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Serine Hydroxymethyl Transferase from *Streptococcus thermophilus* and L-Threonine Aldolase from *Escherichia coli* as Stereocomplementary Biocatalysts for the Synthesis of β-Hydroxy-α,ω-diamino Acid Derivatives

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Abstract: A novel serine hydroxymethyl transferase from *Streptococcus thermophilus* (SHMT) and a L-threonine aldolase from *Escherichia coli* (LTA) were used as stereocomplementary biocatalysts for the aldol addition of glycine to *N*-Cbz amino aldehydes and benzyloxyacetaldehyde (Cbz = benzyloxycarbonyl). Both threonine aldolases were classified as low-specific L-*allo*-threonine aldolases, and by manipulating reaction parameters, such as temperature, glycine concentration, and reaction media, SHMT yielded exclusively L-*erythro* diastereomers in 34–60% conversion, whereas LTA gave L-*threo* diastereomers in 30:70 to 16:84 diastereomeric ratios and with 40–68% conversion to product. SHMT is among the most stereoselective L-threonine aldolases described. This is due, among other things, to its activity–temperature dependence: at 4°C SHMT has high synthetic activity but negligible retro-aldol activity on L-threonine. Thus, the kinetic L-*erythro* isomer was largely favored and the reactions were virtually irreversible, highly stereoselective, and in turn, gave excellent conversion. It was also found that treatment of the prepared *N*-Cbz-γ-amino-β-hydroxy-α-amino acid derivatives with potassium hydroxide (1 M) resulted in the spontaneous formation of 2-oxazolidinone derivatives of the β-hydroxyl and γ-amino groups in quantitative yield. This reaction might be useful for further chemical manipulations of the products.

Keywords: aldol reaction · aldolases · asymmetric synthesis · biotransformations · lyases

Introduction

Aldolases are an interesting class of lyases that have received much attention due to their ability to catalyze asymmetric carbon–carbon coupling reactions, and their chiral-induction capacity leads to stereochemically pure products.[1] Among them, the glycine-dependent family[2] are interesting catalysts for β-hydroxy-α-amino acid synthesis and for the chemical resolution of β-hydroxy-α-amino acid racemates.[3–5] β-Hydroxy-α-amino acids are an important class of natural products with biological activity on their own and also as constituents of many naturally occurring complex compounds, such as antibiotics and immunosupressants.[2,3,6] They also constitute excellent intermediates for the synthesis of biologically relevant compounds, such as idulonic acid mimetics,[7] the immunosuppressive lipid mycestericin D,[8] acyclic sugar analogues,[9] 3,4-dihydroxyprolines,[10] and antibiotics,[5,11] among others. Furthermore, these polyfunctional compounds might be useful building blocks for peptidomimetics and other nonproteinogenic peptide-like structures of biological interest. Threonine aldolases reversibly catalyze

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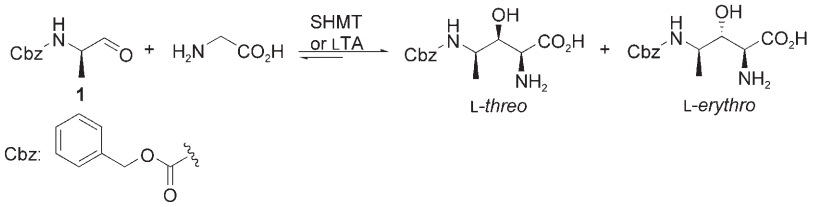
the aldol addition of glycine to a variety of acceptor aldehydes with complete control of the α-carbon stereochemistry, but with low stereoselectivity at the β-carbon atom.[12]

Our ongoing project on the synthesis of new polyfunctional derivatives of 4-aminobutyric acid (i.e., 2,4-diamino-3-hydroxyacid derivatives)[13] has led us to investigate a stereoselective chemoenzymatic approach. The key step of this approach consisted of the aldol addition of glycine to N-protected amino aldehydes, which is catalyzed by threonine aldolases. We have recently cloned and characterized a novel serine hydroxymethyltransferase (SHMT; EC 2.1.2.1), with threonine aldolase activity, from *S. thermophilus*.[14] This aldolase and the L-threonine aldolase from *E. coli*,[3] were overexpressed in *E. coli* M15 as recombinant His6-tagged proteins. In previous preliminary work,[14] SHMT was assayed as a catalyst in aldol additions of glycine to *N*-Cbz-3-aminopropanal, *N*-(*R*)-Cbz-alaninal, and benzyloxyacetaldehyde (Cbz = benzyloxycarbonyl). These aldehydes were accepted as substrates with 24–27% conversions to product and 60–40% diasteromeric excesses, which were rather unpractical for synthetic purposes.[14] This low stereoselectivity at the β-carbon atom is commonly found in most of threonine aldolases that have been investigated.[3,6,15]

In pursuit of a better enzymatic reaction performance and to further exploit the synthetic utility of these enzymes, we investigated the effect of reaction variables, such as temperature, reaction media, and glycine concentration on the reaction conversion and diasteromeric ratio. Aldehydes, such as *N*-(*R*)-, *N*-(*S*)-Cbz-alaninal, *N*-Cbz-3-aminopropanal, *N*-Cbz-2-aminoethanal (*N*-Cbz-glycinal), and benzyloxyacetaldehyde were assayed as acceptor substrates. The products that were obtained have four functionalities of utmost interest, and which could be applied towards the synthesis of intermediates of statine derivatives,[13,16] protease inhibitors,[17] antivirals (i.e., HIV),[18] and peptide mimetics,[19] among other bioactive compounds. For instance, the aldol addition of glycine to *N*-Cbz-3-aminopropanal furnishes a stereoisomeric precursor of β-hydroxyornithine, which is a relevant building block for the β-lactamase inhibitor clavulanic acid and the antibiotic and anticancer agent acivicin.[20]

Results and Discussion

Influence of reaction conditions on the SHMT- and LTA-catalyzed aldol additions: We selected the aldol addition of glycine to *N*-(*R*)-Cbz-alaninal (Scheme 1) in emulsion,[21,22,23] which is catalyzed by SHMT and LTA aldolases at 35 and 25 °C, respectively, as a model for our initial investigation. The reaction temperatures were selected according to the activity–temperature profiles that were obtained in previous studies for both aldolases (see the Supporting Information).[14,24]



Scheme 1. SHMT- and LTA-catalyzed aldol additions of glycine to (*R*)-*N*-Cbz-alaninal.

Analysis of the crude reaction mixtures by HPLC revealed the presence of two new peaks with close retention times; these were assigned unambiguously to the L-*threo* or L-*erythro* diastereomers by NMR spectroscopy (see herein and the supporting information). Inspection of the reaction progress curves revealed that in both SHMT and LTA catalysis, the L-*erythro* diastereomer was kinetically preferred. Then, after a prolonged reaction time, it was converted to the more stable L-*threo* diastereomer, which is an equilibrium product (Figure 1). For LTA catalysis, the reaction under the selected conditions was too fast to detect the interconversion point and a quasi-equilibrium situation was already attained after short reaction times (Figure 1b). It is worth noting that the diastereoselectivity for LTA was good (L-*threo*/L-*erythro*, 86:14), although in both cases the reaction conversion was rather low (ca. 20 and 40%, respectively). As Figure 1 shows after 20–24 h, no significant changes were observed in the yield and stereoselectivity; this indicates an equilibrium state.

