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Highly Efficient Stereoconservative Amidation and Deamidation of α-Amino Acids

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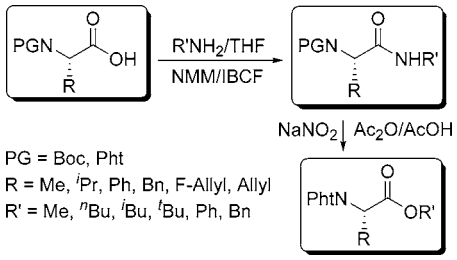
ABSTRACT



An overall stereoconservative protection and deprotection method of amino and carboxyl groups is presented. *N*-Phthaloyl *N*′-alkyl secondary amides of α-amino acids can be generated from corresponding *N*-phthaloyl amino acids by coupling reaction of *N*-alkylamines using mixed anhydride method. These secondary amides can be transformed by thermal rearrangement of intermediate nitrosoamides to *O*-alkyl esters with retention of configuration and excellent yields.

We required a variety of amino acid amides for peptidomimetic studies. For this purpose, it was necessary to develop procedures for amidation as well as deamidation of enantiopure α-amino acids. Also, we had the problem of stereoconservative deamidation of chiral α-amino acid secondary amides in our recent synthesis of fluorinated amino acids.[1] We could not find any chemical transformation of optically active secondary amino acid amides to the corresponding esters or acids without partial racemization. There are few reports on strong basic deamidation of cyclopropyl amides[2] and specific enzymatic amidation and deamidation of primary amides.[3]

For synthetic peptides (peptide design), several protection and deprotection steps are required. The dipeptide **VII** can be synthesized by coupling reaction of N-protected amino acid (AA) **II** and C-terminal *N*-alkyl amide **VI**. Further, the peptide chain can be extended to the left side (N-terminal) to **XIV** by repeating N-deprotection at **VII** and coupling reaction with **IX**. However, it is always difficult to add a new AA to the right side (C-terminal), which needs selective secondary amide hydrolysis of **VII** to acid **XI**. Then, the peptide **XIII** can be synthesized by coupling reaction of **XI** and **XII**. Figure 1 shows the necessity of protection and deprotection of the N-terminus as well as the C-terminus of the corresponding amino acid building blocks.

Several decades ago, an impressive and convincing work by White[4] showed that *N*-alkyl secondary amides of simple carboxylic acids can be transformed to *N*-nitrosoamides, which were then thermally rearranged to *O*-alkyl esters with

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(1) Shendage, D. M.; Fröhlich, R.; Bergander, K.; Haufe, G. *Eur. J. Org. Chem.* **2004**, submitted.

(2) Zhang, X.; Hodgetts, K.; Rachwal, S.; Zhao, H.; Wasley, J. W. F.; Craven, K.; Brodbeck, R.; Kieltyka, A.; Hoffman, D.; Bacolod, M. D.; Girard, B.; Tran, J.; Thurkauf, A. *J. Med. Chem.* **2000**, *43*, 3923−3932.

(3) For enzymatic amidation/deamidation, see:(a) Trauthewein, H.; May, O.; Dingerdissen, U.; Buchholz, S.; Drauz, K. *Tetrahedron Lett.* **2003**, *44*, 3737−3739. (b) Wolf, L. B.; Sonke, T.; Tjen, K. C. M. F.; Kaptein, B.; Broxterman, Q. B.; Schoemaker, H. E.; Rutjes, F. P. J. T. *Adv. Synth. Catal.* **2001**, *343*, 662−674. (c) Cerovsky, V.; Kula, M.-R. *Angew. Chem.* **1998**, *110*, 1986−1989; *Angew. Chem., Int. Ed.* **1998**, *37*, 1885−1887. (d) Steinke, D.; Kula, M.-R. *Angew. Chem.* **1990**, *102*, 1204−1206; *Angew. Chem., Int. Ed.* **1990**, *29*, 1139−1140.

