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A Cross-Metathesis Route to Functionalized α-Methyl α-Substituted Amino Acids

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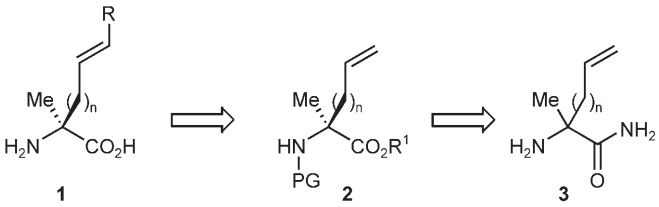
Abstract: A chemoenzymatic approach to the synthesis of functionalized α-methyl α-substituted amino acids is detailed. This involves amidase-mediated enzymatic resolution of α-methyl α-substituted side-chain ω-unsaturated amino acids followed by functionalization *via* cross-metathesis.

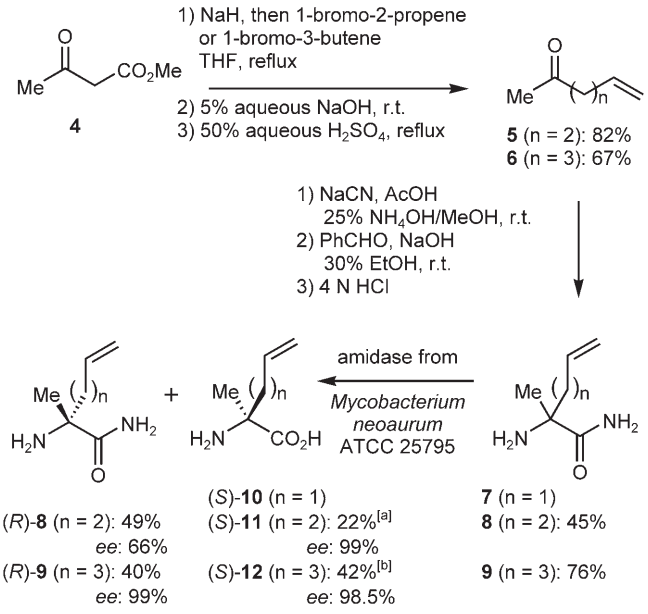
Keywords: amino amidase; cross-metathesis; enzyme catalysis; α-methyl α-substituted amino acids; side-chain ω-unsaturated α-amino acids

Enantiomerically pure α,α-disubstituted α-amino acids, especially α-methyl α-substituted amino acids, are of increasing interest for the agrochemical and pharmaceutical industry.[1] Compared to their α-H counterparts, peptides containing one or more such amino acids are less prone to epimerization, display enhanced metabolic stability and possess different folding behavior.[2] In line with our research on metathesis applications of side-chain ω-unsaturated α-H-amino acids,[3] and inspired by successful ring-closing metathesis examples of α-methyl α-substituted amino acids from our own[4] and other groups,[5] we set out to explore the viability of a cross-metathesis[6] route to prepare functionalized α-methyl α-substituted amino acids. As a result, we herewith report that by combining enzymatic resolution of the racemic α,α-disubstituted side-chain ω-unsaturated amino acid amides **3**[4,7] with subsequent cross-metathesis on the terminal olefin functions, the functionalized α-amino acids **1** are readily accessible (Scheme 1). Although we will not discuss any follow-up chemistry of the functionality introduced in the final products, it will be clear that the resulting functional group provides ample opportunity for further synthetic derivatization.[8]



Scheme 1. Retrosynthesis of enantiopure side-chain ω-unsaturated α-methyl α-amino acids.

Enantiopure α-methyl α-allylglycine amide (*R*)-**7** and the corresponding acid (*S*)-**10** were synthesized previously in our lab *via* a chemoenzymatic strategy.[4,7] The synthesis of the homologous α-amino acids **11** and **12** proceeded in a different manner as shown in Scheme 2, but also involved an enzymatic resolution to obtain the (*S*)-α-amino acids in enantiopure form. The strategy commenced with methyl acetoacetate (**4**), which *via* standard alkylation with the required olefin, hydroxide-mediated saponification and subsequent acidic decarboxylation was converted into ketones **5** and **6** in good overall yields. Subjection to Strecker conditions, followed by partial nitrile hydrolysis (NaOH, PhCHO, MeOH) and subsequent Schiff base hydrolysis (4 N HCl) provided the α-amino acid amides **8** and **9** in good overall yields. They were made salt-free *via* extraction from a basic water layer (pH 10) with CH_2Cl_2 and after concentration subjected to whole cells from *Mycobacterium neoaurum* ATCC 25795 in an aqueous solution starting at pH 8.3. After 18 h, the reaction mixtures were worked up, the products were separated and purified *via* ion exchange chromatography. Chiral HPLC analysis showed excellent *ee*s for α-amino acids (*S*)-**11** and (*S*)-**12** of 99 and 98.5%, respectively. These num-

