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Synthesis of γ-halogenated and long-chain β-hydroxy-α-amino acids and 2-amino-1,3-diols using threonine aldolases

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Abstract—The L- and D-threonine aldolase catalyzed formation of γ-halogenated and long-chain L- and D-3-alkylserine-derivatives **1**–**12**, respectively, was shown starting from glycine and halogenated C_2– or C_4–C_{12} aldehydes. LTA from *Pseudomonas putida* accepted all tested aldehydes with strongly varying diastereoselectivity (de up to 93%). Only aldehydes smaller than decanal were converted by DTA from *Alcaligenes xylosoxidans* with good selectivities (de up to 73%). Utilizing isobutanal enantio- and diastereopure D-*syn*-2-amino-3-hydroxy-4-methylpentanoic acid was obtained (ee>99%, de>95%), which was converted to the corresponding 2-amino-1,3-diol.

1. Introduction

Sphingolipids are a class of lipids derived from the amino alcohol sphingosine. They bear at neutral pH only a small positive charge as a consequence of intramolecular hydrogen bonding.[1] This property enables them to cross membranes or move between membranes with ease.[2] The lipid backbone is O-linked to ethanolamine, serine or choline, and amide-linked to fatty acids (Fig. 1). Sphingolipid metabolites such as ceramides, sphingosine, and sphingosine-1-phosphate are lipid second messengers involved in diverse cellular processes.[3–7] Sphingosine consists of a long unsaturated hydrocarbon chain and is synthesized in vivo from palmitoyl-CoA and serine in a three-step procedure.[8] Natural sphingoid bases occur in the D-*anti* (2*S*,3*R*) configuration. Among the three unnatural bases, D-*syn* (2*S*,3*S*) is of particular interest due to its anticancer activity (safingol).[9]

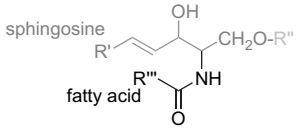

Figure 1. Sphingolipids consists of sphingosine, a polar group (R: ethanolamine, serine or choline) and a fatty acid.

Keywords: Enzymes; Threonine aldolase; Biocatalysis; Amino acid.
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The interest in sphingolipids has increased in recent years, due to the finding that D-*anti*-sphingosines modulate immune response (immunosuppressants, e.g., Plakoside A[10] and activators of the immune system, e.g., KRN7000[11]).

Both, sphingosines and dihydrosphingosines (sphinganines) were shown to be highly active. They have been targets of intense interest for synthetic chemists due to the lack of readily available natural sources. Despite their structural diversity, the key for the preparation of these compounds is the stereoselective construction of the 2-amino-1,3-diol part of the molecule. According to reviews[12] of recent literature about the synthesis of sphingosine[13] different approaches were chosen, either from the chiral pool (A: β-hydroxy-α-amino acids: starting from serine/reduction of the carboxylate to the aldehyde/Wittig olefination/asymmetric epoxidation,[14] serine derived 1,5-dioxyspiro[3.2]hexane and organocuprates,[11] starting with serine-derived Weinreb amides and ensuing stereoselective reduction of the ketone;[15] B: carbohydrates: starting from D-glucose and utilizing olefin cross-metathesis[16]) or involving asymmetric induction to control the stereochemistry (via a Henry reaction utilizing 2-nitroethanol followed by catalytic hydrogenation,[17] via reduction of enantiopure diprotected β-hydroxy-α-aminonitriles,[18] via a regioselective iodoamination of allylic alcohols with subsequent hydrolysis,[19] a palladium-catalyzed isomerization of 5-vinyloxazolines followed by Suzuki-coupling,[20] via α,β-epoxy carboxylic esters and consecutive nucleophilic attack of azide and reduction of the ester,[21] by an aldol condensation between

an iminoglycinate bearing a chiral group and an appropriate aldehyde,[22] asymmetric pinacol-type coupling of chiral imines with aldehydes,[23] via β-keto-sulfoxide or sulfone intermediates for C–C bond formation[24] or OsO_4-catalyzed asymmetric dihydroxylation and regioselective azide-substitution).[25]

