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A Versatile Chemo-Enzymatic Route to Enantiomerically Pure β-Branched α-Amino Acids

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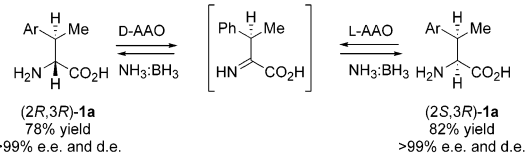
Enantiomerically pure β-branched α-amino acids constitute valuable building blocks for the synthesis of modified peptides possessing activity as enzyme inhibitors.[1] The incorporation of bulky β-substituents into an amino acid leads to conformational restriction in peptides and allows for the development of high affinity ligands for receptors. Existing methods for the synthesis of the individual stereoisomers of β-methyl α-amino acids generally rely upon either stereoselective approaches employing chiral auxiliaries[2] or alternatively nonselective synthesis followed by separation of the isomers by fractional recrystallization of diastereomeric salts.[3] Although high enantiomeric excesses can be achieved via the latter process, the limiting factor is the maximum yield of 50%. An attractive, recently developed alternative is the catalytic asymmetric hydrogenation of β,β-disubstituted didehydroamino acids using chiral bis-phosphine ligands.[4] However, it has been found that although the (*Z*)-aryl-didehydroamino acids undergo fast and highly selective hydrogenation, the corresponding (*E*)-isomers react more slowly and with much lower selectivities, resulting in access to only two of the four possible stereoisomers.[5] Herein, we report a combined chemobiocatalytic method for the synthesis of all four stereoisomers of a series of enantiomerically pure β-methyl-β-arylalanine analogues.

We have recently developed a method for the deracemization of α-amino acids[6] and amines[7] via a cyclic oxidation−reduction sequence and have shown that it can be extended to the stereoinversion of β- and γ-substituted α-amino acids.[8] The catalytic cycle involves the combined action of an enantioselective amino acid oxidase (AAO), which oxidizes the α-amino acid to the corresponding imine, together with a nonselective reducing agent (e.g., ammonia−borane), which effects reduction back to the starting material. For example, the (2*R*,3*R*)- and (2*S*,3*R*)-isomers of β-methylphenylalanine **1a** were successfully interconverted by this method in high yield and enantio/diastereoselectivity (Scheme 1).[8] Since the (2*S*,3*R*) and (2*R*,3*S*) pair of diastereomers are easily accessed via catalytic asymmetric hydrogenation of the (*Z*)-didehydroamino acids as described above, we envisaged using the AAO stereoinversion procedure to convert them, respectively, to the less accessible (2*R*,3*R*)- and (2*S*,3*S*)-diastereoisomers.

Methyl (*Z*)-2-(*N*-acetylamino)but-2-enoate **3** was prepared by dehydration of L-threonine methyl ester **2** according to the method of Nugent et al.[9] Subsequent bromination using *N*-bromosuccinimide followed by triethylamine gave the vinyl bromide **4** (X = Br) in 48% yield as a 1:1 mixture of (*E*/*Z*)-isomers[10] which could be separated (Scheme 2). However, the subsequent Suzuki couplings were generally found to proceed in higher yields with the (*E*)- rather than (*Z*)-isomers, and hence, we decided to examine the more reactive vinyl iodide, which to our knowledge has not previously been reported.[11]

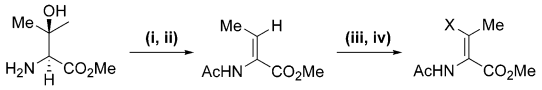
Scheme 1. Stereoinversion of β-Methylphenylalanine **1a** (Ar = Ph)



(2*R*,3*R*)-**1a**
78% yield
>99% e.e. and d.e.

(2*S*,3*R*)-**1a**
82% yield
>99% e.e. and d.e.

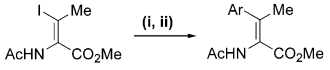
Scheme 2. Preparation of *Z*-**5** from L-Threonine Methyl Ester **2**[a]



2 (*Z*)-**3** (*Z*)-**4** (X = Br) (*Z*)-**5** (X = I)

[a] Reagents and conditions: (i) Ac_2O, NaOAc. (ii) Et_3N, MeOH. (iii) NXS, CH_2Cl_2. (iv) Et_3N.

