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An Efficient and High Yield Method for the *N-tert*-Butoxycarbonyl Protection of Sterically Hindered Amino Acids

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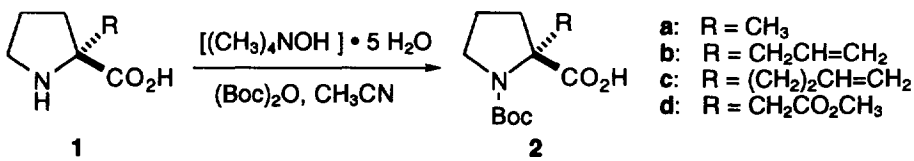
Abstract: An improved method for the *N-tert*-butoxycarbonyl protection of the amino functionality of α-alkylated prolines and other sterically hindered α,α-disubstituted amino acids has been developed in which the lipophilic base tetramethylammonium hydroxide is used to solubilize the otherwise insoluble zwitterionic amino acid in acetonitrile, thereby obviating the need for an aqueous medium.

The use of $C^{\alpha,\alpha}$-disubstituted amino acids has proved to be a valuable tool in the design of peptidomimetics. The incorporation of these conformationally restricted building blocks into bioactive peptides provides a means for controlling their conformational flexibility and for studying their biologically relevant conformation.[1-5] Due to the sterically hindered nature of $C^{\alpha,\alpha}$-disubstituted amino acids, problems are often encountered in protecting their amino group and in affecting their coupling into peptides.[6-8] For example, the preparation of *N-tert*-butoxycarbonyl-α-aminoisobutyric acid (Boc-Aib-OH) and *N-tert*-butoxycarbonyl-1-aminocyclopentane-1-carboxylic acid (Boc-Ac_5c-OH) using standard conditions [NaOH, dioxane, H_2O and di-*tert*-butyl dicarbonate (Boc_2O)] has been reported to go in a yield of only 51% and 61%, respectively, as compared with the greater than 90% yields generally obtained with typical α-amino acids under the same reaction conditions.[6] In another example where *tert*-butoxycarbonylazide was used with tetramethylguanidine in DMSO, *N-tert*-butoxycarbonyl-α-methylphenylalanine and *N-tert*-butoxycarbonyl-α-methylvaline were prepared in 70% and 13% yield, respectively. Although this method was reported as an improvement over other attempted methods, it suffered from a very long reaction time (3 weeks) and inconsistent results.[7] More recently, the synthesis of *N-tert*-butoxycarbonyl-2,3-methano arginine was carried out using excess Boc_2O and NaOH in a mixture of t-BuOH/H_2O in a 40-60% yield.[8]

In trying to prepare *N-tert*-butoxycarbonyl-α-allyl proline (**2b**) and other *N-tert*-butoxycarbonyl-α-alkylated prolines (Scheme 1) as key intermediates in the synthesis of conformationally constrained analogs of the dopamine receptor modulatory peptide Pro-Leu-Gly-NH_2,[9] we observed yields (40-60%) comparable to those cited above when standard reaction conditions (NaOH, dioxane, H_2O, and Boc_2O) were used. Since the effort invested in preparing the enantiomerically pure α-alkylated prolines **1a-d** was considerable,[10] these yields were deemed unsatisfactory, thereby prompting us to develop a more efficient method. We initially experimented with adding a several fold excess (3-5 equivalents) of Boc_2O under the standard reaction

conditions, but observed only a modest improvement in yield. We suspected that because of the aqueous conditions and the slow course of the reaction (3-4 days) the Boc_2O was being hydrolyzed before it could react with the α-alkylated proline. We felt that the lifetime of Boc_2O in the reaction mixture could be prolonged by carrying out the reaction in an organic solvent. However, it was recognized that the poor solubility of the zwitterionic amino acid in aprotic solvents would be a limiting factor. To overcome the solubility problem, the lipophilic base tetramethylammonium hydroxide (TMAH) was employed. Formation of the tetramethylammonium salt of an α-alkylated proline amino acid enhanced the amino acid's solubility in organic solvents and therefore allowed for the reaction to be carried out in dry acetonitrile. These reaction conditions[11] afforded excellent yields of the *tert*-butoxycarbonyl-protected α-substituted prolines **2a-d** (Scheme 1) as summarized in Table 1. The yields obtained when the reaction was scaled up to as much as 45 mmoles of amino acid were comparable to those reported herein at 1 mmole.

Scheme 1.