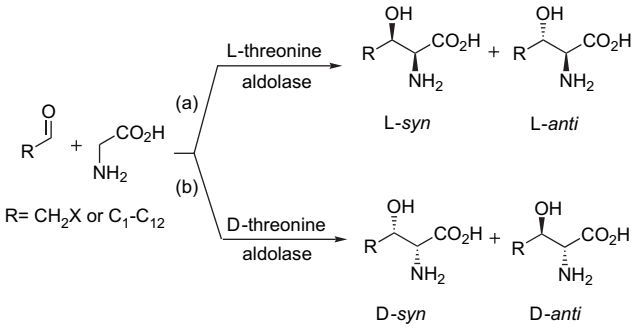
γ-Halogenated β-hydroxy-α-amino acids constitute an important class of compounds due to their unique metabolic and physiological activities. They have been applied as biological tracers, enzyme inhibitors, and for medical applications such as control of blood pressure, allergies, and tumor growth.[26] Enzymatic synthesis of 4-fluorothreonine was reported using a pyridoxal-5′-phosphate (PLP) dependent threonine transaldolase (TTA) from *Streptomyces cattleya* in a whole cell process. TTA is involved in 4-fluorothreonine biosynthesis and uses fluoroacetaldehyde and threonine to generate 4-fluorothreonine **1** and acetaldehyde.[27]

Recently, L- and D-threonine aldolases (LTA/DTA) have been studied for the non-physiological synthesis of β-hydroxy-α-amino acids using aldehydes and glycine. The reaction proceeded with complete stereoselective control at the α-carbon.[28–30] However, these protocols suffer from limited substrate range being glycine the only donor, modest yields and low diastereoselectivities. The latter obstacles were overcome for β- and γ-benzyloxy aldehydes as was shown for the synthesis of monobactams[31] and long-chain products—mycestericin D and F.[32,33] Recently, the product range was widened to several phenylserine derivatives with partly excellent enantio- and diastereoselectivities. This was achieved[34,35] by using DTA from *Alcaligenes xylosoxidans*[36] and LTA from *Pseudomonas putida*.[37] Herein we give a more detailed overview of the TA-catalyzed synthesis of γ-halogenated β-hydroxy-α-amino acids and long-chain aldehydes.

2. Results and discussion

2.1. Synthesis of β-hydroxy-α-amino acids

We examined different steric-demanding aldehydes such as halogenated acetaldehydes and long-chain aldehydes to test the applicability of both LTA and DTA (Scheme 1).

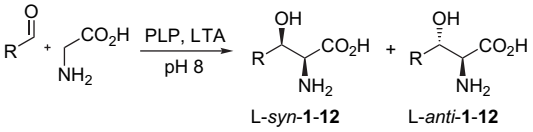


Scheme 1. TA-catalyzed synthesis of (a) L- and (b) D-serine derivatives.

2.1.1. L-Threonine aldolase. We showed that LTA from *P. putida* catalyzes the stereoselective formation of γ-halogenated threonine using glycine and the corresponding halogenated aldehydes (Table 1, entries 2–4). The product formation was confirmed by HPLC, ^{19}F NMR and ^{1}H NMR. The highest diastereoselectivity was obtained for L-**1** (entry 2). For the reaction with bromoacetaldehyde and glycine low conversion was obtained (entry 4) compared to reactions with the F- and Cl-substrates (entries 2 and 3). Surprisingly, the diastereoselectivity for the synthesis of halogenated threonine was from moderate to good, whereas the reaction with acetaldehyde showed no stereoselectivity for the β-carbon, which resulted in the formation of an equimolar mixture of L-*syn*/*anti*-threonine **2** (entry 1), which is in line with the classification as L-*low specificity* threonine aldolase.[38] The more steric-demanding isobutanal showed the anticipated effects—a high increase in selectivity up to a de of 42% but also lower yield of L-*syn*-**5** (entry 5). On the contrary, isovaleraldehyde gave high yield but lower diastereoselectivity of L-*syn*/*anti*-**6** (entry 6).

