Amino Acids (2010) 38:747–751
DOI 10.1007/s00726-009-0279-y

ORIGINAL ARTICLE

Preparation of *N*-acetyl, *tert*-butyl amide derivatives of the 20 natural amino acids

A. R. Ekkati · A. A. Campanali · A. I. Abouelatta · M. Shamoun · S. Kalapugama · M. Kelley · Jeremy J. Kodanko

Received: 14 January 2009 / Accepted: 11 March 2009 / Published online: 31 March 2009
© Springer-Verlag 2009

Abstract *N*-Acetyl-AA(amino acid)-NHtBu derivatives of all 20 naturally occurring amino acids have been synthesized. Syntheses were performed via solution-phase methodology with yields that allow for access to gram quantities of substrates, in most cases. Syntheses include the coupling of a hindered amine, *tert*-butylamine, with each amino acid, either directly or in two steps using an activated ester isolated as an intermediate. The introduction of protecting groups was necessary in some cases. The development of synthetic sequences to access challenging substrates, such as the one derived from asparagine, are discussed.

Keywords Amino acid substrate synthesis · *N*-Boc-amino acid · *N*-Fmoc-amino acid · Amino acid protection · Amino acid deprotection

Abbreviations

Ac	Acetyl
AA	Amino acid
OBn	Benzyl ester
Cbz	Benzyloxycarbonyl
Boc	*tert*-Butoxycarbonyl
tBu	*tert*-Butyl
DCC	1,3-Dicyclohexylcarbodiimide
EDAC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
DMF	Dimethylformamide
Fmoc	9-Fluorenylmethyloxycarbonyl
HOBt	1-Hydroxybenzotriazole
IBCF	Isobutylchloroformate
NMM	4-Methylmorpholine
PNP	*p*-Nitrophenol
HBTU	*O*-(Benzotriazol-1-yl)-*N,N,N,N′*-tetramethyluronium hexafluorophosphate
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
trityl or Trt	Triphenylmethyl

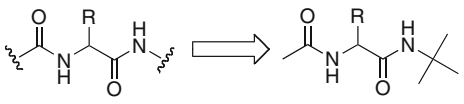
Electronic supplementary material The online version of this article (doi:10.1007/s00726-009-0279-y) contains supplementary material, which is available to authorized users.

A. R. Ekkati · A. A. Campanali · A. I. Abouelatta · M. Shamoun · S. Kalapugama · M. Kelley · J. J. Kodanko (✉)
Department of Chemistry, Wayne State University, 5101 Cass Ave., Detroit, MI 48202, USA
e-mail: jkodanko@chem.wayne.edu

Introduction

Reactive oxygen species, such as singlet oxygen or hydroxy radical, can damage proteins (Garrison 1987; Stadtman 1993). Due to the fact that this oxidative damage has a causative role in many disease states, as well as in aging, protein oxidation has been the focus of intense investigations and is well understood today (Berlett and Stadtman 1997). On the contrary, little is known about the reactivity of proteins with metal-based oxidants, wherein the reactive oxygen atom is bound to a metal center. In particular, there is not much information available about how metal-based oxidants react with individual amino acid residues (Ekkati and Kodanko 2007). This is far from ideal, because metal-based oxidants have been implicated in the targeted oxidation of proteins (Cuenoud et al. 1992; Brown et al. 1995) and have the potential to be widely useful in applications such as protein inactivation or footprinting.

In order to study the ability of metal-based oxidants to modify amino acid residues of polypeptides, we have



1 Ala	**8** Met	**15** Ser
2 Val	**9** Tyr	**16** Thr
3 Phe	**10** Trp	**17** His
4 Leu	**11** Gln	**18** Arg
5 Pro	**12** Lys	**19** Asn
6 Gly	**13** Glu	**20** Ile
7 Cys	**14** Asp	

Fig. 1 Structure of Ac-AA-NHtBu substrates that mimic amino acid residues of polypeptides

designed substrates of the general formula Ac-AA-NHtBu (Fig. 1, AA = amino acid) that mimic amino acid residues in a polypeptide chain. These substrates mimic residues by virtue of having their N- and C-termini blocked as amides, specifically, acetyl and *tert*-butyl, respectively. In this paper, we describe the synthesis of the aforementioned amino acid substrates derived from each of the 20, naturally occurring, amino acids (**1**–**20**). The synthetic routes to access substrates **1**–**20** rely on solution-phase methodology, and can be used to furnish substrates on multi-gram scale, leading to their ready availability for reactivity studies with metal-based oxidants.