It has been reported that lowering the reaction temperature might favor the kinetic product and improve the diastereoselectivity.[3,12] Moreover, this also might affect the equilibrium position of the reaction, and, therefore, the final conversions to aldol adducts. Hence, the model aldol addition that is catalyzed by SHMT and LTA was studied at 4, 10, and 25 °C in both emulsions and a conventional DMF/water 1:4 cosolvent mixture.

In the case of SHMT, by reducing the reaction temperature from 35 to 4 °C, the kinetic product, the L-*erythro* diastereomer, was strongly favored, whereas the L-*threo* diastereomer was extremely minimized; one single diastereomer was detected (Figure 2). Moreover, the conversion rose remarkably from 20 to 70–75% probably because of the alteration in the equilibrium position in favor of the aldol adduct. Thus, for the aldol addition of glycine to *N*-(*R*)-Cbz-alaninal, SHMT at either 4 °C in emulsion or at 10 °C in DMF/H_2O 1:4 provides complete stereocontrol and good product conversions. These observations are in a good agreement with the biochemical characterization data of SHMT.[14] The activity–temperature profile revealed that the retroaldol activity of SHMT is undetectable at 4 °C and negligible at 20 °C, whereas at 4 °C significant synthetic activity towards the L-*erythro* isomer was achieved (Figure 2). Moreover, the retroaldol activity of SHMT for L-*allo*-threonine is two orders of magnitude higher than that for L-threonine (V_{max}/K_m=7.8 vs. 0.061 $U\,mg^{-1}\,mM^{-1}$).[14] Hence a

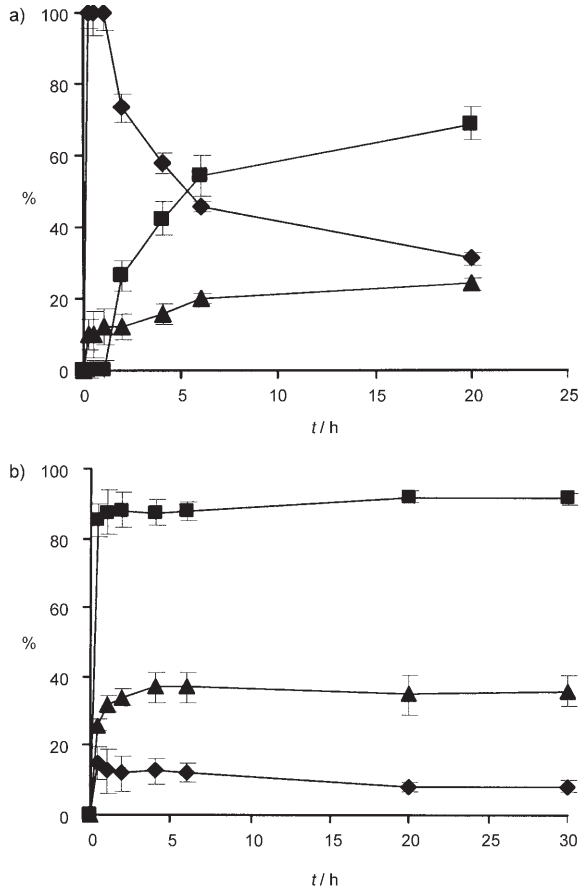


Figure 1. Time-course profiles for the aldol addition of glycine to **1** catalyzed by SHMT at 35 °C (a) and LTA at 25 °C (b) in emulsions. ◆: L-*erythro* isomer, ■: L-*threo* isomer, ▲: the reaction conversion to both products. The data that is shown corresponds to the mean values that were obtained with three emulsion formulations: $H_2O/C_{14}E_4$/tetradecane, $H_2O/C_{14}E_4$/hexadecane, and $H_2O/C_{14}E_4$/squalane always in a ratio of 90:4:6 (w/w/w), in which $C_{14}E_4$ is a technical grade tetra(ethyleneglycol)tetradecyl ether surfactant ($C_{14}H_{29}(OCH_2CH_2)_4OH$), with an average of 4 moles of ethylene oxide per surfactant molecule.[22,23] Experiments were performed in triplicate and the estimated standard error for each point is represented by the corresponding error bars. For the rest of the reaction conditions, see the Experimental Section.

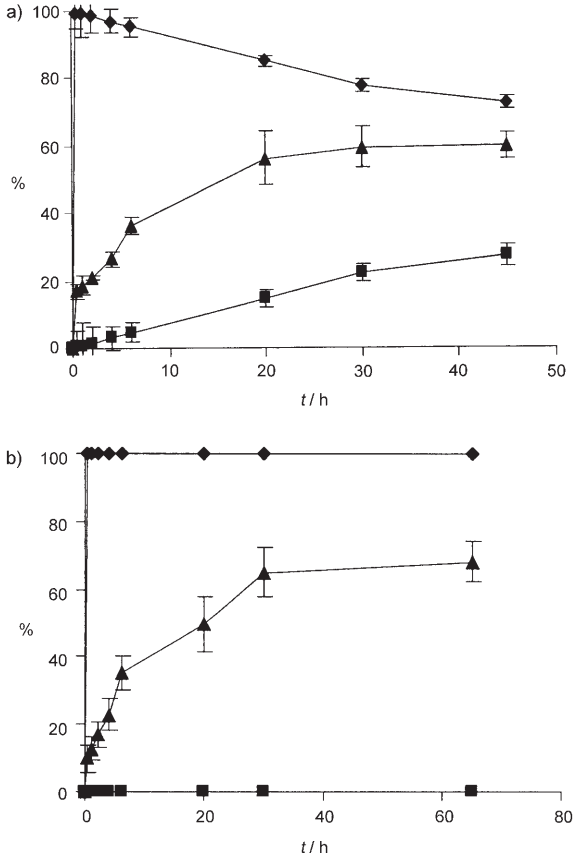


Figure 2. Time-course profiles for the aldol addition of glycine to **1** catalyzed by SHMT at 25 (a) and 4 °C (b) in emulsion systems. Similar profiles to those shown in (b) were obtained in DMF/buffer at 10 and 4 °C. For the reaction conditions, see the Experimental Section. ◆: L-*erythro* isomer, ■: L-*threo* isomer, ▲: the reaction conversion to both products. Experiments were performed in triplicate and the estimated standard error for each point is represented by the corresponding error bars.

plausible explanation for the high stereoselectivity of SHMT at 4 °C lies in both the reduced retroaldol activities at low temperatures and a clear kinetic preference for the L-*erythro* configuration. Upon increasing the temperature, the retroaldol activity increased and, consequently, a decrease in the diasteromeric ratio in favor of the equilibrium product, which was cleaved at a lower rate, was observed. The results that were obtained for SHMT were in good agreement with those that were found in the aldol addition of glycine to benzaldehyde catalyzed by DTA from *Alcaligenes xylosoxidans*.[12]

For the LTA catalyst, the kinetic product was rapidly consumed at any temperature that was assayed, and always yielded L-*threo* as the major diastereomer (L-*threo*/L-*erythro* 86:14; Figure 3). The reaction conversion to product improved to 60% in DMF/H_2O 1:4 at either 10 or 4 °C, which were the best reaction conditions for this particular example (Figure 3). Reactions that were conducted at 0 °C resulted in impractical synthetic rates, and time profiles that were similar to those at 4 °C. The activity–temperature profile of LTA revealed significant retroaldol activity at 20 °C (2.0 vs. 0.1 $\mu M\,min^{-1}$ for SHMT; see the Supporting Information) and detectable activity at 4 °C (0.5 $\mu M\,min^{-1}$). The retroaldol activity of LTA for L-*allo*-threonine is also two orders of magnitude higher than that for L-threonine ($V_{max}/K_m = 248$ vs. 1.5 $U\,mg^{-1}\,mM^{-1}$; Supporting Information); this reflects the preference for the L-*erythro* aldol adduct as the kinetic product.[24] Hence, it is likely that the temperature profile can help us to understand the different behavior of both aldolases, and that the reaction temperature could play a critical role in controlling their stereoselectivities. Most interestingly, SHMT and LTA turned out to be stereocomplementary biocatalysts for the aldol addition of glycine to *N*-(*R*)-Cbz-alaninal with good product conversions.