Similar effects were obtained for butanal and slightly higher de for hexanal (entries 7 and 8a). DMSO and DMF, which had been shown to be compatible with LTA[35] did not improve the outcome of the reaction (entries 8a–c). They were tested for hexanal and did not have a positive effect for the conversion at comparable selectivity. Similar effects were obtained for all long-chain aldehydes. Surprisingly, the diastereoselectivity switches to *anti* for octanal and larger aldehydes (entries 9–11). We assume, switching the stereoselectivity occurs due to the limiting size of the hydrophobic pocket, where the hydrophobic part of the β-hydroxy-α-amino acid is usually located in. That is why favorable interactions between the residues of the hydrophobic pocket and the

Table 1. LTA catalyzed formation of long-chain serine derivatives



Entry	R	Yield[a]/%	de[b]/%
1	CH_3 L-**2**	n.d.	4 (*anti*)
2[c]	CH_2F L-**1**	50	93 (*syn*)
3	CH_2Cl L-**3**	65	40 (*syn*)
4[c]	CH_2Br L-**4**	20	73 (*syn*)
5	$CH(CH_3)_2$ L-**5**	55	42 (*syn*)
6	$CH_2CH(CH_3)_2$ L-**6**	94	10 (*syn*)
7	$(CH_2)_2CH_3$ L-**7**	71[g]	28 (*syn*)
8a[d]	$(CH_2)_4CH_3$ L-**8**	92	31 (*syn*)
8b[e]		66	25 (*syn*)
8c[f]		63	28 (*syn*)
9	$(CH_2)_6CH_3$ L-**9**	25	9 (*anti*)
10	$(CH_2)_8CH_3$ L-**10**	29	23 (*anti*)
11	$(CH_2)_9CH_3$ L-**11**	33	15 (*anti*)
12	$(CH_2)_{10}CH_3$ D-**12**	11	15 (*anti*)

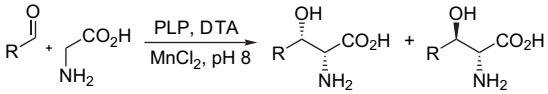
Conditions: 1 mL solution containing glycine (1 M), aldehyde (100 mM), PLP (50 μM), and LTA (77 U) at 25 °C; 60–180 min; ee>99% (L) for all reactions.

[a] Determined by HPLC.
[b] Determined by HPLC or ^{1}H NMR.
[c] Aldehyde (10 mM).
[d] No co-solvent.
[e] 30 vol % DMSO.
[f] 20 vol % DMF.
[g] Isolated yield.

long alkyl group cannot be achieved. Similar results were obtained for decanal and undecanal (entries 10 and 11). Dodecanal gave the corresponding L-*anti*-**9** (C_{14}) with lower yield (entry 12).

2.1.2. D-Threonine aldolase. Yamada et al.[36] showed that neither the physiological degradation of D-*syn*/*anti*-2 forming glycine and acetaldehyde nor the reversed synthesis reaction gave significant selectivity for the β-position. Applying our optimized conditions we obtained similar results (Table 2, entry 1). However, DTA shows high specificity in the aldol reactions when halogenated acetaldehydes are used. D-*syn*-**1** (entry 2) was obtained with a de of 97%, whereas the diastereoselectivity in the synthesis of D-*syn*-**3** and D-*syn*-**4** was 78% and 82%, respectively. Bromoacetaldehyde was converted to D-*syn*-**4** to a lower extent as compared to D-*syn*-**1** and D-*syn*-**3** (entry 4 vs 2 and 3). The use of isobutanal gave the product D-*syn*-**5** with high de and low yield (entry 5) whereas DTA-catalyzed reactions showed significantly lower de for butanal and isovaleraldehyde (entries 6 and 7). Hexanal was converted with high selectivity implying that more than four aldehyde carbons were needed for convenient diastereoselective induction using non-branched aldehydes (entry 8a). Regarding longer chains already octanal showed low conversion and for decanal, undecanal, and dodecanal conversion was below detection limit (entries 9–12). Thus, DMSO and DMF as co-solvents were tested on hexanal to improve the performance (entries 8a–c). DMSO and DMF, gave slightly increased yield. Hence, the co-solvents were tested on larger aldehydes (octanal–dodecanal) but no improved yield was obtained.

