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ARTICLE TYPE

Expanding Dynamic Kinetic Protocols: Transaminase-Catalyzed Synthesis of α-Substituted β-Amino Ester Derivatives

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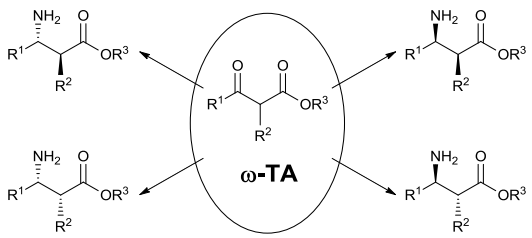
Several α-alkylated β-amino esters have been obtained *via* DKR processes employing a kit of transaminases and isopropylamine as amino donor in aqueous medium at mild conditions. Thus, while acyclic α-alkyl-β-keto esters afforded excellent conversions and enantioselectivities, although usually low diastereoselectivities, with more constrained cyclic β-keto esters high to excellent inductions were obtained.

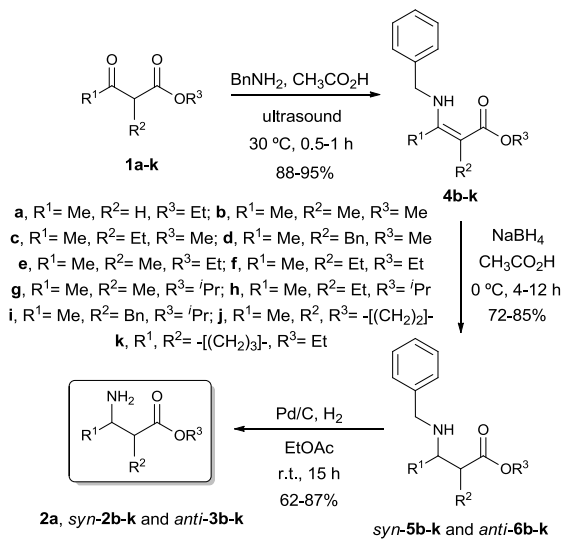
The development of enzymatic strategies that enable access to 100% theoretical yield of stereoisomerically enriched compounds starting from easily available racemic or prochiral derivatives, has recently attracted many efforts due to the simpler isolation and purification techniques required.[1] In this sense, recent advances achieved in dynamic systems to get access to enantio- or diastereomerically pure compounds have been described. Thus, performing a kinetic resolution on a substrate which simultaneously can undergo racemization has led to efficient dynamic kinetic resolutions (DKRs).[2] While this methodology has extensively been employed with hydrolases (usually in combination with a metal-based racemization)[3] and oxidoreductases (often combined with a racemization in basic conditions),[4] for other enzymes it has been scarcely exploited.

For instance, with ω-transaminases (ω-TAs, EC 2.6.1.x),[5] one of the most promising biocatalysts applied to the synthesis of chiral amines, it is remarkable that only one example described by Kroutil and co-workers can be recognized as a dynamic kinetic resolution.[6] In this contribution, the authors proposed the DKR of an α-chiral aldehyde through spontaneous racemization combined with the use of an ω-TA to afford, after ring closure, 4-phenylpyrrolidin-2-one with 92% isolated yield and 68% *ee*.



Scheme 1 General overview for the synthesis of enantioenriched α-alkylated β-amino esters through ω-TA-catalyzed dynamic amination.

Due to our previous experience with the synthesis of several α-alkylated β-keto esters,[4a,d] and since these substrates can racemize at neutral pH, it was decided to study the DKR of these compounds through ω-TA-catalyzed amination to obtain the corresponding α-substituted β-amino esters (Scheme 1). These compounds have a great relevance since they are present in biologically active peptides,[7] and are also valuable for the preparation of peptidomimetics,[8] substituted β-lactams[9] and β-amino acids.[10] The selective synthesis of these unprotected synthons is challenging,[11] and no general strategy comprising the direct asymmetric reductive amination of the α-substituted β-keto ester precursors has been described until now.[12]



Scheme 2 Synthesis of racemic aliphatic α-substituted β-amino ester derivatives through enamine formation-reduction protocol.