Synthetic applications of the SHMT and LTA catalysts: The results that were obtained thus far prompted us to establish

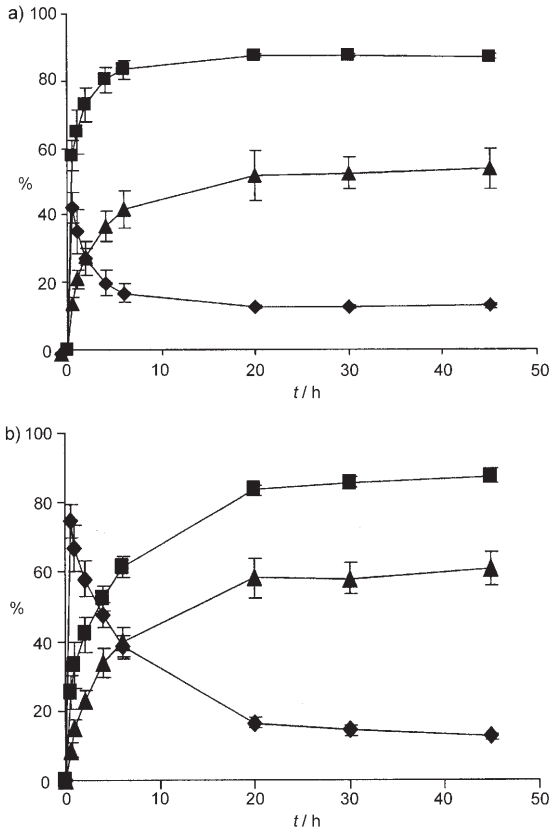
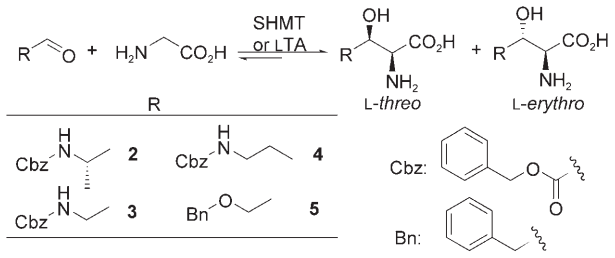


Figure 3. Time-course profiles for the aldol addition of glycine to **1** catalyzed by LTA in emulsion (a) and DMF/H_2O 4:1 (b) at 4°C. Similar profiles were monitored at 10°C. For the reaction conditions see the Experimental Section. ◆: L-*erythro* isomer, ■: L-*threo* isomer, ▲: the reaction conversion to products. Experiments were performed in triplicate and the estimated standard error for each point is represented by the corresponding error bars.

whether the reaction conditions could have a similar effect on a range of selected acceptor aldehydes, such as *N*-(*S*)-Cbz-alaninal (**2**), *N*-Cbz-glycinal (**3**), *N*-Cbz-3-aminopropanal (**4**), and benzyloxyacetaldehyde (**5**; Scheme 2). In this case, the concentration of glycine was also included in the study in addition to the reaction media and temperature. It has been reported that an excess of glycine helps in shifting the equilibrium to the aldol adducts,[3] but might complicate purification procedures. Thus, the stereoselectivities and conversions of the SHMT- and LTA-catalyzed aldol additions of glycine to each selected aldehyde were studied as a function of the reaction temperature (4 and 25°C), reaction media (emulsion and DMF/H2O 1:4) and aldehyde/glycine ratio (1:1.2, 1:2, and 1:5).



Scheme 2. SHMT- and LTA-catalyzed aldol additions of glycine to *N*-Cbz-aminoaldehydes and benzyloxyacetaldehyde.

Thus, experiments under 12 defined conditions were conducted for each aldehyde by using either SHMT or LTA catalysts (24 experiments per aldehyde) and the conversions to products and diastereoselectivities of each reaction were monitored by HPLC (representations of both the conversion to product and diasteromeric ratio versus reaction time that were obtained for aldehydes **2**, **3**, and **5** are reported in the Supporting Information). The stereochemistry of the L-*erythro* and L-*threo* isomers was unequivocally assigned by NMR spectroscopy on the isolated products upon transformation to their 2-oxazolidone derivatives (see further discussions in the text and the Supporting Information).

Aldehydes **2**–**5** were found to be acceptable as substrates and produced the corresponding *N*-Cbz-ω-amino- and *N*-Cbz-ω-hydroxybenzyl-β-hydroxy-α-amino acid derivatives. Table 1 shows the conversions and diasteromeric ratios obtained after each change in reaction conditions for aldehydes **2** and **5** as significant examples. The outcome of this study revealed that some general trends can be drawn on the stereoselectivity and conversion as a function of the reaction parameters, but the best synthetic conditions depended on each acceptor aldehyde.

Stereoselectivity: Time-course profiles for the acceptor aldehydes that were studied were rather similar to those obtained with the model aldehyde (*R*)-*N*-Cbz-alaninal (**1**). In all cases, the kinetic product of the enzymatic aldol addition was the L-*erythro* isomer, whereas the thermodynamic product was the L-*threo* isomer. Overall, reactions that were conducted in emulsion favored the formation of the L-*threo* isomer. This might be explained by the fact that reactions were faster in emulsion than in DMF/H_2O 1:4, which is probably due to the inactivation of the biocatalyst by DMF, which enables rapid L-*erythro* to L-*threo* interconversion (Figure 3). Furthermore, the solvation effect, that is, the substrate and product partition in the different phases of the emulsion media, might be considered to influence both reaction rate and diasteromeric distribution.

Temperature was also a useful variable for controlling the stereochemical outcome of the reactions with other aldehydes. As expected, stereoselectivity towards the kinetic isomer (i.e., L-*erythro*) increased at 4°C, whereas the contrary was true at room temperature. Overall, at 4°C, SHMT provides the L-*erythro* isomer with high diasteromeric ratios (91:9, 100:0), whereas at 25°C, LTA gave the L-*threo* isomer with moderate diastereoselection (18:82, 40:60). Raising the temperatures above 25°C to force the formation of L-*threo* isomer with either LTA and SHMT leads to a strong decrease in the reaction conversion; this hampers their synthetic applicability at a preparative level (results not shown).[14]

Glycine concentration also influenced the stereochemical outcome of the reactions because it is involved in shifting

Table 1. Aldol addition of glycine to **2** and **5** catalyzed by SHMT and LTA. Substrate conversion to product and diasteromeric ratio after 20 h as a function of the reaction conditions tested.[a]