1 **2**

Table 1. Yields and chemical properties of *tert*-butoxycarbonyl-protected amino acids.[a]

no.	compound	% yield	m.p.,°C	$[\alpha]_D^{25}$
2a	Boc-L-α-Me-Pro-OH	98[b]	129-132	-41.4° (*c* 1.45, $CHCl_3$)
2b	Boc-L-α-Allyl-Pro-OH	91[b,c], 61[d]	116-118[e]	+68.8° (*c* 0.78, MeOH)[e]
2c	Boc-L-α-(3-Butenyl)-Pro-OH	97[b,c]	90-91	+36.2° (*c* 2.13, MeOH)
2d	Boc-L-α-($CH_2CO_2CH_3$)-Pro-OH	100[f]	oil	+50.5° (*c* 0.52, $CHCl_3$)
3	Boc-Ac_3c-OH	92[f]	179-180[g]	
4	Boc-Ac_5c-OH	88[b,c]	133-135[h]	
5	Boc-*N*-Me-Aib-OH	95[f]	147-148[i]	
6	(±)-Boc-Pip-OH	100[f]	129-131[j]	

[a] All compounds gave satisfactory analytical and ^1H NMR and ^{13}C NMR spectroscopic data. Analytical data the for newly synthesized compounds **2a**, **2c**, and **2d** are given in ref. 12. [b] Yield of recrystallized product. [c] Yield reported is an average of two runs. [d] Yield obtained using TMAH as a 25% solution in MeOH. [e] Lit.[9] m.p. 118-119°C, $[\alpha]_D$ +72.5° (*c* 1.2, MeOH). [f] Yield obtained after extensive drying of the reaction product *in vacuo*. [g] Lit.[13] m.p. 176-177°C. [h] Lit.[6] m.p. 130.5-131.5°C. [i] Lit.[6] m.p. 153.0-153.5°C. [j] Lit.[14] m.p. 126-127°C.

Optimal results were obtained when 1.5 equivalents of $(Boc)_2O$ were used initially followed by an additional 0.5 equivalents of $(Boc)_2O$ two days later. When lower amounts of $(Boc)_2O$ were used lower yields would result. For example, when **2c** was made using only 1.2 equivalents of $(Boc)_2O$ a yield of 82% was obtained. This is in contrast to the 97% yield obtained when a total of 2 equivalents was used. Also, experiments were conducted using TMAH either as the solid pentahydrate or as a 25% solution in methanol (both reagents were purchased from the Aldrich Chemical Company). As can be seen by entry **2b** in Table 1, better results were obtained when the pentahydrate form was used. Accordingly this form of TMAH was used in the preparation of all the other *tert*-butoxycarbonyl-protected sterically hindered amino acids.

Because of the success in preparing *tert*-butoxycarbonyl-α-substituted prolines through the use of the new method described above, the method was tried on other α-substituted amino acids in which difficulties previously had been encountered in protecting the amino group. Thus, 1-aminocyclopropane-1-carboxylic acid (Ac_3c), 1-aminocyclopentane-1-carboxylic acid (Ac_5c), *N*-methyl-α-aminoisobutyric acid (*N*-Me-Aib), and pipecolic acid (Pip) were all reacted with $(Boc)_2O$ and TMAH to give the *tert*-butoxycarbonyl-protected derivatives **3-6**, respectively. As shown in Table 1, very high yields were achieved in each case.

In summary, a new, simple, and efficient method for the *tert*-butoxycarbonyl protection of hindered amino acids has been developed. As illustrated in Table 2 much improved yields were obtained with this new method in comparison to yields reported previously with other methods. As demonstrated by the variety of hindered amino acids investigated in this study, this method should find application with other sterically hindered amino acids.

Table 2. Comparison of the yields for *tert*-butoxycarbonyl-protection of sterically hindered amino acids.

	% yield	
compound	present work	previously reported
2b	91	64[9]
3	92	85[13]
4	88	61[6]
6	100	60[14]