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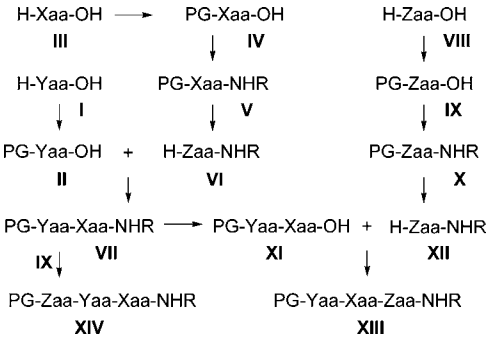
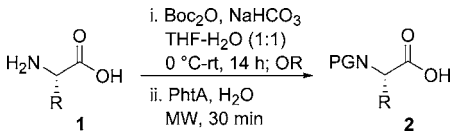


Figure 1. Synthetic strategy for polypeptides; PG = protecting group; Xaa = Yaa = Zaa = AA = amino acids.

elimination of nitrogen. There is also a recent report on transformation of similar *N*-aryl amides to *O*-aryl esters.[5] The issue of competing modes of decomposition of *N*-nitrosoamides has been debated for decades and was recently summarized by Darbeau and co-workers, who concluded that the nitrosoamides decompose principally along deaminative pathways ($-N_2$), leading to rearranged products, and denitrosative pathways ($-$"NO^+"), leading to starting material, depending on pH and temperature.[6]

This methodology has not yet been applied to chiral amino acids. Thus, several amino acids **1** have been N-protected by *tert*-butyloxycarbonyl (Boc) or phthaloyl (Pht) protecting groups using $Boc_2O/NaHCO_3/THF-H_2O$ or $PhtA/H_2O$ in nearly quantitative yields (Table 1).

Table 1. N-Protections of Amino Acids **1**



entry	(*S*)-**1**	R	PG	(*S*)-**2**	% yield[a]
1	**f**	F-allyl[b]	Boc	**f**	>99
2	**g**	allyl[b]	Boc	**g**	>99
3	**h**	Me	Pht	**h**	>99
4	**i**	^{i}Pr	Pht	**i**	95
5	**j**	Bn	Pht	**j**	>98

[a] Yields of isolated products. [b] Racemate; PhtA = phthalic anhydride; MW = microwave; F-allyl = 2-fluoroallyl.

The *N*′-alkyl amides **3** were synthesized by selective *N*′-alkyl amidation of protected amino acids **2** followed by mixed anhydride coupling reaction[7] (Figure 2) using *N*-methyl morpholine (NMM) and isobutyl chloroformate (IBCF) in THF at -20 °C in quantitative yields. Several different Boc-protected (Table 2, entries 1−6) and phthalimide-protected (Table 2, entries 7−9, and Table 3, entries 1−5) amino acids **2** were derivatized to the respective *N*′-alkyl amides **3** in practically quantitative yields using corresponding *N*-alkylamines.[8]

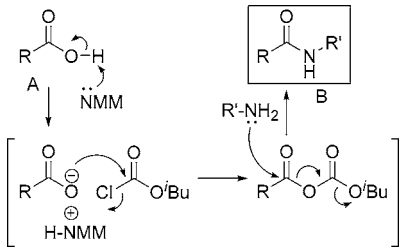
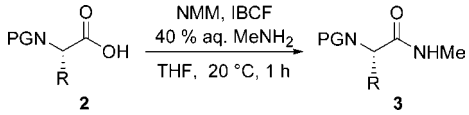


Figure 2. Mechanism of mixed anhydride coupling reaction.

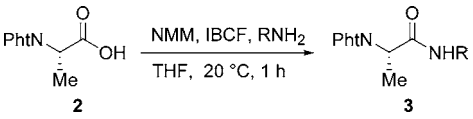
Table 2. *N*′-Methyl Amidation of Protected Amino Acids **2**



entry	(*S*)-**2**	PG	R	(*S*)-**3**	% yield[a]
1	**a**	Boc	Me	**a**	>98
2	**b**	Boc	*N*-$(CH_2)_3$−[b]	**b**	>99
3	**d**	Boc	Ph[c]	**d**	>99
4	**e**	Boc	Bn	**e**	>99
5	**f**	Boc	F-allyl[d]	**f**	>99
6	**g**	Boc	allyl[d]	**g**	>99
7	**h**	Pht	Me	**h**	>99
8	**i**	Pht	^{i}Pr	**i**	>98
9	**j**	Pht	Bn	**j**	>99

[a] Yields of isolated products. [b] (*S*)-Proline. [c] (*R*)-Phenylglycine. [d] Racemate.

Table 3. *N*′-Alkyl Amidation of *N*-Phthalimido Alanine (**2h**)



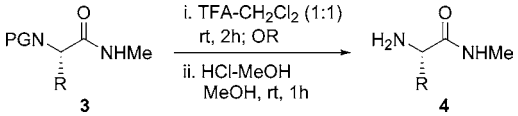
entry	(*S*)-**2**	R	(*S*)-**3**	% yield[a]
1	**k**	^{n}Bu	**k**	>99
2	**l**	^{i}Bu	**l**	>98
3	**m**	^{t}Bu	**m**	>99
4	**n**	Ph	**n**	>99
5	**o**	Bn	**o**	>99

[a] Yields of isolated products.