Table 2. DTA catalyzed formation of long-chain serine derivatives



Entry	R	Yield[a]/%	de[b]/%
1	CH_3 D-**2**	n.d.	2 (*anti*)
2[c]	CH_2F D-**1**	30	97 (*syn*)
3	CH_2Cl D-**3**	26	78 (*syn*)
4[c]	CH_2Br D-**4**	6	82 (*syn*)
5	$CH(CH_3)_2$ D-**5**	24	>95 (*syn*)
6	$CH_2CH(CH_3)_2$ D-**6**	65	54 (*syn*)
7	$(CH_2)_2CH_3$ D-**7**	52	9 (*syn*)
8a[d]	$(CH_2)_4CH_3$ D-**8**	33	73 (*syn*)
8b[e]		36	59 (*syn*)
8c[f]		42	68 (*syn*)
9	$(CH_2)_6CH_3$ D-**9**	12	55 (*syn*)
10	$(CH_2)_8CH_3$ D-**10**	<1	—
11	$(CH_2)_9CH_3$ D-**11**	<1	—
12	$(CH_2)_{10}CH_3$ D-**12**	<1	—

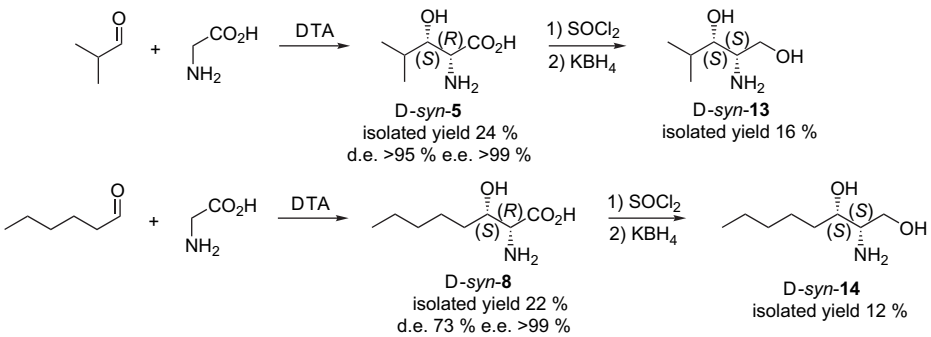
Conditions: 1 mL solution containing glycine (1 M), aldehyde (100 mM), PLP (50 μM), $MnCl_2$ (50 μM), and DTA (23 U) at 25 °C; 180–240 min; ee>99% (D) for all reactions.
[a] Determined by HPLC.
[b] Determined by HPLC or ^1H NMR.
[c] Aldehyde (10 mM).
[d] No co-solvent.
[e] 30 vol % DMSO.
[f] 20 vol % DMF.

2.2. Follow-up chemistry

After the aldol reaction, D-*syn*-3-isopropylserine **3** and D-*syn*-3-pentylserine **5** were reduced to the corresponding 2-amino-1,3-diols (Scheme 2). The low yields can be explained by the laborious work-up of the crude 2-amino-1,3-diols **13** and **14**. The reaction conditions were not optimized and the reaction was done only at small scale.