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11. **General procedure:** Free α,α-disubstituted amino acid (1.0 mmol) and TMAH (1.0 mmol) were added to CH_3CN (5-10 ml) which had been freshly distilled from CaH_2. The mixture was stirred at room temperature until a solution was formed (generally, solutions formed within 30 minutes with the exception of entry **4** which never dissolved completely). Boc_2O (1.5 mmol) was then added and stirring was continued for 2 days. On the third day, another 0.5 mmol of Boc_2O was added and the mixture stirred for another day. The CH_3CN was removed *in vacuo* and the residue was partitioned between H_2O and Et_2O. The aqueous layer was washed with an additional portion of Et_2O and then acidified with solid citric acid to pH 3-4. The aqueous solution was extracted three times with EtOAc. The combined organic extracts were washed with H_2O, dried ($MgSO_4$) and the EtOAc then removed *in vacuo* to give the *tert*-butoxycarbonyl-protected amino acid as a white solid that was recrystallized from Et_2O (compounds **2a-c** and **4**). In the case of compounds **3**, **5**, and **6**, recrystallization was not carried out as the material obtained in each case had a m.p. that matched a previously reported m.p. (see Table 1) and the spectral data was consistent with the proposed structure. Compound **2d** was obtained as a clear oil and attempts at crystallization were unsuccessful. However, the material was shown to be pure by ^1H and ^{13}C NMR and elemental analysis.
12. Rotamers are observed about the carbamate bond in both ^1H and ^{13}C NMR spectra for **2a**, **2c**, and **2d**.
***N-tert*-Butoxycarbonyl-L-α-methyproline (2a).** ^1H NMR (300MHz, $CDCl_3$): δ 1.39, 1.42 (s, 9H, Boc); 1.49, 1.57 (s, 3H, α-CH_3); 1.82-1.94 (m, 3H, β-CH_2 and γ-CH_2); 2.20-2.45 (m, 1H, β-CH_2); 3.38-3.62 (m, 2H, δ-CH_2); 11.6-11.8 (br s, 1H, COOH). ^{13}C ($CDCl_3$): δ 22.85, 23.66 (γ-C); 23.45 (α-CH_3); 28.93, 29.06 (Boc CH_3); 39.56, 40.96 (β-C); 48.42, 48.99 (δ-C); 65.40, 66.40 (α-C); 81.09, 81.28 (Boc C-O); 154.31, 155.82 (Boc C=O); 179.40, 181.69 (COOH). Anal. Calcd for **2a** ($C_{11}H_{19}NO_4$): C, 57.62; H, 8.35; N, 6.11. Found: C, 57.86; H, 8.65; N, 6.19.
***N-tert*-Butoxycarbonyl-L-α-(3-butenyl)-proline (2c).** ^1H NMR (300MHz, $CDCl_3$): δ 1.42, 1.49 (s, 9H, Boc); 1.7-2.3 (m, 7H, β-CH_2, γ-CH_2, and $CH_2CH_2CH{=}CH_2$); 2.70-2.78 (m, 1H, $CH_2CH_2CH{=}CH_2$); 3.26-3.78 (m, 2H, δ-CH_2); 4.94-5.06 (m, 2H, $CH{=}CH_2$); 5.70-5.86 (m, 1H, $CH{=}CH_2$); 11.05 (br s, 1H, COOH). ^{13}C ($CDCl_3$): δ 23.35 (γ-C); 28.56, 29.02 (Boc CH_3); 34.20, 34.66, 35.74, 38.15, and 38.20 (β-C and $CH_2CH_2CH{=}CH_2$); 49.22, 49.95 (δ-C); 70.92 (α-C); 82.73 (Boc C-O); 115.31, 115.83 ($CH{=}CH_2$); 137.87, 138.69 ($CH{=}CH_2$); 157.73 (Boc C=O); 175.39 (COOH). Anal. Calcd for **2c** ($C_{14}H_{23}NO_4$): C, 62.43; H, 8.61; N, 5.20. Found: C, 62.52; H, 8.46; N, 5.46.
***N-tert*-Butoxycarbonyl-L-α-(methoxycarbonylmethyl)-proline (2d).** ^1H NMR (300MHz, $CDCl_3$): δ 1.42, 1.44 (s, 9H, Boc); 1.8-2.1 (m, 2H, γ-CH_2); 2.3-2.8 (m, 2H, β-CH_2); 2.9-3.3 ($CH_2CO_2CH_3$); 3.35-3.61 (m, 2H, δ-CH_2); 3.65 (s, 3H, OCH_3); 10.99 (br s, 1H, COOH). ^{13}C ($CDCl_3$): δ 23.29, 23.68 (γ-C); 28.89, 29.00 (Boc CH_3); 36.52, 38.37, 38.09, and 39.77 (β-C and CH_2CO_2Me); 48.69, 49.12 (δ-C); 52.38 (OCH_3); 66.20, 67.05 (α-C); 81.56, 81.66 (Boc C-O); 153.9, 155.8 (Boc C=O); 171.60 (CO_2Me); 177.72, 179.89 (COOH). Anal. Calcd for **2d** ($C_{13}H_{21}NO_6$): C, 54.35; H, 7.37; N, 4.88. Found: C, 54.17; H, 7.25; N, 4.60.
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