(4) (a) White, E. H. *J. Am. Chem. Soc.* **1954**, *76*, 4497−4498. (b) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6008−6010. (c) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6011−6014. (d) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6014−6022. (e) White, E. H.; Elliger, C. A. *J. Am. Chem. Soc.* **1967**, *89*, 165−167. (f) White, E. H. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, pp 336−339. (g) Le Noble, W. J.; White, E. H.; Dzadzic, P. M. *J. Am. Chem. Soc.* **1976**, *98*, 4020−4221.

The N-protected amides **3** were subsequently hydrolyzed to free amides **4** (peptide building blocks) using TFA/CH_2Cl_2 (Table 4, entries 1−2) or to the corresponding HCl−salts (Table 4, entries 3−4) by HCl−MeOH in anhydrous MeOH with high yields.

Table 4. N-Deprotection of Amides **3**

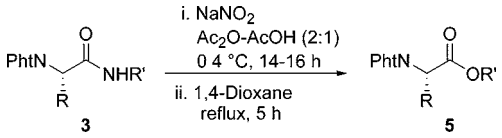


entry	(*S*)-**3**	PG	R	(*S*)-**4**	% yield[a]
1	**a**	Boc	Me	**a**	98
2	**b**	Boc	*N*-$(CH_2)_3$-[b]	**b**	>98
3	**f**	Boc	F-allyl[c]	**f**	98
4	**g**	Boc	allyl[c]	**g**	82

[a] Yields of isolated products. [b] (*S*)-Proline. [c] Racemate.

Several lipases and esterases have been screened in order to hydrolyze racemic amides **4f** and **4g** to the corresponding free acids, e.g., **1f** and **1g** enzymatically, however, without success.[9] Thus, the *N*′-methyl amide (*S*)-**4p**[1] was protected[10] to PhtN-L-Fap-NHMe **3p** (PG = Pht, R = F-allyl) and converted[11] to its methyl ester **5p** (Table 5, entry 9) by

Table 5. Deamidation of Amides **3**



entry	(*S*)-**3**	R	R′	(*S*)-**5**	% yield[a]
1	**h**	Me	Me	**h**	96
2	**i**	^{i}Pr	Me	**i**	>98
3	**j**	Bn	Me	**j**	>98
4	**k**	Me	^{n}Bu	**k**	>98
5	**l**	Me	^{i}Bu	**l**	97
6	**m**	Me	^{t}Bu	**m**	0
7	**n**	Me	Ph	**n**	0
8	**o**	Me	Bn	**o**	98
9	**p**	F-allyl	Me	**p**	91

[a] Yields of isolated products; F-allyl = 2-fluoroallyl.

nitrosoamide decomposition reaction[4] in 91% yield. The stereochemistry of compound **3p** was confirmed from X-ray crystal structure (Figure 3).[12]

The deamidation procedure[11] was developed and applied for a variety of phthaloyl-protected amino acid amides **3** to

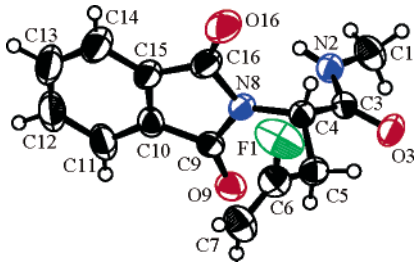


Figure 3. ORTEP diagram of **3p**.

obtain corresponding methyl esters **5** in good to excellent yields (Table 5). The rearrangement of *N*′-*tert*-butyl or *N*′-phenyl amide[5] **3m**−**n** (Table 5, entries 6−7) did not occur, and most of the starting material was recovered. This might be due to decomposition of the intermediary nitrosoamide following the denitrosative pathway.[6]

Interestingly, also the *N*′-methyl amide **3b** was similarly deamidated[11] (86% yield), demonstrating the utility of the procedure for *N*,*N*-dialkyl carbamates (Scheme 1).

No racemization was observed for this deamidation procedure by comparison of specific rotation of **2j** ([α]$_D$

(5) Glatzhofer, D. T.; Roy, R. R.; Cossey, K. N. *Org. Lett.* **2002**, *4*, 2349−2352 and references therein.

(6) Darbeau, R. W.; Perez, E. V.; Sobieski, J. I.; Rose, W. A.; Yates, M. C.; Boese, B. J.; Darbeau, N. R. *J. Org. Chem.* **2001**, *66*, 5679−5686 and references therein.

(7) Perich, J. W.; Johns, R. B. *J. Org. Chem.* **1988**, *53*, 4103−4105.