T [°C]	Conditions[b] concn of glycine [mM]	Medium	Aldehyde	SHMT		LTA	
				Conv. [%][c]	dr *erythro*/*threo*	Conv. [%][c]	dr *erythro*/*threo*
25	70	cosol.	**2**	19	21:79	20	12:88
25	70	emul.	**2**	18	20:80	20	9:91
25	140	cosol.	**2**	54	20:80	26	43:57
25	140	emul.	**2**	41	16:84	41	10:90
25	280	cosol.	**2**	63	23:74	21	27:73
25	280	emul.	**2**	41	17:83	50	13:87
4	70	cosol.	**2**	18	80:20	33	40:60
4	70	emul.	**2**	17	85:15	24	20:80
4	140	cosol.	**2**	35	97:3	25	50:50
4	140	emul.	**2**	30	76:24	26	24:76
4	280	cosol.	**2**	32	99:1	29	55:45
4	280	emul.	**2**	36	84:16	38	28:72
25	70	cosol.	**5**	80	66:33	84	56:44
25	70	emul.	**5**	90	50:50	80	37:63
25	140	cosol.	**5**	45	43:57	35	53:47
25	140	emul.	**5**	68	41:59	55	40:60
25	280	cosol.	**5**	50	46:54	44	60:40
25	280	emul.	**5**	65	40:60	40	40:60
4	70	cosol.	**5**	55	97:3	33	82:18
4	70	emul.	**5**	63	91:9	33	60:40
4	140	cosol.	**5**	45	95:5	20	75:25
4	140	emul.	**5**	44	87:13	20	45:55
4	280	cosol.	**5**	50	96:4	22	76:24
4	280	emul.	**5**	57	88:12	33	54:46

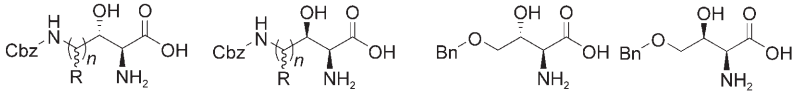
[a] Time-course plots of the conversion to product and the *erythro*/*threo* ratio for the aldol addition to aldehydes **2**, **3**, and **5** are included in the Supporting Information. [b] Cosol.: DMF/buffer 1:4; emul.: $H_2O/C_{14}E_4$/hexadecane 90/4/6 (wt%). [c] Molar percentage conversions to the aldol adducts with respect to the starting aldehyde concentration were determined by HPLC from the crude reaction mixtures by using purified standards.

the equilibrium towards the condensation products.[12,25] The best stereoselectivity was obtained at high glycine concentrations. An excess of glycine appears to inhibit the formation of the L-*threo* isomer, and shifts the diasteromeric interconversion point towards higher reaction times (Figure 4).

Comparing the performance of both aldolases for the reactions studied, SHMT from *S. thermophilus* was by far more stereoselective than LTA from *E. coli*, and thus constitutes a promising and robust biocatalyst for aldol additions of glycine.

Substrate conversion to product: Overall, the best conversions to aldol adducts were achieved at 4°C and/or at high glycine concentration (Figure 4), but they depended mostly on the aldehyde acceptor. Importantly, the conditions for increasing the substrate conversion were fully compatible with the ones that achieved high stereoselectivity. Concerning the reaction media, the best conversions with SHMT were achieved in DMF/H_2O 1:4, whereas emulsion was the best medium for the LTA-catalyzed reactions.

Table 2. Synthesis of *N*-Cbz-ω-amino- and ω-benzyloxy-β-hydroxy-α-amino acid derivatives by aldol additions of glycine to *N*-Cbz-aminoaldehydes and benzyloxyacetaldehyde catalyzed by SHMT and LTA.



6a: *n*=1, R: CH_3(*R*) **6b**
7a: *n*=1, R: CH_3(*S*) **7b**
8a: *n*=1, R: H **8b**
9a: *n*=2, R: H **9b**
10a **10b**

Acceptor aldehyde	Aldolase ($U\,mL^{-1}$)	Product	Conv. [%] (*t* [h])	*T* [°C]	Gly [mM][c] ([mL])[d]	Isolated yield [%][e]	dr L-*erythro*/ L-*threo*
1	SHMT (8)	**6a**	60 (30)[a]	4	70 (34)	30	100:0
1	LTA (12)	**6a/6b**	40 (20)[a]	4	70 (37)	20	16:84
2	SHMT (8)	**7a**	48 (20)[a]	4	280 (58)	30	100:0
2	LTA (12)	**7a/7b**	54 (20)[b]	25	140 (42)	27	18:82
3	SHMT (8)	**8a/8b**	35 (20)[a]	4	70 (39)	13	86:14
3	LTA (12)	**8a/8b**	60 (20)[b]	25	140 (54)	18	30:70
4	SHMT (6)	**9a**	20 (8)[a]	4	70 (25)	3	100:0
4	SHMT (8)	**9a/9b**	34 (25)[a]	25	70 (36)	10	50:50
4	LTA (12)	**9a/9b**	49 (25)[b]	25	70 (42)	11	50:50
5	SHMT (8)	**10a**	68 (20)[a]	4	70 (37)	40	97:3
5	LTA (12)	**10a/10b**	45 (20)[b]	25	140 (30)	30	40:60

[a] DMF/H_2O 1:4 (v/v). [b] High H_2O content emulsions: H_2O/hexadecane/$C_{14}E_4$ 90/6/4 (wt%). [c] [Aldehyde] = 60 mM [d] Reaction volume. [e] Purification procedures were not optimized.

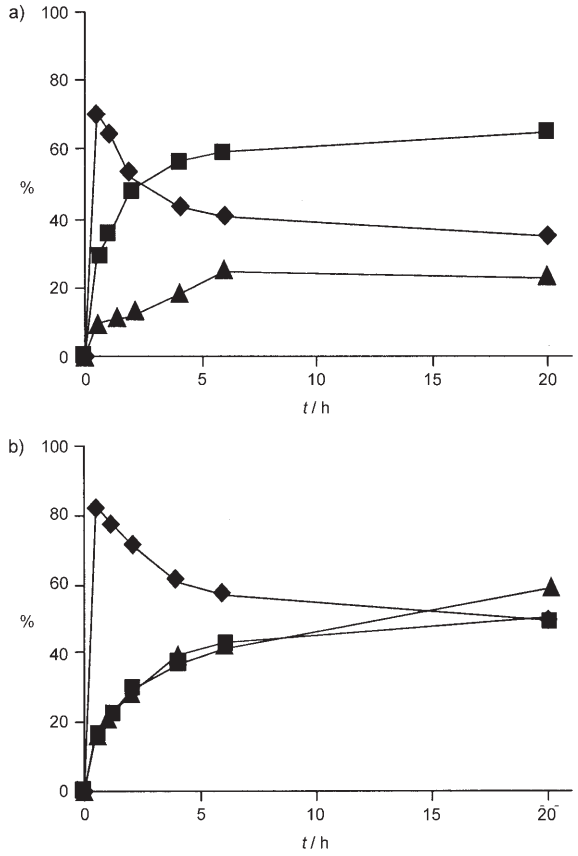
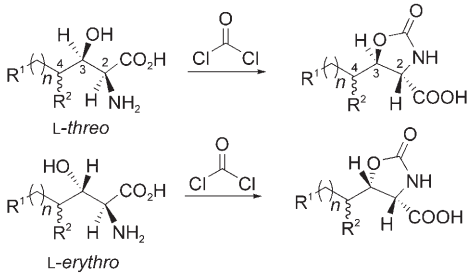


Figure 4. LTA-catalyzed aldol addition of glycine to *N*-Cbz-glycinal (**3**) at 70 (a) and 280 mM (b) glycine concentration at 25 °C in emulsion. ■: L-*threo* isomer, ◆: L-*erythro* isomer, ▲: the reaction conversion to both products.