Materials and methods

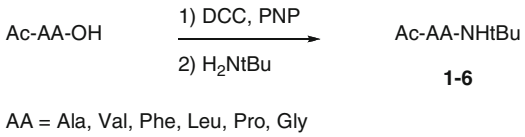
All reagents were purchased from commercial sources and used as received unless otherwise noted. *N*-acetyl amino acids and some protected amino acids were prepared according to previously reported literature (Chenault et al. 1989; Reddy and Ravindranath 1992; Ray et al. 2006). Other protected amino acids were purchased: Boc-Asp(OBn)-OH, Fmoc-Asn(Trt)-OH, Boc-His(Tos)-OH, and Boc-Arg(Tos)-OH. All experimental procedures can be found in the Supporting Information. Due to the fact that amino acids can racemize during *N*-acetylation (Reddy and Ravindranath 1992) and enantiopure substrates were not required for oxidation studies, optical rotation data, and measurements of enantiopurity were not collected. NMR spectra were recorded on a Varian FT-NMR Unity-300, Mercury-400 or 500 MHz Spectrometer. Mass spectra were recorded on a Waters ZQ2000 single quadrupole mass spectrometer using an electrospray ionization source. IR spectra were recorded on a Nicolet FT-IR spectrophotometer.

Results and discussion

Although the synthetic target for substrates **1**–**20** are identical, the methods used to access these substrates varied depending on the particular amino acid. The *tert*-butylamide was chosen because it prevents oxidation adjacent to the nitrogen atom of the C-terminal amide. This necessity presented an additional challenge in forming amide bonds with some substrates, due to the steric hindrance of *tert*-butylamine. To overcome this challenge, four general methods were used to access substrates **1**–**20**, Methods A, B, C, and D. All methods involve the condensation of *tert*-butylamine with some form of the amino acid. Method A involves the condensation of an *N*-acetyl amino acid with a *p*-nitrophenol ester. Method B employs EDAC or DCC/HOBt to achieve condensation. Method C starts with *tert*-butoxycarbonyl protected *N*-terminal amino acid, instead of an *N*-acetyl, and utilizes benzyl and tosyl protecting groups for the side chains. Substrates **19** and **20** required unique syntheses and are discussed separately in Method D.

Method A

N-Acetylated amino acids of Ala, Val, Phe, Leu, Pro, and Gly were condensed with *p*-nitrophenol using DCC as the coupling reagent to form activated *p*-nitrophenol esters (Scheme 1). Each activated ester was isolated and purified by recrystallization before treatment with excess *tert*-butylamine, which furnished the corresponding C-terminal amides. This procedure was advantageous because the salt $(tBuNH_3)(OC_6H_4NO_2)$ was removed easily by filtration after the condensation was complete, allowing access to the final substrates in good to moderate yields (Table 1).



Scheme 1 Syntheses of substrates **1**–**6** via an isolated *p*-nitrophenol ester

Table 1 Conditions and yields for formation of *p*-nitrophenol esters and condensation products for **1**–**6**

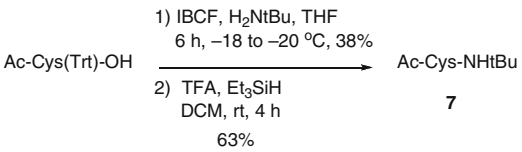
Ac-AA-NHtBu AA=	Ac-AA-OPNP yield[a] (%)	Condensation yield[a]
Ala (**1**)	42	71
Val (**2**)	79[b]	40
Phe (**3**)	63[b]	62[b]
Leu (**4**)	60[b]	61
Pro (**5**)	73[b]	66
Gly (**6**)	44[b]	61

[a] rt unless otherwise stated

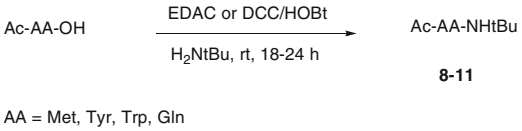
[b] 50°C to rt

Method B

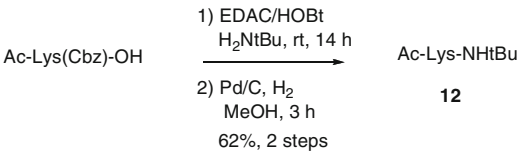
In this method, the *N*-acetyl amino acids of Cys, Met, Tyr, Trp, Gln, and Lys were converted to substrates **7–12** by direct condensation with *tert*-butylamine using DCC, EDAC, IBCF, or EDAC/HOBt as coupling reagents (Schemes 2, 3, 4). Synthesis of Cys derived substrate **7** began with the condensation of Ac-Cys(Trt)-OH and *tert*-butylamine utilizing the coupling reagent isobutylchloroformate (Scheme 2). After amide bond formation, the trityl protecting group was removed in moderate yield to produce the desired Ac-Cys-NHtBu substrate **7**. Final substrates **8–11** were purified by recrystallization. The lower isolated yields observed in some cases resulted from the recrystallization process, not from inefficient condensation reactions (Table 2).