Scheme 3. Suzuki Couplings with Vinyl Iodide *Z*-**5**[a]



(*Z*)-**5** (*Z*)-**6a-f**

[a] Reagents and conditions: (i) $ArB(OH)_2$, $Pd(OAc)_2$. (ii) Na_2CO_3, EtOH.

Treatment of (*Z*)-**3** with *N*-iodosuccinimide followed by Et_3N yielded **5** as a 1:1 mix of (*E*/*Z*)-isomers. However, addition of 2% TFA to the solvent CH_2Cl_2[12] gave **5** in 48% yield predominantly as the (*Z*)-isomer (*Z*/*E* = 5:1). The (*Z*)-isomer was separated by chromatography and then subjected to a range of Suzuki couplings (Scheme 3), which proceeded in good yields (Table 1) to give a range of (*Z*)-didehydroamino acids (**6a**−**f**).

Asymmetric hydrogenation of the (*Z*)-didehydroamino acids **6a**−**f** was performed at 100 psi hydrogen for 18 h in methanol using either the [Rh(*R*,*R*)-Et-DuPhos(COD)]BF_4 or [Rh(*S*,*S*)-Et-DuPhos(COD)]BF_4 catalyst to yield the (2*R*,3*S*)-**7a**−**f** or (2*S*,3*R*)-**7a**−**f** diastereoisomers respectively (Scheme 4). Purification through a short column of silica resulted in quantitative yields of the products **7a**−**f** with ee's >98%, with the exception of (2*R*,3*S*)-**7f** (ee = 96%) and (2*S*,3*R*)-**7f** (ee = 93%).[13] Deprotection by refluxing in 4 M HCl gave the enantiomerically pure amino acids **1a**−**f** as the hydrochloride salts in high yields and >99% ee after trituration with acetone (Table 2).

We initially screened the (2*R*,3*S*)-isomers of **1a**−**f** against D-AAO from pig kidney, which we had previously shown to be effective for the stereoinversion of (2*R*,3*S*)-**1a** to (2*S*,3*S*)-**1a**.[8]

However, this particular enzyme was unable to transform all of the substrates, and thus, we turned to the D-AAO from *Trigonopsis*

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Table 1. Yields of **6a**−**f** from Suzuki Couplings

product	Ar	yield %
6a	C_6H_5	81
6b	*p*-F−C_6H_4	77
6c	*p*-CF_3−C_6H_4	84
6d	2-naphthyl	72
6e	2,5-di-Me-C_6H_3	82
6f	*p*-MeO−C_6H_4	73

Scheme 4. Asymmetric Hydrogenation of *Z*-**6a**−**f**

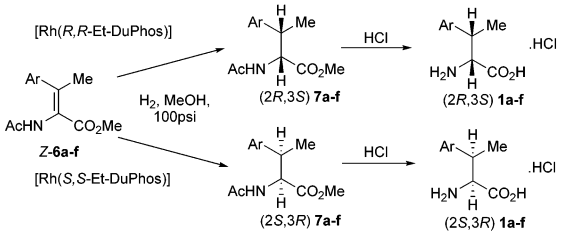


Table 2. Yields and Enantiomeric Excesses of (2*R*,3*S*)-**1a**−**f** and (2*S*,3*R*)-**1a**−**f**

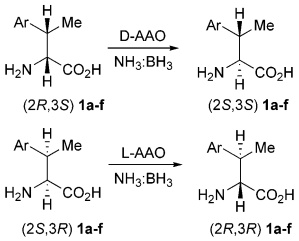
substrate	Ar	product	yield %	ee %
(2*R*,3*S*)-**7a**	C_6H_5	(2*R*,3*S*)-**1a**	88	>99
(2*S*,3*R*)-**7a**	C_6H_5	(2*S*,3*R*)-**1a**	89	>99
(2*R*,3*S*)-**7b**	4-F−C_6H_4	(2*R*,3*S*)-**1b**	78	>99
(2*S*,3*R*)-**7b**	4-F−C_6H_4	(2*S*,3*R*)-**1b**	76	>99
(2*R*,3*S*)-**7c**	4-CF_3−C_6H_4	(2*R*,3*S*)-**1c**	71	>99
(2*S*,3*R*)-**7c**	4-CF_3−C_6H_4	(2*S*,3*R*)-**1c**	84	>99
(2*R*,3*S*)-**7d**	2-naphthyl	(2*R*,3*S*)-**1d**	68	>99
(2*S*,3*R*)-**7d**	2-naphthyl	(2*S*,3*R*)-**1d**	87	>99
(2*R*,3*S*)-**7e**	2,5-di-Me-C_6H_3	(2*R*,3*S*)-**1e**	89	>99
(2*S*,3*R*)-**7e**	2,5-di-Me-C_6H_3	(2*S*,3*R*)-**1e**	73	>99
(2*R*,3*S*)-**7f**	4-MeO−C_6H_4	(2*R*,3*S*)-**1f**	66	>99
(2*S*,3*R*)-**7f**	4-MeO−C_6H_4	(2*S*,3*R*)-**1f**	67	>99

