, *J. Am. Chem. Soc.* **1995**, *117*, 2363–2364.
[13] L. Kurti, B. Czakó, *Strategic Applications of Named Reactions in Organic Synthesis*, Elsevier, **2005**.
[14] Q. Xu, J. Yu, F. Han, J. Hu, W. Chen, L. Yang, *Tetrahedron: Asymmetry* **2010**, *21*, 156–158.
[15] D. Evans, K. Chapman, E. Carreira, *J. Am. Chem. Soc.* **1988**, *110*, 3560–3578.
[16] S. E. Bode, M. Wolberg, M. Mueller, *Synthesis* **2006**, 557–588.
[17] K. Narasaka, H. C. Pai, *Chem. Lett.* **1980**, *9*, 1415–1418.
[18] K. M. Chen, G. E. Hardtmann, K. Prasad, O. Repič, M. J. Shapiro, *Tetrahedron Lett.* **1987**, *28*, 155–158.
[19] B. Samuelsson, *Science* **1983**, *220*, 568–575.
[20] O. Rådmark, O. Werz, D. Steinhilber, B. Samuelsson, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2015**, *1851*, 331–339.

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