Scale-up: Syntheses of the β-hydroxyamino acid derivatives were scaled up to 100–300 mg of aldol adduct. The reaction conditions for each aldehyde were selected to obtain high product yields of both the L-*threo* and L-*erythro* isomers, and, therefore, to generate the maximum configuration diversity of the aldol products (Table 2). Moderate to excellent conversions (30–70 %) and diasteromeric ratios (60:40–100:0) were achieved. Interestingly, LTA and SHMT were nonselective towards the (*R*)-*N*-Cbz- and (*S*)-*N*-Cbz-alaninal, with both aldehydes yielding good conversions and stereoselectivity (Table 1). However, (*S*)-*N*-Cbz-alaninal, needed four times the glycine concentration than (*R*)-*N*-Cbz-alaninal to achieve similar results. When both aldehydes were tested with 70 mM glycine at 4 °C, the reaction conversion ratios (*R*/*S*) were 80:18 and 62:33, respectively; the equilibrium conversion to product was clearly in favor of the *R* enantiomer. *N*-Cbz-3-aminopropanal gave an L-*erythro*/L-*threo* ratio of 1:1 with either SHMT or LTA at conversions of around 34 and 50 %, respectively. Excellent diasteromeric excess for the L-*erythro* isomer was obtained, but only under kinetic conditions with conversions of around 20 %. It is noteworthy that both SHMT and LTA from *Candida humicola* gave the same L-*erythro* stereochemical preference for the aldol addition of glycine to benzyloxyacetaldehyde.[15]

Assignment of the stereochemistry of the L-*threo*/L-*erythro* products. Formation of 2-oxazolidinone derivatives: Absolute configurations were obtained based on the premise that L- and D-threonine aldolases are highly selective for the stereochemistry at the C-α carbon atom.[2,6,12] Moreover, it has been reported that SHMT did not hydrolyze either D-threonine or D-*allo*-threonine.[14] The assignment of the relative stereochemistry of both isomers was performed by NMR spectroscopy. It has been shown that the coupling constant ($^3J_{HH}$) between H1 and H2 for the L-*threo* isomer is slightly larger than for the L-*erythro* isomer. However, according to Futagawa et al.[26] the contrary might be true. Hence, neither the $^3J_{H2H3}$ coupling constant nor the H1 chemical shift from the aldol adducts of this study allowed us to unequivocally assign the signals to the L-*threo* and L-*erythro* isomers. This was because the experimental $^3J_{H2H3}$ coupling constant differences can be attributed to the presence of variable and alternatively weighted conformations that arise from relatively fast C–C bond rotations.[26,27] Hence, the aldol adducts that were obtained by both threonine aldolases were converted to the 2-oxazolidone derivatives (Table 3), which eliminates the free C2–C3 rotation. Then, the assignment that was based upon both the $^3J_{H2H3}$ coupling constants and their chemical shifts was unequivocal.[6,26] As expected, the $^3J_{H2H3}$ coupling constant for the L-*erythro* isomer was larger than for the L-*threo* isomer, and very characteristic down-

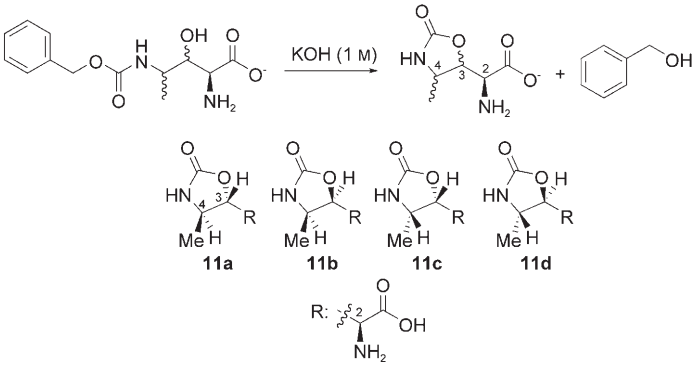
Table 3. The most relevant ^{1}H and ^{13}C NMR spectroscopic chemical shifts [ppm] and H,H-coupling constants [Hz] in [D$_6$]DMSO of stereoisomeric 2-oxazolidone derivatives from compounds **6a**–**10b**.



			L-*Threo*					L-*Erythro*				
n	R_1	R_2	δ(H_2)	δ(H_3)	$^3J_{H2H3}$	δ(C_4)	δ(CO_2H)	δ(H_2)	δ(H_3)	$^3J_{H2H3}$	δ(C_4)	δ(CO_2H)
0	Cbz-NH	CH_3 (*R*)	4.14	4.38	3.8	48.7	172.4	4.25	4.49	7.7	46.6	171.2
0	Cbz-NH	CH_3 (*S*)	4.11	4.45	4.3	49.1	172.4	4.35	4.60	8.7	46.6	171.4
0	Cbz-NH	H	4.09	4.50	4.4	43.6	172.1	4.38	4.72	8.8	41.3	171.0
1	Cbz-NH	H	4.01	4.50	4.5	35.8	172.0	4.28	4.70	8.4	31.3	171.4
0	BnO	H	4.14	4.67	5.0	70.3	172.1	4.39	4.91	9.2	68.0	171.1

field shift effects were observed for the H2 and H3 protons, whereas upfield effects for C4 and the COOH chemical shifts were also evident due to the gamma-gauche effect (Table 3).

The reaction of aldol adducts with phosgene for the preparation of 2-oxazolidinone derivatives requires a base. It was found that sodium bicarbonate was the best suited for this purpose. By treating the *N*-Cbz-γ-amino-β-hydroxy-α-amino acid derivatives with potassium hydroxide (1 M), 2-oxazolidinone derivatives were formed in quantitative yield (Scheme 3). This is probably explained by the attack



Scheme 3. 2-Oxazolidone derivatives (**11a–11d**); formation from compounds **6a–7b**.

of the β-hydroxylate on the carbonyl function of the urethane group with elimination of benzyl alcohol. The reaction was exemplified at the analytical level by using the diastereomers of 2-amino-4-(benzyloxycarbonylamino)-3-hydroxypentanoate aldol adducts. The concerted analysis of ^{1}H and ^{13}C chemical shifts, the observed gamma effects, mainly on δ(H3) (i.e., about $\delta = 4.8$ for the *cis* and 4.5 ppm for the *anti* configuration) and $\delta(CH_3)$ (around $\delta = 21$ for the *cis* and 16 ppm for the *anti* configuration), and NOE enhancements (see the Supporting Information) confirmed the assignment of the relative configurations of compounds (**11a–11d**; Scheme 3).

Conclusion

Glycine-dependent aldolases are emerging as useful tools for the synthesis of polyfunctional molecules, such as β-hydroxy-α-amino acid derivatives. However, in many instances, the narrow substrate tolerance, low yield, and sometimes poor steroselectivity are important issues that must be addressed.

From the synthetic data that was obtained in this work, it may be concluded that medium engineering is a useful way to improve the yield and stereoselectivity of SHMT and LTA aldolases, and, therefore, it might be possible to achieve the synthesis of both the L-*threo* and L-*erythro* diastereomers of each aldehyde. Temperature, glycine excess, and reaction media (i.e., emulsion or DMF/H_2O cosolvent systems) are important parameters for controlling both conversions to product and stereoselectivities. In particular, temperature was an important parameter when working with SHMT. Thus, for glycine-dependent aldolases with similar temperature profiles, it might be synthetically useful to work at low temperatures because the retroaldol activity is strongly inhibited, whereas a high synthetic capacity is maintained; this makes the reaction virtually irreversible. This strategy might be extended to thermophillic aldolases, and work is currently underway in our lab in this direction. Overall, at low temperatures, both yield and reaction conversion improved; a similar effect was also observed upon increasing the excess of glycine. Reactions were normally faster in emulsion than in DMF/H_2O 1:4. Therefore, the latter was the best for the SHMT catalyst, whereas emulsions at 25 °C were the conditions of choice for LTA in which high reaction rates were needed to accumulate the L-*threo* isomer in the reaction medium. Under the selected reaction conditions, the aldol additions furnished the desired products in yields that are useful on a preparative scale. However, similar to other syntheses with these aldolases,[3,6] the isolated yields are still low from the perspective of a potential industrial application. Therefore, there remain challenges in regards to improving both the conversion to product of the enzymatic aldol reaction and the purification procedures.