Scheme 2 Synthesis of substrate **7** via direct condensation employing the coupling reagent isobutylchloroformate; starting from *N*-acetylated and trityl protected cysteine



Scheme 3 Synthesis of substrates **8–11** via direct condensation; starting from *N*-acetyl amino acids



Scheme 4 Synthesis of **12** via direct condensation of *N*-acetyl-Lys(Cbz)-OH with *tert*-butylamine followed by hydrogenation of the CBz protecting group

Table 2 Yields and conditions for the synthesis of substrates **8–11**

Ac-AA-NHtBu AA=	Condensation yield (%)
Met (**8**)	45[a]
Tyr (**9**)	37[b]
Trp (**10**)	48[c]
Gln (**11**)	56[c]

[a] DCC

[b] EDAC/HOBt

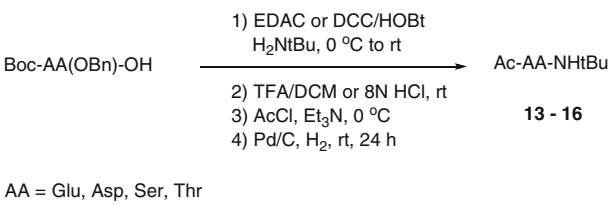
[c] EDAC

For Lys derived substrate **12**, the amine side chain was protected with a Cbz group. After condensation of Ac-Lys(Cbz)-OH with *tert*-butylamine, the Cbz protecting group was removed by catalytic hydrogenation over Pd/C (Scheme 4). It is interesting that low yields were obtained during the condensation when the base employed was Et_3N, but increased when the base was changed to NMM (LeTiran et al. 2002).

Method C

Functional groups present in the side chains of Glu, Asp, Ser, and Thr created the potential for undesired side reactions such as diacetylation or ring formation. For example, during initial attempts to synthesize **15** and **16** from *N*-acetyl derivatives, low yields were observed in the condensation reaction when the hydroxyl groups of the Ser and Thr substrates were not protected. This complication lead to the use of protected amino acids (Boc-AA(OBn)-OH) that are commercially available as the starting point. Each protected amino acid in this series was condensed with *tert*-butylamine using either EDAC or DCC/HOBt at 0°C to rt (Scheme 5) to form the corresponding C-terminal amide in good to moderate yields (Table 3). Subsequent removal of the *N*-Boc group with either TFA/CH_2Cl_2 or 8 N HCl/THF followed by treatment with acetyl chloride furnished the *N*-acetyl amino acids. To complete the sequence, the benzyl groups were removed by catalytic hydrogenation over Pd/C, yielding substrates **13–16**. It is important to note that a single diastereomer of **16** was formed using this synthetic pathway, consistent with no epimerization of the α-center occurring under these coupling conditions.

Syntheses of substrates **17** and **18** derived from His and Arg, respectively, began from *N*-Boc amino acids that possessed *N*-toluenesulfonyl protection on the side chains. These Boc-AA(Tos)-OH amino acid derivatives were condensed with *tert*-butylamine using HBTU (Scheme 6) to provide the C-terminal amide in good to moderate yields. The *N*-Boc groups were removed and the resultant amines were acetylated. Removal of the *N*-toluenesulfonyl



Scheme 5 Synthesis of substrates **13–16** via Boc-AA(OBn)-OH

Table 3 Yields for condensation, acetylation, and removal of side chain protecting group for the formation of substrates **13–16**

Ac-AA-NHtBu AA=	Boc-AA(OBn) NHtBu yield (%)	Ac-AA(OBn)-NHtBu yield, two steps[c] (%)	Ac-AA(OBn)-NHtBu deprotection yield (%)
Glu (**13**)	53[a]	61[d]	96
Asp (**14**)	53[a]	60[d]	96
Ser (**15**)	56[a]	42[e]	96
Thr (**16**)	87[b]	62[e]	95