3. Conclusion

We have shown that TAs are enzymes highly capable for the synthesis of γ-halogenated-threonine. LTA and DTA catalyze the formation of fluorothreonine with high enantio- and diastereoselectivities. The drawback of these reactions is the low yield for the reactions utilizing chloro- and especially bromoacetaldehyde. The systematic study of eight long-chain aldehydes as substrates for the LTA and DTA catalyzed synthesis of 3-alkylserines gave varying results. Regarding LTA, all aldehydes (C_2–C_{12}) were converted at low to high yields. Selectivity was improved for hexanal and the steric demanding isobutanal. Surprisingly, for octanal up to dodecanal the opposite diastereoselectivity (*anti*) was obtained. For DTA, enantiopure 3-isopropylserine **5** and also 3-pentylserine **8** showed a high de. Octanal was converted at a low rate whereas larger aldehydes were not accepted by the enzyme. Only *syn*-selectivity was obtained for DTA. Finally, the 2-amino-1,3-diols, 2-amino-4-methylpentane-1,3-diol **13** and 2-aminooctan-1,3-diol **14** were synthesized from the corresponding serine-derivatives opening the field for the synthesis of short-chain sphingosine-derivatives.



Scheme 2. Two-step synthesis of 2-amino-1,3-diols **13** and **14** starting from glycine and the corresponding aldehydes.

4. Experimental

4.1. General experimental procedures

All reagents and solvents were obtained from commercial sources and appropriately purified, if necessary. All enzymes were overexpressed using published procedures.[35] ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Varian INOVA 500 (^{1}H 499.82 MHz, ^{13}C 125.69 MHz, ^{19}F 470.95 MHz) or on a Varian GEMINI 200 (^{1}H 199.98 MHz, ^{13}C 50.29 MHz) using the residual peaks of D_2O (^{1}H: δ 4.79) or DMSO-d_6 (^{1}H: δ 2.50, ^{13}C δ 40.2) as references. H_2O/D_2O NMR samples were taken directly from the aqueous solution, diluted with D_2O (1:1) and recorded using H_2O presaturation.[39] Analytical HPLC was carried out with a Hewlett Packard Series 1100 HPLC using a G1315A diode array detector. If not otherwise noted, a LiChrospher® RP-18 (250 mm, 5 μm) column was used for analysis. The yields of β-hydroxy-α-amino acids were determined by HPLC after derivatization with *ortho*-phthaldialdehyde/2-mercaptoethanol (OPA/MCE, achiral derivatization, de determination, compared to NMR results) or OPA/*N*-acetyl cysteine (OPA/NAC, chiral derivatization, ee determination).[40,41]

4.2. General procedure for the synthesis of halogenated acetaldehydes

4.2.1. Synthesis of fluoroacetaldehyde.[42] 2-Fluoroethanol (0.5 mL, 8.6 mmol) was added to a suspension of pyridinium dichromate (1.0 g, 2.7 mmol) in dichloromethane (15 mL) and the reaction mixture was stirred under reflux for 16 h. The reaction solution including product, substrate, and solvent was distilled into a flask containing water (3 mL). The organic layer was separated and extracted with a further volume of water (3 mL). The combined aqueous layer and the organic layer were analyzed by ^{19}F NMR and ^{1}H NMR analysis. Fluoroacetaldehyde hydrate was present in the aqueous layer at a final concentration of 30 mM. The sample also contained residual 2-fluoroethanol (60 mM). NMR (D_2O, 500 MHz): fluoroacetaldehyde δ −231.5 (dt, $^{2}J_{HF}$=46 Hz, $^{3}J_{HF}$=5.1 Hz); fluoroethanol −225.0 (tt, $^{2}J_{HF}$=47.5 Hz, $^{3}J_{HF}$=32.0 Hz).

4.2.2. Synthesis of bromoacetaldehyde. Bromoacetaldehyde hydrate was present in the aqueous layer at a final concentration of 20 mM. The sample also contained residual bromoethanol (70 mM).