By considering the aldol and retroaldol activities[14,24] of the SHMT and LTA catalysts that are used in this work, it can be concluded that both aldolases are low-specific L-*allo*-threonine aldolases.

Both medium and enzyme-engineering methods might be an excellent complement to alter the stereochemical outcome of aldolases.[28] Modification of the existing aldolases and related enzymes is also of paramount importance to alter substrate tolerance, and in turn, the enzymes catalytic properties.[29] Work is currently in progress in our lab to elucidate the three-dimensional structure of SHMT with the aim of modifying it to improve its tolerance towards the widest structural diversity of aldehyde acceptors.

From the synthetic point of view, we have found that treatment of *N*-Cbz-γ-amino-β-hydroxy-α-amino acid derivatives with potassium hydroxide (1 M) caused the spontaneous formation of 2-oxazolidinone derivatives between the γ-amino and β-hydroxy groups in quantitative yield. This reaction might be useful for further chemical manipulations of the products.

Experimental Section

Materials: L-Threonine, L-*allo*-threonine, and yeast alcohol dehydrogenase (ADH) were purchased from Sigma. Pyridoxal-5-phophate, glycine, and benzyloxyacetaldehyde (**1**) were purchased from Aldrich. *N*-Benzyloxycarbonyl-3-amino propanal (*N*-Cbz-3-aminopropanal), *N*-benzyloxycarbonyl-2-aminoethanal (*N*-Cbz-glycinal), and (*R*)- and (*S*)-*N*-benzyloxycarbonyl-alaninal (**3**) were synthesized in our lab by previously described procedures.[22,30] Serine hydroxymethyl transferase from *S. thermophilus* (SHMT) and L-threonine aldolase from *E. coli* (LTA) were pro-

duced and purified in our lab and in a fermentation plant facility by following previously described methodologies.[14] All other chemicals and reagents that were used in this work were of analytical grade.

Enzyme activity: Threonine aldolase activity was measured spectrophotometrically as described in a previous work.[14] One unit of TA activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of acetaldehyde (1 μmol of NADH oxidized) per minute at 37 °C.

Aldol additions catalyzed by SHMT and LTA

Enzymatic aldol additions in emulsions: Aldol additions of glycine to aldehydes in emulsion systems were conducted in 10 mL screw-caped test tubes as follows:[22] The aldehyde (0.15 mmol), oil (0.3 g), and surfactant (0.2 g) were mixed with a vortex mixer for 15 s (2400 rpm). Then, a glycine solution (2 mL; 0.175, 0.350, or 0.700 mmol, depending on the experiment) at pH 6.5 for SHMT or pH 8 for LTA was added dropwise while stirring at 4, 10, or 25 °C with a vortex mixer. This was followed by the addition of a solution of pyridoxal-5-phosphate (190 μL, 0.3 μmol). Finally, the reaction was started by adding the enzyme (20 U SHMT or 30 U LTA). The final reaction volume was 2.5 mL. The test tubes were placed on a horizontal shaking bath (100 rpm) at constant temperature. The reactions were followed by HPLC until the peak of the product reached a maximum.

Enzymatic aldol additions in DMF/H_2O 1:4: The aldehyde (0.15 mmol) was dissolved in DMF (0.5 mL). Then, the glycine solution (2 mL; 0.175, 0.350, or 0.700 mmol, depending on the experiment) at pH 6.5 for SHMT or pH 8 for LTA was added while stirring at 4, 10, or 25 °C with a vortex mixer. This was followed by the addition of a solution of pyridoxal-5-phosphate (190 μL, 0.3 μmol). The rest of the experimental procedure was the same as above.

HPLC analyses: HPLC analyses were performed on a Lichrograph HPLC system (Merck, Germany) fitted with XBridge C18 5 μm (Waters). Samples (50 mg, for reactions in emulsions or 50 μL for reactions in DMF/H_2O 1:4) were withdrawn from the reaction medium, dissolved in methanol (1 mL), which contained HOAc (2.5% v/v) to stop any enzymatic reaction, and then subsequently analyzed by HPLC. The solvent system was as follows: solvent A: 0.1% (v/v) trifluoroacetic acid (TFA) in H_2O, solvent B: 0.095% (v/v) TFA in H_2O/CH_3CN 1:4. HPLC conditions for reaction monitoring with underivatized adducts were: gradient elution from 0 to 70% solvent B in 30 min was used; flow rate: 1 mL min^{-1}; detection at 215 nm. Retention factors for each compound are provided.

The L-*erythro*/L-*threo* aldol adducts from *N*-Cbz-glycinal (**3**) and *N*-Cbz-3-aminopropanal (**4**) could not be separated by HPLC under the conditions described above. Baseline separation of both diasteroisomers was accomplished after derivatization of the free α-amino function with a *tert*-butyloxycarbonyl group. Samples (50 mg) were withdrawn from the reaction medium and dissolved in di-*tert*-butyl dicarbonate solution (0.6 M; 200 μL). Then Et_3N (6 M; 50 μL) was added to this solution and the mixture was incubated for 1 h at 25 °C in a horizontal shaker. After this time, the total volume was transferred to an HPLC vial with MeOH/HOAc (250 μL, 1:0.025) and analyzed subsequently by HPLC. HPLC conditions were as follows: gradient elution from 10 to 80% solvent B in 35 min, the eluents and other conditions were as described above. Retention factors for each derivatized compound are provided.

Scale-up: Enzymatic reactions on the 100 mg scale were conducted in screw-capped flasks (250 mL), which were placed in a reciprocal shaker (50 rpm) and thermostated at the required temperature (25 or 4 °C). The procedure was similar to that described for the analytical reactions and substrate concentrations, temperature, enzyme units, and incubation times were selected based upon the acceptor aldehyde and are summarized in Table 1.

The enzymatic reactions were stopped by the addition of MeOH/HOAc 39:1 (v/v) and the crude was purified and filtrated through Celite/activated charcoal (95:5); the filtration cake was cleaned with MeOH. The filtrate was evaporated under vacuum to eliminate the MeOH. The residue that was obtained was dissolved in H_2O and the unreacted aldehyde and other hydrophobic impurities were extracted with EtOAc (3×150 mL). The aqueous layer that contained the product was freeze dried and then purified by semipreparative RP-HPLC on a Perkin–Elmer 250×25 mm column, filled with C_{18}, 5 μm-type stationary phase, by using the following procedure. First, the column was equilibrated with 0.1% (v/v) TFA in H_2O. Then, the sample was adjusted to pH 2–3 with TFA and loaded onto the column. The salts were eliminated by washing with of 0.1% (v/v) TFA in H_2O (100 mL). Then, the product was eluted with a gradient of 0.1% (v/v) TFA in CH_3CN (8 to 48% v/v in 30 min). The flow rate was 10 mL min^{-1}, and the products were detected at 215 nm. The pure fractions were pooled and lyophilized for further characterization by NMR spectroscopy.