(8) **General Procedure for *N*-Alkyl Amidation.** *N*-Methyl morpholine (10 mmol) and isobutyl chloroformate (10 mmol) were successively added to a solution of Boc-Xaa-OH **2a**−**g** (10 mmol) or PhtN-Xaa-OH **2h**−**n** (10 mmol) in THF (20 mL) at −20 °C. After an activation period of 3 min, 40% aqueous methylamine (12−50 mmol) or *N*-alkylamine (13 mmol) in THF (5 mL) was added to above solution, and the resulting solution was stirred for 1 h at −20 °C prior to the addition of 5% $NaHCO_3$ (20 mL). After 30 min at room temperature, the aqueous phase was extracted with CH_2Cl_2 (three times). The combined organic layer was washed with 5% $NaHCO_3$ (two times) and dried (Na_2SO_4). Evaporation of solvents under reduced pressure gave Boc-Xaa-NHMe **3a**−**g** or PhtN-Xaa-NHR **3h**−**n**. The crude products were purified by flash chromatography (EtOAc/cyclohexane, 1:2) or by crystallization (EtOAc/pentane or CH_2Cl_2/pentane).

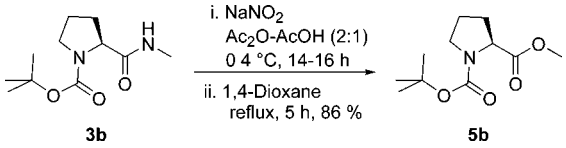
(9) In collaboration with Prof. Dr. A. Liese, Institute of Biochemistry, University of Münster, Germany, and Prof. Dr. F. P. J. T. Rutjes, Department of Organic Chemistry, University of Nijmegen, Nijmegen, The Netherlands.

(10) To a solution of H-L-Fap-NHMe **4** (2 mmol) in $CHCl_3$/MeOH (2:1, 20 mL) was added phthalic anhydride (2.4 mmol, freshly recrystallized from $CHCl_3$) at 0 °C. After 10 min, oxalyl chloride (3 mmol) was added dropwise at 0 °C. Then, the solution was refluxed for 5 h and cooled to room temperature. The solvent was evaporated under vacuum, and the residue was recrystallized from CH_2Cl_2/pentane in a freezer.

(11) **General Procedure for Deamidation.** To a solution of the desired *N*-phthaloyl *N*′-alkyl amide **3** (5 mmol) in a 2:1 mixture of Ac_2O and AcOH (25 mL) was added granular $NaNO_2$ (100 mmol) in portions over 2 h at 0−4 °C. Evolution of a brown gas occurred, and the solution changed color; sometimes a solid precipitated. After 14−16 h, the mixture was warmed to room temperature within 20 min, added into ice−water (25 mL), and extracted with Et_2O or CH_2Cl_2 (three times). The combined organic layer was washed (carefully!) with 5% Na_2CO_3 (three times) and then H_2O and dried (Na_2SO_4). Evaporation of solvents gave a yellowish liquid. GC showed complete conversion of phthalimido-amides. To this residue was added anhydrous 1,4-dioxane (25 mL), and the solution was refluxed. The yellowish color of the solution disappeared in the first 1 h, but reflux was continued for 5 h. Then, the solution was cooled to room temperature and the solvent was removed under vacuum to give colorless oils. GC of the crude product as such shows high purity.

(12) X-ray crystal structure analysis for **3p**: formula $2C_{14}H_{13}FN_2O_3 \times CH_2Cl_2$, M = 637.45, colorless crystal 0.45 × 0.30 × 0.10 mm, a = 7.778(1), b = 17.538(1), c = 22.402(1) Å, V = 3055.9(5) Å^3, ρ_{calcd} = 1.386 g cm^{-3}, μ = 24.37 cm^{-1}, empirical absorption correction (0.407 ≤ T ≤ 0793), Z = 4, orthorhombic, space group $P2_12_12_1$ (No. 194), λ = 1.54178 Å, T = 223 K, ω and φ scans, 13 926 reflections collected (±h, ±k, ±l), [(sin θ)/λ] = 0.59 Å^{-1}, 5039 independent (R_{int} = 0.042) and 4564 observed reflections [$I \geq 2\,\sigma(I)$], 396 refined parameters, R = 0.047, wR_2 = 0.137, Flack parameter −0.03(2), max residual electron density 0.51 (−0.43) e Å^{-3}; hydrogens at nitrogen atoms N2 were obtained from difference Fourier calculations, others were calculated and all refined as riding atoms.