Thus, it was planned the synthesis of racemic β-keto esters **1b-k** and the corresponding mixture of *syn* and *anti* diastereoisomers of the α-substituted β-amino esters **2b-k** and **3b-k** (Scheme 2), to optimize the analytical methods to measure the enzymatic conversions and the enantio- and diastereoselectivities. Compounds **1b-k** were achieved through treatment of the β-keto ester precursors with the corresponding alkyl halide in basic medium.[4a,d] In a subsequent step, adapting a protocol described by Brandt and coworkers,[13] ultrasonication of these derivatives under the presence of benzylamine at 30 ºC smoothly afforded the (*Z*)-*N*-benzylated enamine esters **4b-k** in excellent yields. Then, the corresponding *N*-protected β-amino esters *syn*-**5b-k** and *anti*-**6b-k** were obtained as diastereoisomeric mixtures by reduction of the C=C double bond with $NaBH(OAc)_3$ at 0 ºC in high yields. As previously

described, the *syn* isomers were preferentially formed.[11d,14] In a last step, deprotection of these compounds under hydrogenation conditions afforded the racemic α-substituted β-amino esters *syn*-**2b**-**k** and *anti*-**3b**-**k** in high yields. A series of alkyl derivatives were synthesized changing the α-alkyl chain (R^2= Me, Et, Bn) and the ester alkyl moiety (R^3= Me, Et, ^{i}Pr). Furthermore, cyclic compounds **1j** and **1k**, were also tried as substrates to study the effect of constrained systems in these dynamic processes.

To perform these biocatalyzed transformations, a series of 24 commercially available transaminases, Codex® Transaminase Screening Kit, was tried as most of them are able to work under the very convenient conditions using isopropylamine in molar excess as amino donor.[15] In a first set of experiments, unsubstituted ethyl acetoacetate **1a** was studied as substrate for these TAs (Tables S2-S3 in ESI) using alanine or isopropylamine as amino donors. Better conversions were achieved in the second case utilizing an excess (1 M) of the amine in phosphate buffer 100 mM pH 7.5, and in the presence of PLP (1 mM) and DMSO (2.5% v v^{-1}) for solubility reasons. Various (*S*)- and (*R*)-selective enzymes were detected showing very high to excellent conversions and stereoselectivities. Especially the last ones are interesting since not many (*R*)-selective TAs are described in the literature.[16]

Then, different reaction conditions were explored to optimize the transamination process using α-ethylated keto ester **1c** as substrate. After enzymatic screening, a few TAs were chosen as suitable catalysts since they afforded a diastereomeric mixture of **2c** and **3c** with high conversions, excellent *ee* and moderate to high *de*. Some parameters such as pH, temperature, biocatalyst loading and addition of basic resins were studied to improve the DKR conditions (see Tables S4-S5 in ESI), but no positive effect was observed. Therefore, 30 ºC and pH 7.5 were selected as the best conditions to perform these transformations.

As a further step, the TA-catalyzed reactions with α-alkylated β-keto esters **1b-i** were attempted to have an overview of the effect that could present: a) the alkyl group at α-position, and b) the substitution in the ester moiety. The results are shown in Table 1 and Tables S6-S13 in ESI. In all cases it was found that 12 TAs showed (*S*)-stereopreference while other 12 were (*R*)-selective. It was remarkable that with just few exceptions (ATA-007, ATA-009, and ATA-117), these biocatalysts afforded high conversions for these substrates and were highly specific for the amination of the carbonyl group, showing excellent selectivities at position 3, just obtaining in most cases 2 out of 4 diastereoisomers.

Regarding the (*S*)-selective family of transaminases, generally the *syn*-(2*R*,3*S*) isomers were provided in slight excess (up to 60/40) and very high conversions (around 90%). Among them, ATA-224, ATA-234, TA-P1-F12, and TA-P1-G05 were the most active. (*S*)-selective enzymes TA-P1-A06, TA-P1-G06 and ATA-103 afforded preferentially *anti*-(2*S*,3*S*)-**3b**, **3c**, **3f**, and **3i** with moderate to high diastereomeric ratios. (*R*)-Selective biocatalysts also showed a slight stereopreference for the formation of the enantiopure *syn*-(2*S*,3*R*) amino esters (usually up to 60/40) with excellent conversions (>90%), especially ATA-015, ATA-016, ATA-024, ATA-025, and ATA-033. Although showing lower conversions, TA-P2-A07 was the most selective enzyme for most of the keto esters. In contrast, ATA-301 presented an opposite diastereopreference for *anti*-(2*R*,3*R*), in some cases higher than 80/20. For some TAs, *e.g.* TA-P1-A06 and ATA-301, it remained clear that the presence of a benzyl moiety at α-position could largely influence the diastereoselectivity of the process (Figures S1 and S2 in ESI).