4.3. General procedure for the synthesis of γ-halogenated threonine derivatives

4.3.1. L-4-Fluorothreonine L-2. To a solution of LTA (77 U) or DTA (23 U) and pyridoxal-5′-phosphate (13 ng, 50 nmol) in 0.5 mL buffer (KH_2PO_4, 50 mM, pH 8.0) glycine (75 mg, 1.0 mmol) and fluoroacetaldehyde hydrate (0.5 mL, 0.01 mmol) were added. The reaction mixture was stirred at rt for 3 h to give L-**2**: yield 50%; de 93% (*syn*); ee *syn*>99% (L), *anti*>99% (L); HPLC: OPA/MCE derivatization; KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=80:20, 0.8 mL/min, t_{syn}=17.1 min, t_{anti}=24.7 min.

4.3.2. L-4-Chlorothreonine L-3. HPLC: OPA/MCE derivatization; KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=81:19, 0.8 mL/min, t_{syn}=19.7 min, t_{anti}=33.4 min.

4.3.3. L-4-Bromothreonine L-4. HPLC: OPA/MCE derivatization; KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=80:20, 0.8 mL/min, t_{syn}=16.1 min, t_{anti}=23.3 min.

4.4. General procedure for the synthesis of L-serine derivatives

4.4.1. L-2-Amino-3-hydroxy-4-methylpentanoic acid L-5. To a solution of LTA (77 U) and pyridoxal-5′-phosphate (13 ng, 50 nmol) in 1 mL buffer (KH_2PO_4, 50 mM, pH 8.0) isobutanal (7 mg, 0.1 mmol) and glycine (75 mg, 1.0 mmol) were added. The reaction mixture was stirred at rt for 100 min to give L-*syn*-**5**; yield 55%; de 42% (*syn*); ee *syn*>99% (L), *anti*>99 % (L); HPLC: OPA/MCE derivatization; KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=69:31, 0.75 mL/min, t_{syn}=7.0 min, t_{anti}=11.4 min; ^{1}H NMR (500 MHz, D_2O): δ 0.77 (m, 6H), 1.73 (m, 1H), 3.55 (dd, 0.75H, *syn*, *J*=3.5 Hz, *J*=7.0 Hz), 3.62 (m, 0.25H), 3.62 (d, 0.75H, *syn*, *J*=3.5 Hz), 3.72 (d, 0.25H, *anti*, *J*=3.5 Hz).[30]

4.4.2. L-2-Amino-3-hydroxy-5-methylhexanoic acid L-6. HPLC: OPA/MCE derivatization; KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=69:31, 0.75 mL/min, t_{syn}=10.3 min, t_{anti}=15.9 min; ^{1}H NMR (500 MHz, D_2O): δ 0.70 (m, 6H), 1.21 (m, 2H), 1.50 (m, 1H), 3.40 (d, 0.54H, *syn*, *J*=5.0 Hz), 3.63 (d, 0.46H, *anti*, *J*=3.5 Hz), 3.95 (dt, 0.54H, *syn*, *J*=5.0 Hz, *J*=10.0 Hz), 4.00 (dt, 0.46H, *anti*, *J*=3.5 Hz, *J*=8.0 Hz).