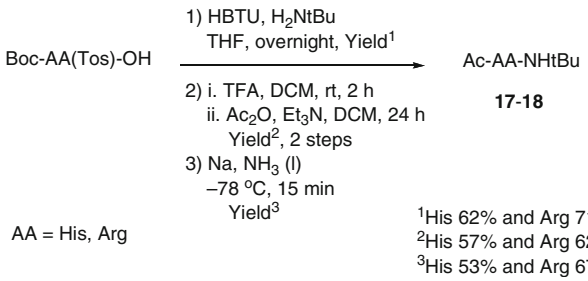
[a] EDAC

[b] HOBt/DCC

[c] Boc deprotection followed by acetylation

[d] TFA/CH_2Cl_2

[e] 8 N HCl/THF



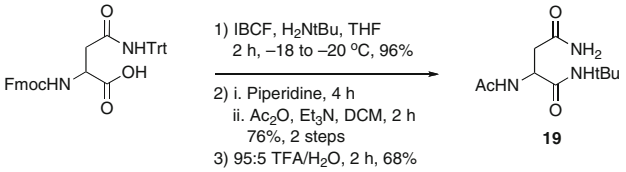
Scheme 6 Synthesis of substrates **17** and **18** via Boc-AA(Tos)-OH

protecting groups of Ac-His(Tos)-NHtBu and Ac-Arg(Tos)-NHtBu was straightforward using Na and liquid ammonia to produce **17** and **18** with moderate to excellent yields; **18** was isolated as the HCl salt.

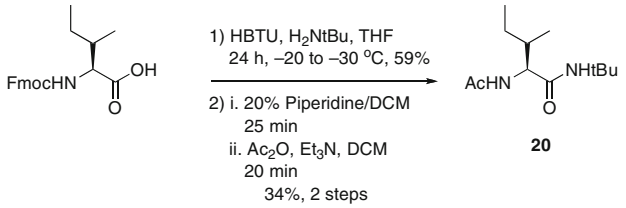
Method D

Syntheses of substrates derived from Asn and Ile, **19** and **20**, respectively, proved to be particularly challenging. The final sequences to these two substrates are included below, along with a description of dead-end routes that did not allow access to these substrates.

The initial synthetic route for Asn derived substrate **19** followed the same pathway as that of the Gln substrate **11** (Scheme 3). However, the condensation reactions with Ac-Asn-OH and *tert*-butylamine using EDAC furnished only trace amounts of product under a variety of conditions, presumably due to complications of ring formation (Jones 2002). The second route toward substrate **19** made use of the availability of Ac-Asp(OBn)-NHtBu, an intermediate in the synthesis of **14**. In this route, transformation of the benzyl ester into an amide using methanolic ammonia was attempted (Swayze and Townsend 1995; Ramasamy et al. 1987). It was noted that the benzyl ester also converted to a



Scheme 7 Synthesis of Asn derivative substrate **19** via *N*-Fmoc and trityl protection



Scheme 8 Synthesis of Ac-Ile-NHtBu via *N*-Fmoc protection

methyl ester in addition to desired amide **19**. This methyl ester gave incomplete conversion to the amide, even upon subjection to higher temperatures and longer reaction times. Attempts to isolate **19** from the methyl ester in pure form were unsuccessful. The third and final route to **19** started from Fmoc-Asn(Trt)-OH (Scheme 7). After careful optimization, condensation of *tert*-butylamine with Fmoc-Asn(Trt)-OH using isobutyl chloroformate, NMM, and a reaction temperature of −20°C furnished the amide product Fmoc-Asn(Trt)-NHtBu in 96% yield. Removal of the *N*-Fmoc group using piperidine and acetylation of the resultant primary amine with acetic anhydride furnished Ac-Asn(Trt)-NHtBu. To complete the synthesis of **19**, the *N*-trityl group was removed using 95:5 TFA/H_2O, which was advantageous because the triphenylmethanol byproduct was easily removed by precipitation upon the addition of H_2O.