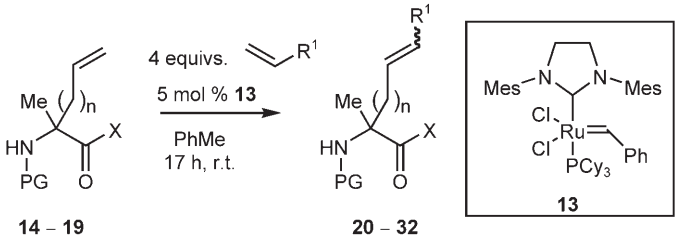


Scheme 2. Chemoenzymatic synthesis of α-amino acids **11** and **12**.[a] Conversion 40%, E-ratio > 250.[b] Conversion 51%, E-ratio > 200.

bers, combined with those of the corresponding amides result in enantiomeric ratios (E) of > 250 and > 200, respectively, which are in line with previous observations.[4,7]

Next, we screened a series of differently protected racemic α-methyl α-amino acid derivatives (**14**–**17**) in combination with olefinic precursors in cross-metathesis reactions (Table 1). Based on various examples of cross-metathesis on α-H-amino acids,[9] the reactions were conducted in the presence of 5 mol% of the second generation Grubbs catalyst (**13**) and four equivalents of the cross-metathesis partner in toluene at room temperature. In the case of Boc-derivative **14** reasonable yields were obtained with all five olefins to provide the α-amino acids **20**–**24** (entries 1–5). Besides the desired product, which in all entries was isolated as an inseparable mixture of *E*/*Z*-isomers (generally ranging from 3:1 to 6:1, based on ^{1}H NMR), in all cases trace amounts of dimeric α-amino acid metathesis products were obtained (< 5%). Slightly lower cross-metathesis yields were obtained with tosyl and acetyl protecting groups in combination with styrene (entries 6 and 7). In addition, a series of olefins was reacted with the industrially interesting formyl-protected α-amino acid **17**, which provided α-amino acids **27**–**30** in satisfactory yields (entries 8–11). Unlike the other metathesis products, the acrylate adduct **30** was obtained as a single *E*-isomer.[10] Finally, the racemic Boc-protected α-amino acid amides **18** and **19** were subjected to cross-metathesis in combination with 2-vinyl-1,3-dioxolane as the metathesis partner, leading to the desired products **31** and **32** in 74 and 71% yield, respectively (entries 12 and 13).

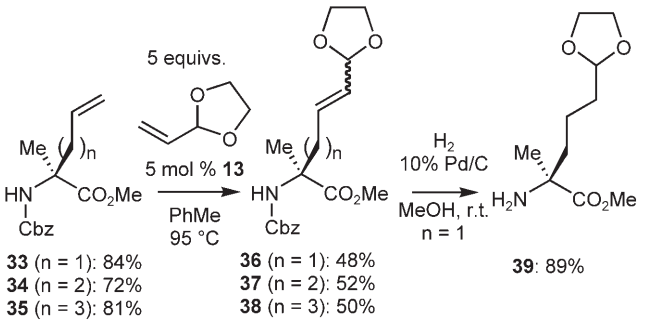
Table 1. Cross-metathesis reactions.



Entry	Amino acid	PG	X	n	R^1	Product	Yield [%][a]
1	**14**	Boc	OMe	1	$CH(OC_2H_4O)$	**20**	45 (69)[b]
2	**14**	Boc	OMe	1	CH_2SiMe_3	**21**	70
3	**14**	Boc	OMe	1	$CH(OEt)_2$	**22**	66
4	**14**	Boc	OMe	1	Ph	**23**	64
5	**14**	Boc	OMe	1	$CH_2CH(CO_2Et)_2$	**24**	55 (64)[c]
6	**15**	Ts	OMe	1	Ph	**25**	42[c]
7	**16**	Ac	OMe	1	Ph	**26**	46
8	**17**	CHO	OMe	1	$CH(OC_2H_4O)$	**27**	49
9	**17**	CHO	OMe	1	Ph	**28**	49[c]
10	**17**	CHO	OMe	1	*n*-hexyl	**29**	55
11	**17**	CHO	OMe	1	CO_2Et	**30**	70
12	**18**	Boc	NH_2	1	$CH(OC_2H_4O)$	**31**	74
13	**19**	Boc	NH_2	2	$CH(OC_2H_4O)$	**32**	71

[a] Isolated yields. In all cases, trace amounts (< 5%) of homo-dimerized amino acids were also obtained.
[b] Yield determined by GC.
[c] Yield determined by ^{1}H NMR.

To further underline the viability of this strategy, a series of experiments was carried out on a somewhat larger scale with the enantiopure α-amino acid derivatives **33**–**35**, which were obtained *via* esterification ($SOCl_2$, MeOH) and subsequent *N*-protection (Cbz-OSu, MeCN) of α-amino acids (*S*)-**10**–**12** (Scheme 3).



Scheme 3. Synthesis of dioxolane-substituted side-chain ω-unsaturated α-methyl α-amino acids.