4.4.3. L-3-Propylserine L-7. To solution of glycine (0.75 g, 5 mmol), pyridoxal-5′-phosphate (65 μg, 0.2 μmol) and LTA (193 U) in 4.5 mL buffer (KH_2PO_4, 50 mM, pH 8.0) butanal was added (45 μL, 0.5 mmol) and stirred at rt. The opaque, slightly yellow solution was diluted with 50 mL MeOH after 6 h and incubated at 4 °C over night. Glycine precipitated and was filtered off (0.240 g, 64% of used glycine) and the filtrate was evaporated. The product was purified on a short silica column (CH_2Cl_2/MeOH/NH_3 (30% in H_2O) gradient 75:20:5 to 10:10:1) to yield 54 mg L-**7**; yield 71%; de 28% (*syn*); ee *syn*>99% (L), *anti*>99% (L); HPLC: OPA/MCE derivatization; KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=69:31, 0.75 mL/min, t_{syn}=7.0 min, t_{anti}=11.4 min; OPA/NAC derivatization; KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=80:20, 0.8 mL/min, $t_{D\text{-}syn}$=4.5 min, $t_{L\text{-}syn}$=5.1 min, $t_{D\text{-}anti}$=6.8 min, $t_{L\text{-}anti}$=8.6 min; ^{1}H NMR (500 MHz, D_2O): δ 0.73 (m, 3H), 1.25 (m, 4H), 3.42 (d, 0.64H, *syn*, *J*=4.4 Hz), 3.62 (d, 0.36H, *anti*, *J*=3.6 Hz), 3.89 (m, 1H);[30] ^{13}C NMR (50 MHz, D_2O): *syn*+*anti*: δ 13.0, 13.5, 18.4, 18.8, 33.1, 35.4, 59.4, 59.6, 69.3, 69.5, 172.0, 173.2.

4.4.4. Synthesis of L-3-pentylserine L-8 using co-solvents. To a stirred solution of glycine (0.75 g, 5 mmol), pyridoxal-5′-phosphate (65 μg, 0.2 μmol) and LTA (1.0 mL, 386 U/threonine) in 3.5 mL buffer (KH_2PO_4, 50 mM, pH 8.0) were added hexanal (60 μL, 0.5 mmol) dissolved in DMSO (0.44 mL) and stirred for 3.5 h at rt. HPLC: OPA/MCE derivatization, KH_2PO_4 buffer, (50 mM, pH 8)/CH_3CN=80:20; 0.8 mL/min; t_{syn}=12.8 min, t_{anti}=20.2 min; OPA/NAC derivatization, KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=75:25; 0.8 mL/min; $t_{D\text{-}syn}$=5.7 min, $t_{L\text{-}syn}$=6.8 min, $t_{D\text{-}anti}$=9.3 min, $t_{L\text{-}anti}$=10.8 min; ^{1}H NMR (200 MHz, D_2O): δ 0.71 (t, 3H, *J*=5.6 Hz), 1.30 (m, 8H), 3.47 (dd, 0.79H, *syn*,

J=0.8 Hz, J=4.4 Hz), 3.67 (d, 0.21H, *anti*, J=3.8 Hz), 3.91 (m, 1H).[30]

4.4.5. L-3-Heptylserine L-9. HPLC: OPA/MCE derivatization, KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=61:39; 0.8 mL/min; t_{syn}=10.5 min, t_{anti}=14.8 min; OPA/NAC derivatization, KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN= 72:28; 0.8 mL/min; $t_{D\text{-}syn}$=11.2 min, $t_{L\text{-}syn}$=15.2 min, $t_{D\text{-}anti}$= 17.1 min, $t_{L\text{-}anti}$=20.8 min.

4.4.6. L-3-Nonylserine L-10. HPLC: OPA/MCE derivatization, KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=56:44; 0.8 mL/min; t_{syn}=12.9 min, t_{anti}=17.5 min.

4.4.7. L-3-Decylserine L-11. HPLC: OPA/MCE derivatization, KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=53:47; 0.8 mL/min; t_{syn}=13.4 min, t_{anti}=17.9 min.

4.4.8. L-3-Undecylserine L-12. HPLC: OPA/MCE derivatization, KH_2PO_4 buffer (50 mM, pH 8)/CH_3CN=50:50; 0.8 mL/min; t_{syn}=14.4 min, t_{anti}=19.1 min.