The second challenging substrate to synthesize was **20**, a derivative of Ile. In the synthesis of **20**, amide bond formation was facile, but epimerization at the α-stereocenter and formation of diastereomeric mixtures proved to be challenging to suppress. Initial acetylation of Ile was carried out using acetic anhydride and acetic acid; these reaction conditions gave mixtures of diastereomers due to epimerization at the α-carbon, presumably proceeding through an oxazolone intermediate. It is known that under basic conditions, amino acids can be acetylated without epimerization (Reddy and Ravindranath 1992). Therefore, Ile was acetylated using acetic anhydride with the addition of NaOH at low temperature to keep the reaction at alkaline pH. Although this procedure gave the single diastereomer Ac-Ile-OH, upon condensation with *tert*-butylamine, epimerization was again noted. Different coupling reagents and conditions were screened in an attempt to generate

a single diastereomer from Ac-Ile-OH, including EDAC, EDAC/HOBt, and HBTU. All conditions were found to result in formation of diastereomeric mixtures. Condensation of *tert*-butylamine with the succinimide ester Ac-Ile-OSu or with Boc-Ile-OH gave similar results (Ray et al. 2006). Fortunately, condensation of Fmoc-Ile-OH and *tert*-butylamine using the coupling reagent HBTU at −20°C furnished, exclusively, a single diastereomer (Scheme 8). Upon removal of the *N*-Fmoc group with 20% piperidine/CH_2Cl_2, the resultant primary amine was acetylated with acetic anhydride in the presence of triethylamine, furnishing **20** in a moderate yield over two steps. The difficulty encountered in suppressing epimerization at the α-center with the Ile substrate was likely due to oxazolone formation and deprotonation, which can result in facile epimerization, becoming competitive with amide bond formation. This effect could be due to a slower condensation with the bulky nucleophile *tert*-butylamine, with respect to other amines that have been used successfully in the past.

Conclusion

In summary, synthetic routes to access Ac-AA-NHtBu derivatives **1**–**20**, where AA represents each of the natural amino acids, have been developed. Due to the fact that these derivatives posses amides on the C- and N-termini, they are good models for residues in a peptide chain. Unlike solid-phase methods, the solution-phase syntheses described herein provide ready access to gram quantities of these amino acid derivatives in most cases as well as single diastereomers in some cases. With synthetic access to substrates **1**–**20** developed, their reactivity with a variety of metal-based oxidants can now be defined. Studies of this type are currently underway in our laboratory and will be reported in due course.

References

Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272:20313–20316. doi:10.1074/jbc.272.33.20313

Brown KC, Yang S-H et al (1995) Highly specific oxidative crosslinking of proteins mediated by a nickel-peptide complex. Biochemistry 34:4733–4739. doi:10.1021/bi00014a030

Chenault HK, Dahmer J et al (1989) Kinetic resolution of unnatural and rarely occurring amino acids: enantioselective hydrolysis of *N*-acyl amino acids catalyzed by acylase I. J Am Chem Soc 111:6354–6364. doi:10.1021/ja00198a055

Cuenoud B, Tarasow TM et al (1992) A new strategy for directed protein cleavage. Tetrahedron Lett 33:895–898. doi:10.1016/S0040-4039(00)91569-7

Ekkati AR, Kodanko JJ (2007) Targeting peptides with an iron-based oxidant: cleavage of the amino acid backbone and oxidation of side chains. J Am Chem Soc 129:12390–12391. doi:10.1021/ja075075i

Garrison WM (1987) Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. Chem Rev 87:381–398

Jones J (2002) Amino acid and peptide synthesis. Oxford University Press, New York

LeTiran A, Stables JP et al (2002) Design and evaluation of affinity labels of functionalized amino acid anticonvulsants. J Med Chem 45:4762–4773. doi:10.1021/jm020225f

Ramasamy K, Kini GD et al (1987) Nucleoside peptides. 8. Synthesis of certain peptide derivatives of ribavirin and tiazofurin. Nucleosides Nucleotides 6:901–911. doi:10.1080/15257778708073436

Ray S, Drew MGB et al (2006) The role of terminal tyrosine residues in the formation of tripeptide nanotubes: a crystallographic insight. Tetrahedron 62:7274–7283. doi:10.1016/j.tet.2006.05.042

Reddy AV, Ravindranath B (1992) Acetylation under ultrasonic conditions: convenient preparation of *N*-acetyl amino acids. Synth Commun 22:257–264. doi:10.1080/00397919208021300

Stadtman ER (1993) Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu Rev Biochem 62:797–821. doi:10.1146/annurev.bi.62.070193.004053

Swayze EE, Townsend LB (1995) The synthesis of substituted imidazo[4, 5-d]isothiazoles via the ring annulation of isothiazole diamines: an investigation of the chemical, physical, and biological properties of several novel 5:5 fused analogs of the purine ring system. J Org Chem 60:6309–6317. doi:10.1021/jo00125a016