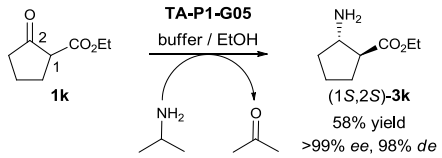
Next, it was envisaged that the employment of more constrained derivatives could improve the diastereoselectivity of the process while maintaining the excellent *ee*. Therefore, two cyclic compounds (**1j** and **1k**) were tried as possible amino acceptors for these TAs (Tables S14-S15 in ESI).

Table 1. Selected results from the transamination of racemic acyclic α-alkyl-β-keto esters **1b-i** using isopropylamine as amino donor (*t*= 24 h).[a]

		(*S*)-Selective					(*R*)-Selective				
Entry	**1b-i**	Enzyme	*c* (%)[b]	Ratio **2**/**3**	*ee* **2** (%)[c]	*ee* **3** (%)[c]	Enzyme	*c* (%)[b]	Ratio **2**/**3**	*ee* **2** (%)[c]	*ee* **3** (%)[c]
1	**1b**	TA-P1-A06	88	34/66	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	ATA-301	25	17/83	n.d.	>99 (2*R*,3*R*)
2		ATA-113	89	57/43	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	TA-P2-A07	61	58/42	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
3	**1c**	TA-P1-A06	87	16/84	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	ATA-301	21	20/80	n.d.	98 (2*R*,3*R*)
4		ATA-231	88	54/46	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	TA-P2-A07	47	62/38	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
5	**1d**	TA-P1-A06	98	58/42	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	ATA-024	>99	56/44	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
6	**1e**	--	--	--	--	--	ATA-301	39	36/64	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
7		TA-P1-G05	88	56/44	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	TA-P2-A07	79	55/45	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
8	**1f**	TA-P1-A06	93	29/71	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	ATA-301	41	32/68	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
9		ATA-224	93	55/45	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	TA-P2-A07	50	58/42	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
10	**1g**	--	--	--	--	--	ATA-301	47	32/68	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
11		TA-P1-G05	94	57/43	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	ATA-025	95	54/46	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
12	**1h**	--	--	--	--	--	ATA-301	35	38/62	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
13		ATA-103	88	54/46	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	TA-P2-A07	53	54/46	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)
14	**1i**	ATA-103	94	23/77	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	--	--	--	--	--
15		TA-P1-F12	98	58/42	>99 (2*R*,3*S*)	>99 (2*S*,3*S*)	ATA-301	89	59/41	>99 (2*S*,3*R*)	>99 (2*R*,3*R*)

[a] For other enzymatic results and experimental conditions, see ESI. [b] Measured by GC. [c] Measured by chiral GC.

For **1j** generally lower conversions (<40%) were observed, but several (*S*)-selective biocatalysts could afford preferentially the *anti*-(2*R*,1'*S*) isomer, while (*R*)-TAs did not show good selectivities. Better results were obtained for keto ester **1k**, finding that ATA-113 and TA-P1-G05 were able to produce *anti*-(1*S*,2*S*)-**3k** with very high conversions, *ee* (>99%) and *de* (94-96%). Thus, a 50 mg-scale reaction was achieved with TA-P1-G05 obtaining by simple acid-base extraction the chiral amino ester (1*S*,2*S*)-**3k** with a good yield and excellent *ee* and *de* (Scheme 3).[17]



Scheme 3 Synthesis of (1*S*,2*S*)-**3k** through a DKR process with a transaminase using isopropylamine as amino donor.

Herein we show our first results about the application of a set of commercially available ω-TAs to provide several α-alkylated β-amino esters using a dynamic protocol. These highly interesting targets are difficult to synthesize by other methodologies and in most cases the synthetic routes involve several steps or the employment of harsh conditions. Thus, a series of acyclic α-alkyl-β-keto esters were synthesized and used as substrates for these enzymes, finding that although excellent conversions and *ee* were achieved for many of them, diastereoselectivities remained modest with few exceptions. As an additional extension, the application of two constrained cyclic derivatives was performed to study their effect in the transamination reactions. Gratifyingly, racemic ethyl 2-oxocyclopentanecarboxylate showed excellent *ee* and *de* under our dynamic conditions, so the corresponding amino ester *anti*-(1*S*,2*S*)-**3k** could be synthesized at higher scale. With these preliminary results in hand, the application of these biocatalysts over new constrained substrates will be done, and due to the development of highly efficient tools in the Molecular Biology field,[16b,18] the design of novel transaminases that could provide specifically each diastereoisomer in enantiomerically pure form seems highly feasible in a near future.

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† Electronic Supplementary Information (ESI) available: [experimental procedures, enzymatic results, analytics and copies of ^{1}H- and ^{13}C-NMR of the novel compounds are shown]. See DOI: 10.1039/b000000x/

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