variabilis, which in combination with ammonia−borane complex gave good yields (68−81%) of the desired (2*S*,3*S*)-diastereomers of **1a**−**f** with excellent selectivity (>99% de) (Table 3).[13] The corresponding L-β-arylphenylalanine analogues (2*S*,3*R*)-**1a**−**f** were converted to the remaining set of (2*R*,3*R*)-diastereomers using snake venom L-AAO and ammonia−borane complex with good to excellent yields (80−92%) and again very high selectivity (de > 99%) (Table 3). One reaction, namely that involving (2*S*,3*R*)-**1e** with L-AAO, did not go fully to completion, resulting in only a 71% conversion and 49% de. In this case, an alternative L-amino acid oxidase would need to be identified with the appropriate substrate specificity.

Finally, (2*R*,3*R*)-**1a** was converted to (2*S*,3*R*)-**1a** on a preparative scale, using D-AAO from *T. variabilis* and ammonia−borane complex as the reducing agent, yielding the isolated product in 55% yield. Workup of these reactions is very straightforward, involving simply filtration to remove the enzyme catalyst, followed by evaporation and recrystallization from ethanol/ethyl acetate.

In summary, we have developed a highly versatile and efficient route to all but one of a set of 24 β-methylarylalanine analogues **1a**−**f** in high yields and excellent enantiomeric/diasteroemeric excesses. The vinyl iodide (*Z*)-**5** is used as a unique building block to access all 23 products via asymmetric hydrogenation of the (*Z*)-β,β-disubstituted didehydroamino acids **6a**−**f** followed by stereoinversion using D- and L-AAO's. Further studies aimed at broadening this approach to other classes of chiral compounds are ongoing and will be reported in due course.

Table 3. Stereoinversions of **1a**−**f** Using D- and L-AAO



substrate	Ar	enzyme	product	yield %
(2*R*,3*S*)-**1a**	C_6H_5	D-AAO	(2*S*,3*S*)-**1a**	69
(2*R*,3*S*)-**1b**	C_6H_4-*p*-F	D-AAO	(2*S*,3*S*)-**1b**	68
(2*R*,3*S*)-**1c**	C_6H_4-*p*-CF_3	D-AAO	(2*S*,3*S*)-**1c**	81
(2*R*,3*S*)-**1d**	2-naphthyl	D-AAO	(2*S*,3*S*)-**1d**	80
(2*R*,3*S*)-**1e**	C_6H_3-(2,5-DiMe)	D-AAO	(2*S*,3*S*)-**1e**	72
(2*R*,3*S*)-**1f**	C_6H_4-*p*-OMe	D-AAO	(2*S*,3*S*)-**1f**	81
(2*S*,3*R*)-**1a**	C_6H_5	L-AAO	(2*R*,3*R*)-**1a**	83
(2*S*,3*R*)-**1b**	C_6H_4-*p*-F	L-AAO	(2*R*,3*R*)-**1b**	85
(2*S*,3*R*)-**1c**	C_6H_4-*p*-CF_3	L-AAO	(2*R*,3*R*)-**1c**	92
(2*S*,3*R*)-**1d**	2-naphthyl	L-AAO	(2*R*,3*R*)-**1d**	80
(2*S*,3*R*)-**1e**	C_6H_3-(2,5-DiMe)	L-AAO	(2*R*,3*R*)-**1e**	71
(2*S*,3*R*)-**1f**	C_6H_4-*p*-OMe	L-AAO	(2*R*,3*R*)-**1f**	80

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Supporting Information Available: Experimental procedures for the preparation of vinyl iodide *Z*-**5**, asymmetric hydrogenation reactions, and the stereoinversion procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) All enantiomeric and diastereomeric excesses for the reactions were determined by reverse-phase chiral HPLC (Chirobiotic T column; flow rate = 0.7 mL/min, eluant EtOH/H_2O = 40:60; see Supporting Information for full details).

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