Scheme 1. Deamidation of Boc-L-Pro-NHMe **3b**



−162.8 (*c* 1.01, CH_2Cl_2)) prepared from either **1j** or phenylalanine methyl ester **5j**. Moreover, X-ray structures of the PhtN-L-Ala-NHMe **3h**[13] (Figure 4) and its deamidation

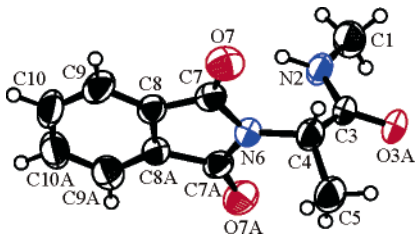


Figure 4. ORTEP diagram of **3h**.

product PhtN-L-Ala-OMe (**5h**)[14] (Figure 5) prove the deamidation reaction without racemization since there is no intermediate in the mechanism,[6] which could force racemization of the α-position.

(13) X-ray crystal structure analysis for **3h**: formula $C_{12}H_{12}N_2O_3$, M = 232.24, colorless crystal 0.40 × 0.15 × 0.10 mm, a = 10.929(1), b = 11.064(1), c = 9.906(1) Å, β = 96.12(1)°, V = 1191.0(2) Å^3, ρ_{calcd} = 1.295 g cm^{-3}, μ = 7.87 cm^{-1}, empirical absorption correction (0.744 ≤ T ≤ 0.925), Z = 4, monoclinic, space group *Cc* (No. 9), λ = 1.54178 Å, T = 223 K, ω and φ scans, 3222 reflections collected (±h, ±k, ±l), [(sin θ)/λ] = 0.58 Å^{-1}, 1594 independent (R_{int} = 0.031) and 1514 observed reflections [$I \geq 2\ \sigma(I)$], 159 refined parameters, R = 0.041, wR_2 = 0.114, Flack parameter 0.4(3), max residual electron density 0.22 (−0.12) e Å^{-3}; hydrogen at nitrogen atom N2 was obtained from difference Fourier calculations, others were calculated and all refined as riding atoms.

(14) X-ray crystal structure analysis for **5h**: formula $C_{12}H_{11}NO_4$, M = 233.22, colorless crystal 0.50 × 0.05 × 0.03 mm, a = 10.300(1), b = 15.373(1), c = 7.235(1) Å, β = 98.49(1)°, V = 1133.0(2) Å^3, ρ_{calcd} = 1.367 g cm^{-3}, μ = 8.74 cm^{-1}, empirical absorption correction (0.669 ≤ T ≤ 0.974), Z = 4, monoclinic, space group $P2_1/c$ (No. 14), λ = 1.54178 Å, T = 223 K, ω and φ scans, 10254 reflections collected (±h, ±k, ±l), [(sin θ)/λ] = 0.59 Å^{-1}, 1609 independent (R_{int} = 0.071) and 928 observed reflections [$I \geq 2\ \sigma(I)$], 156 refined parameters, R = 0.076, wR_2 = 0.208, max residual electron density 0.39 (−0.26) e Å^{-3}, hydrogens were calculated and refined as riding atoms.

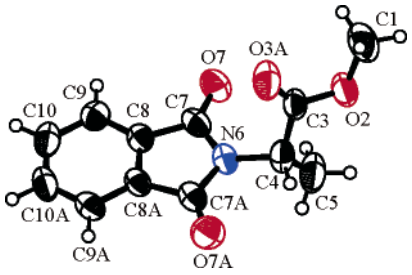


Figure 5. ORTEP diagram of **5h**.

The methyl esters **5** can be hydrolyzed to phthaloyl amino acids **2** under neutral conditions.[15] Finally, the phthaloyl protection can be removed by well-known procedures.[16]

The spectroscopic evidence and corresponding literature for reported compounds is given as Supporting Information.

In conclusion, we developed a highly efficient and stereoconservative amidation/deamidation protocol for chiral α-amino acids. Amidation and N-deprotection procedures lead to important building blocks for synthetic peptides. The deamidation procedure extends the peptide chain at the C-terminus. The mild and efficient nitrosoamide decomposition reaction in the presence of an *N*,*N*-dialkyl carbamate function could extend its use for other functional groups.

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Supporting Information Available: Experimental procedures; complete results of NMR and optimization studies, and full characterization of all compounds; NMR (^{1}H, ^{13}C, ^{19}F) spectra of new compounds and complete X-ray data for **3b**, **3h**, **3p**, **5h**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL048771L

(15) For review, see: Olah, G. A.; Narang, S. C. *Tetrahedron* **1982**, *38*, 2225−2277 and references cited therein.

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