NMR spectroscopy: ^{1}H (500.13 MHz) and ^{13}C (125.76 MHz) NMR spectra were recorded on an AVANCE 500 Bruker spectrometer equipped with a high-sensitivity cryogenically cooled triple-resonance TCI probehead for samples that were dissolved in [D_6]DMSO and CD_3OD solutions. Full structural and stereochemical characterization of all compounds was carried out with the aid of 2D COSY, NOESY, HSQC, and HMBC experiments and from NOE data that was obtained from selective 1D NOESY experiments that were recorded with a mixing time of 500 ms. The ^{13}C NMR spectroscopic peaks were assigned by HSQC and HMBC experiments. For complete signal assignments, see the Supporting Information.

(2*S*,3*R*,4*R*)-2-Amino-4-(benzyloxycarbonylamino)-3-hydroxypentanoic acid ammonium salt (6a): The title compound was obtained according to the general procedure. Yield: 170 mg (30%), 99% pure, as determined by HPLC; retention factor, $k=6.91$; $[\alpha]_D^{20}=-14.8$ ($c=0.49$ in DMSO); ^{1}H NMR (500.13 MHz, [D_6]DMSO): $\delta=7.34$ (s, 1H), 7.25 (d, $J=8.3$ Hz, 1H), 5.03 (s, 1H), 3.73 (s, 1H), 3.54 (dd, $J=8.5$ Hz, 1H), 3.02 (d, $J=8.5$ Hz, 1H), 1.12 ppm (d, $J=6.8$ Hz, 1H); ^{13}C NMR (125.76 MHz, [D_6]DMSO): $\delta=169.4$ (C1), 156.8 (C6), 136.9 (C *ipso*), 128.3 (C arom), 127.7 (C arom), 72.1 (C3), 65.5 (C7), 53.1 (C2), 47.6 (C4), 17.4 ppm (C5)

(2*S*,3*S*,4*R*)-2-Amino-4-(benzyloxycarbonylamino)-3-hydroxypentanoic acid ammonium salt (6b): The title compound was obtained according to the general procedure. Yield: 163 mg (20%); 99% pure and a mixture of diastereomers, as determined by HPLC; retention factor, $k=7.19$; $[\alpha]_D^{20}=-18.0$ ($c=0.47$ in DMSO); ^{1}H NMR (500.13 MHz, [D_6]DMSO): $\delta=7.42$ (d, $J=8.5$ Hz, 1H), 7.36 (m, 5H), 5.01 (s, 2H), 3.8 (dd, $J=7.5$ Hz, 1H), 3.62 (m, 1H), 3.18 (d, $J=4.0$ Hz, 1H), 1.10 (d, $J=6.6$ Hz, 1H), minor signals that corresponded to the L-*erythro* diastereomer: 3.56 (dd, $J=8.2$ Hz, 1H), 3.05 (d, $J=8.1$ Hz, 1H), 1.12 ppm (d, $J=6.8$ Hz, 1H); ^{13}C NMR (125.76 MHz, [D_6]DMSO): $\delta=168.9$ (C1), 155.9 (C6), 137.0 (C *ipso*), 128.0 (C arom), 127.7 (C arom), 72.0 (C3), 65.3 (C7), 55.6 (C2), 48.7 (C4), 16.6 (C5), minor signals that corresponded to the L-*erythro* diastereomer: 169.4 (C1), 156.8 (C6), 53.1 (C2), 47.6 (C4), 17.4 ppm (C5).

(2*S*,3*R*,4*S*)-2-Amino-4-(benzyloxycarbonylamino)-3-hydroxypentanoic acid ammonium salt (7a): The title compound was obtained according to the general procedure. Yield: 406 mg (29%), 99% pure, as determined by HPLC. Retention factor, $k=4.25$, $[\alpha]_D^{20}=+14.3$ ($c=0.56$ in DMSO); ^{1}H NMR (500.13 MHz, [D_6]DMSO): $\delta=7.88$ (d, $J=7.8$ Hz, 1H), 7.33 (s, 5H), 5.01 (s, 2H), 3.75 (s, 1H), 3.68 (t, $J=4.8$ Hz, 1H), 3.29 (d, $J=4.6$ Hz, 1H), 1.07 ppm (d, $J=4.6$ Hz, 3H); ^{13}C NMR (125.76 MHz, [D_6]DMSO): $\delta=168.6$ (C1), 155.6 (C6), 137.2 (C *ipso*), 128.2 (C arom), 127.6 (C arom), 71.9 (C3), 65.0 (C7), 54.2 (C2), 48.7 (C4), 16.1 ppm (C5).

(2*S*,3*S*,4*S*)-2-Amino-4-(benzyloxycarbonylamino)-3-hydroxypentanoic acid amonium salt (7b): The title compound was obtained according to the general procedure; yield: 266 mg (27%), 99% pure and a mixture of diastereomers, as determined by HPLC; retention factor, $k=4.39$; $[\alpha]_D^{20}=+5.4$ ($c=0.48$ in DMSO); ^{1}H NMR (500.13 MHz, [D_6]DMSO): $\delta=7.90$ (d, $J=7.61$ Hz, 5H), 7.34 (m, 1H), 7.23 (d, $J=8.4$ Hz, 1H), 5.01 (s, 2H), 3.81 (t, $J=5.2$ Hz, 1H), 3.76 (m, 1H), 3.22 (d, $J=5.1$ Hz, 1H), 1.08 (d, $J=6.7$ Hz, 3H), minor signals that corresponded to the L-*erythro* diastereomer: 7.90 (d, $J=7.61$ Hz, 1H), 3.68 (t, $J=4.9$ Hz, 1H), 3.27 (d, $J=5.0$ Hz, 1H), 1.06 ppm (d, $J=6.8$ Hz, 3H); ^{13}C NMR (125.76 MHz, [D_6]DMSO): $\delta=168.9$ (C1), 155.6 (C6), 137.1 (C quat), 128.3 (C arom), 127.6 (C arom), 71.0 (C3), 65.1 (C7), 54.8 (C2), 49.6 (C4), 16.6 (C5), minor signals that corresponded to the L-*erythro* diastereomer: 168.6 (C1), 137.2 (C *ipso*), 71.8 (C3), 65.0 (C7), 54.2 (C2), 48.7 (C4), 16.1 ppm (C5).

(2*S*,3*S*)-2-Amino-4-(benzyloxycarbonylamino)-3-hydroxybutanoic acid (8a): The title compound was obtained according to the general procedure. Yield: 117 mg (12%), 99% pure and a mixture of diastereomers, as determined by HPLC. Retention factor, k (*tert*-Boc derivative) = 7.92; $[\alpha]_D^{20} = +11.8$ ($c = 0.43$ in DMSO); ^{1}H NMR (500.13 MHz, [D$_6$]DMSO): $\delta = 7.35$ (s, 5H), 5.01 (s, 1H), 3.83 (dd, $J = 5.1$, 11.1 Hz, 1H), 3.21 (d, $J = 5.2$ Hz, 1H), 3.18 (m, 2H), minor signals that correspond to the L-*threo* diastereomer: 3.93 (s, 1H) 3.08 ppm (s, 1H); ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): $\delta = 168.1$ (C1), 156.4 (C5), 137.1 (C *ipso*), 128.3 (C arom), 127.7 (C arom), 68.5 (C3), 65.2 (C6), 55.8 (C2), 43.2 ppm (C4).