The vinyldioxolane was chosen as the cross-metathesis reactant in view of its versatile reactivity. In contrast to the previous small scale experiments, cross-metathesis on gram scale proceeded sluggishly at room temperature which led us to conduct the reactions at 95 °C. To reach a maximum conversion, the catalyst was added in portions of 1 mol % over 5 h time, leading to products **36**–**38** in reasonable yields. At this temperature, a considerable amount of dimerized vinyldioxolane was also formed and removed *via* chromatography. Finally, hydrogenation of derivative **36** led to the amino ester **39** in 89 %. The latter is a potentially relevant α-Me-derivative of the α-amino acid L-allysine ethylene acetal, which is a useful building block for various synthetic applications.[11]

In conclusion, new examples of enzymatic resolution of α-methyl α-substituted α-amino acids using an amino amidase of *Mycobacterium neoaurum* ATCC 25795 are detailed. The resulting amides and α-amino acids have been evaluated as partners in cross-metathesis reactions in combination with a variety of olefins. Generally, these reactions proceeded reasonably well giving rise to the corresponding functionalized side-chain ω-unsaturated α-amino acids. Finally, the reaction was successfully applied to prepare a series of enantiomerically pure dioxolane-substituted α-amino acids.

Experimental Section

Representative Example of the Enzymatic Resolution:

To a 10 wt % aqueous solution of **9** (10.0 g, 64.1 mmol) at pH 8.3 was added a whole cell suspension of *Mycobacterium neoaurum* ATCC 25795 (1.05 g). The reaction mixture was shaken at 200 rpm and 37 °C for 18 h. After removal of the cells by centrifugation, the acid and amide were separated by strongly basic ion-exchange resin column chromatography (Dowex 1×8) to provide (*S*)-**12** (4.20 g, 42 %) and (*R*)-**9** (4.00 g, 40 %). The enantiopurity of acid and amide was determined by chiral HPLC (Sumichiral OA 5000).

Physical and Spectroscopic Data of the α-Methyl α-Amino Acids

(*S*)-**12**: *ee* 98.5 %; $[\alpha]_D$: +10.8 (*c* 0.83, MeOH); mp >200 °C (dec); IR: ν = 2954, 1584, 1398, 910 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ = 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.03 (d, *J* = 17.1 Hz, 1H), 4.96 (d, *J* = 10.2 Hz, 1H), 2.08 (dt, *J* = 7.0, 7.0 Hz, 2H), 1.91–1.84 (m, 1H), 1.70–1.62 (m, 1H), 1.58–1.36 (m, 2H), 1.44 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ = 176.8, 138.5, 115.0, 61.4, 36.5, 32.6, 23.4, 22.3; HR-MS (ESI+): *m/z* = 158.1197, calcd. for $C_8H_{16}NO_2$ ($M+H^+$): 158.1181.

(*S*)-**11**: *ee* 99 %; $[\alpha]_D$: +13.6 (*c* 1.02, MeOH); mp >200 °C (dec); IR: ν = 2984, 1575, 1403, 1368, 914 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ = 5.82 (ddt, *J* = 16.8, 10.2, 6.5 Hz, 1H), 5.07 (d, *J* = 17.1 Hz, 1H), 4.98 (d, *J* = 10.2 Hz, 1H), 2.19 (m, 1H), 2.07 (m, 1H), 1.96 (m, 1H), 1.74 (m, 1H), 1,46 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ = 175.8, 136.7, 115.0, 61.1, 36.1, 27.5, 22.4; HR-MS (ESI−): *m/z* = 142.0894, calcd. for $C_7H_{12}NO_2$ ($M-H^+$): 142.0868.

Representative Example of a Cross-Metathesis Reaction

To a solution of (*S*)-**33** (1.60 g, 5.80 mmol) and 2-vinyl-1,3-dioxolane (2.88 mL, 28 mmol) in dry and oxygen-free toluene (60 mL) at 95 °C was catalyst **13** (245 mg, 5 mol %) added in five portions. After heating for 17 h, the reaction mixture was concentrated and filtered over a path of silica gel (EtOAc/heptane 1:2) to give (*S*)-**36** in crude form. The crude *E*/*Z*-isomeric mixture of **36** was dissolved in MeOH (50 mL), treated with Pd/C (79 mg of 5 wt %) and stirred under an H_2 atmosphere for 22 h. The mixture was filtrated over Celite, concentrated and purified by column chromatography (CH_2Cl_2/MeOH 9:1) to afford (*S*)-**39** as an amorphous solid; yield: 537 mg (89 %); $[\alpha]_D$: +11.3 (*c* 1.0, CH_2Cl_2); IR: ν = 3620, 2958, 2863, 1735, 1121, 607 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 4.76–4.72 (m, 1H), 3.89–3.71 (m, 4H), 3.62 (s, 1H), 1.64–1.18 (m, 6H), 1.23 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.2, 103.7, 64.4, 57.4, 51.8, 40.7, 33.7, 26.0, 18.5; HR-MS (CI): *m/z* = 218.1394, calcd. for $C_{10}H_{20}NO_4$ ($M+H^+$): 218.1392.

Acknowledgements

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