4.5. General procedure for the synthesis of D-serine derivatives: synthesis of D-2-amino-3-hydroxy-4-methylpentanoic acid D-5

To a solution of DTA (23 U), $MnCl_2$ (6 ng, 50 nmol) and pyridoxal-5′-phosphate (13 ng, 50 nmol) in 1 mL buffer (KH_2PO_4, 50 mM, pH 8.0) isobutanal (7 mg, 0.1 mmol), and glycine (75 mg, 1.0 mmol) were added. The reaction mixture was stirred at rt for 3 h to give D-*syn*-**5**; yield 24%; de 95% (*syn*); ee *syn*>99% (D), *anti*>99% (D).

4.6. General procedure for the synthesis of 2-amino-1,3-diols

4.6.1. Step 1: Synthesis of (2*R*,3*S*) methyl 2-amino-3-hydroxy-4-methylpentanoate. To a mixture of D-*syn*-**5** (135 mg, 0.92 mmol) in methanol (5 mL) thionylchloride (1.0 g, 9 mmol) was added at 0 °C and then stirred for 48 h at rt. The solvent was removed in vacuo to form the hydrochloric salt of (2*R*,3*S*)-methyl 2-amino-3-hydroxy-4-methylpentanoate (153 mg, yield 84%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02 (m, 6H), 1.97 (m, 2H), 3.85 (s, 3H), 4.25 (m, 1H), 4.65 (m, 1H), 8.28 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.7, 19.7, 30.5, 53.9, 56.6, 72.2, 169.5.

4.6.2. Step 2: Synthesis of (2*S*,3*S*)-2-amino-4-methylpentan-1,3-diol D-*syn*-13. (2*R*,3*S*)-Methyl 2-amino-3-hydroxy-4-methylpentanoate was dissolved in ethanol (4 mL) and then KBH_4 (182 mg, 3.4 mmol) dissolved H_2O (2 mL) was added dropwise. The reaction mixture was stirred at rt for about 4 h. The reaction mixture was filtered and the solvent of the filtrate was removed in vacuo. The residue was taken up three times in MeOH (2 mL) and was filtered again. The product was purified on a short silica column (CH_2Cl_2/ MeOH/NH_3 (30% in H_2O) gradient 75:20:5 to 10:10:1) to yield **13** (20 mg, yield 18%); ^{1}H NMR (500 MHz, D_2O): δ 0.72 (d, 3H, J=7.0 Hz), 0.75 (d, 3H, J=6.5 Hz), 1.61 (m, 1H), 2.75 (m, 1H), 3.10 (m, 1H), 3.33 (dd, 1H, J=11.5 Hz, J=7.0 Hz), 3.45 (dd, 1H, J=11.5 Hz, J= 5.5 Hz); ^{13}C NMR (125 MHz, D_2O): δ 17.2, 18.9, 29.6, 52.9, 63.8, 76.6.

4.6.3. Step 1: Synthesis of (2*R*,3*S*) methyl 2-amino-3-hydroxy-octanoate. (2*R*,3*S*)-Methyl 2-amino-3-hydroxyoctanoate (93 mg, yield 90%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, 3H, J=7.0 Hz), 1.32 (m, 4H), 1.49 (m, 2H), 1.69 (m, 2H), 3.83 (s, 3H), 4.12 (m, 1H), 4.26 (m, 1H), 8.29 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.6, 70.1, 58.5, 53.8, 34.0, 31.6, 25.5, 22.8, 14.2.

4.6.4. Step 2: Synthesis of (2*S*,3*S*)-2-aminooctan -1,3-diol D-*syn*-14. D-*syn*-**14** (8 mg, yield 12%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.84 (t, 3H, J=7.0 Hz), 1.23 (m, 8H), 2.43 (m, 1H), 3.20 (dd, 1H, J=11.5 Hz, J=6.0 Hz), 3.30 (m, 1H), 3.50 (dd, 1H, J=10.5 Hz, J=5.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 70.7, 64.4, 57.3, 34.2, 32.2, 26.0, 22.9, 14.7.

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