(2*S*,3*R*)-2-Amino-4-(benzyloxycarbonylamino)-3-hydroxybutanoic acid (8b): The title compound was obtained according to the general procedure. Yield: 232 mg (17%), 99% pure and a mixture of diastereomers, as determined by HPLC; retention factor, k (*tert*-Boc derivative) = 8.44; $[\alpha]_D^{20} = +13.5$ ($c = 0.57$ in DMSO); ^{1}H NMR (500.13 MHz, [D$_6$]DMSO): $\delta = 7.35$ (s, 5H), 5.01 (s, 1H), 3.95 (dd, $J = 6.6$, 10.7 Hz, 1H), 3.25 (d, $J = 4.93$ Hz, 1H), 3.09 ppm (s, 1H), other signals that corresponded to the L-*erythro* diastereomer: 3.85 (dd, $J = 5.2$, 11.2 Hz, 1H), 3.19 ppm (m, 3H); ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): $\delta = 168.$ 8 (C1), 156.3 (C5), 137.1 (C quat), 128.3 (C arom), 127.7 (C arom), 68.6 (C3), 66.3 (C6), 56.1 (C2), 44.2 ppm (C4), other signals that corresponded to the L-*erythro* diastereomer: 168.3 (C1), 156.5 (C5), 137.2 (C *ipso*), 128.3 (C arom), 127.7 (C arom), 68.6 (C3), 65.2 (C6), 56.0 (C2), 43.1 ppm (C4).

(2*S*,3*S*)-2-Amino-5-(benzyloxycarbonylamino)-3-hydroxypentanoic acid (9a) and (2S,3R)-2-Amino-5-(benzyloxycarbonylamino)-3-hydroxypentanoic acid (9b): The title compound was obtained according to the general procedure. Yield: 30 mg (as the pure L-*erythro* isomer, 3%), 83 mg (as a 1:1 L-*erythro*/L-*threo* mixture when using SHMT, 10%), and 109 mg (as a 1:1 L-*erythro*/L-*threo* mixture when using LTA, 11%), 99% pure and a mixture of diasteroisomers or a single isomer, as determined by HPLC; retention factor, k (*tert*-Boc derivative L-*erythro*) = 6.33, k (*tert*-Boc derivative L-*threo*) = 6.53; $[\alpha]_D^{20} = +0.8$ ($c = 0.17$ in DMSO; L-*erythro* isomer); ^{1}H NMR (500.13 MHz, [D$_6$]DMSO): L-*erythro* diastereomer: $\delta = 7.35$ (m, 1H), 5.00 (s, 1H), 3.81 (m, 1H), 3.14 (d, $J = 5.6$ Hz, 1H), 3.01 (m, 1H), 1.55 (m, 1H), 1.48 ppm (m, 1H); ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): L-*erythro* diastereomer: $\delta = 168.3$ (C1), 156.0 (C6), 137.2 (C *ipso*), 128.3 (C arom), 127.7 (C arom), 67.3 (C7), 65.0 (C3), 58.2 (C2), 37.6 (C5), 32.1 ppm (C4); ^{1}H NMR (500.13 MHz, [D$_6$]DMSO): L-*threo* diastereomer: $\delta = 7.35$ (m, 1H), 5.00 (s, 1H), 3.81 (m, 1H), 3.17 (m, 1H), 3.08 (d, $J = 4.8$ Hz, 1H), 1.67 (m, 1H), 1.48 ppm (m, 1H); ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): L-*threo* diastereomer: $\delta = 168.6$ (C1), 156.1 (C6), 137.3 (C *ipso*), 128.3 (C arom), 127.7 (Carom), 67.0 (C7), 65.1 (C3), 57.9 (C2), 37.1 (C5), 33.7 ppm (C4).

(2*S*,3*R*)-2-amino-4-(benzyloxy)-3-hydroxybutanoic acid (10a): The title compound was obtained according to the general procedure. Yield: 296 mg (38%), 99% pure and a mixture of diastereomers, as determined by HPLC; retention factor, $k = 3.32$, $[\alpha]_D^{20} = +13.4$ ($c = 0.50$ in DMSO); ^{1}H NMR (500.13 MHz, [D$_6$]DMSO): $\delta = 7.34$ (m, 5H), 7.29 (m, 1H), 4.49 (s, 1H), 3.99 (dd, $J = 4.9$, 10.3 Hz, 1H), 3.56 (A of an AB system, $J = 4.3$, 10.1 Hz, 1H), 3.50 (B of an AB system, $J = 6.2$, 10.1 Hz, 1H), 3.41 ppm (d, $J = 4.7$ Hz, 1H); ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): $\delta = 168.3$ (TFAC), 168.2 (C1), 138.4 (C *ipso*), 128.1 (C arom), 127.4 (C arom), 72.3 (C5), 71.3 (C4), 68.6 (C3), 55.5 ppm (C2).

(2*S*,3*S*)-2-Amino-4-(benzyloxy)-3-hydroxybutanoic acid (10b): The title compound was obtained according to the general procedure. Yield: 254 mg (37%), 99% pure and a mixture of diastereomers, as determined by HPLC; retention factor, $k = 3.50$, $[\alpha]_D^{20} = +15.0$ ($c = 0.60$ in DMSO); ^{1}H NMR (500.13 MHz, [D$_6$]DMSO): $\delta = 7.34$ (m, 1H), 7.28 (m, 1H), 4.49 (s, 1H), 4.13 (dd, $J = 6.1$, 9.8 Hz, 1H),) 3.47 (m, 2H), 3.24 ppm (d, $J = 3.6$ Hz, 1H), other signals that correspond to the L-*erythro* diastereomer: 4.50 (s, 1H) 3.96 (dd, $J = 5.7$, 9.8 Hz, 1H), 3.53 (m, 2H), 3.28 ppm (d, $J = 5.5$ Hz, 1H); ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): $\delta = 168.7$ (C1), 138.5 (C quat), 128.2 (C arom), 127.4 (C arom), 72.2 (C5), 71.8 (C4), 67.7 (C3), 55.25 ppm (C2), other signals that correspond to the L-*erythro* diastereomer: 168.3 (C1), 138.4 (C *ipso*), 72.4 (C5), 71.6 (C4), 67.7 (C3), 55.3 ppm (C2).

2-Oxazolidinone derivatives: The corresponding pure aldol adduct (10 mg) was dissolved in distilled H_2O (5 mL). Then, $NaHCO_3$ (500 mg) was added. After 15 min, a 20% solution of phosgene in toluene (1.5 mL) was added dropwise while stirring. The reaction was monitored by HPLC. When the starting material was no longer detected, the reaction was stopped by the addition of 5% (v/v) HCl until pH 2–3 was reached. The mixture was successively extracted with EtOAc (3 mL), and then the organic layers were combined and washed with brine (3 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. NMR spectra of the residue were recorded without any further purification. For NMR data and spectra see the text and Supporting Information.

2-Oxazolidinone derivatives (11a–11d): The aldol adduct (10 mg) was dissolved in a solution of 1 M KOH solution (500 μL) and the reaction was followed by HPLC until the peak of the starting material was no longer detected and the benzyl alcohol peak had reached a maximum area. Then, 5% HCl was added to the mixture until pH 2 was reached. The aqueous phase was extracted with EtOAc and lyophilized. The solid that was obtained was suspended in DMSO, the insoluble material was filtered off, and the filtrate was lyophilized. NMR spectra of the residue were recorded without any further purification. For NMR data and spectra see the text and Supporting Information.

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