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# One-pot synthesis of *N*-Cbz-L-BMAA and derivatives from *N*-Cbz-L-serine

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**Abstract**—We report herein an asymmetric synthesis of the modified amino acid *N*-Cbz-L-BMAA and seven of its alkyl derivatives (**2a**–**h**) from *N*-Cbz-L-serine via ring-opening of the β-lactone (formed under modified Mitsunobu conditions) by different amines. This procedure is simple, one-pot and can generate various derivatives that can be investigated for their toxicological effects. In addition, it can be employed to produce analytical standards for water monitoring as well as labeled compounds for biotransformation studies. This toxin has been the focus of serious ecological and public concern since its implication in degenerative disease such as Alzheimer and Parkinsonism dementia.

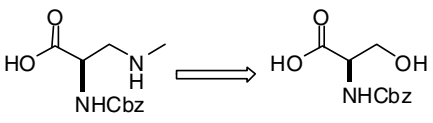

The modified amino acid β-*N*-methylamino-L-alanine (L-BMAA) plays an important role in degenerative diseases.[1] The neurotoxicity of L-BMAA is due to an excitotoxic mechanism, involving elevated intracellular calcium and bicarbonate levels.[2] This amino acid is also a potent glutamate receptor agonist.[3] Its mechanism of action seems to be mediated, in part, by the activation of *N*-methyl-D-aspartate (NMDA) receptors.[4] This non-protein amino acid is neurotoxic and is produced as a secondary metabolite by seeds of the indigenous Guam cycad (*Cycas micronesica*).[5] This effect was evidenced by one study with Chamorro natives of Guam that compared the increase of incidence rates of ALS/PDC (amyotrophic lateral sclerosis/Parkinsonism dementia complex) with the consumption of seeds.[6,7] More recently, it was reported that cyanobacteria of the genus *Nostoc*, which are a root symbiont of cycads, also have the ability to biosynthesize this important metabolite.[7] Human poisonings from cyanobacterial blooms and their toxins (i.e., microcystins, saxitoxins, cylindrospermopsin and others) are well known.[8,9]

The first synthesis reported for this molecule produced a racemic mixture via the methylation of α-acetoamino-β-methylaminopropionic acid, followed by hydrolysis.[10] Later, Ratemi and Vederas[11] employed trimethylsilylamines for the cleavage of lactones in order to obtain the *N*-Cbz-β-amino-L-alanine. In this way, they guaranteed the production of the desired optically pure form.

The aim of this work is the asymmetric synthesis of *N*-Cbz-L-α-amino-β-methylaminopropionic acid and seven of its other *N*-alkyl derivatives from *N*-Cbz-L-serine, with low synthetic cost and significant yields. The retrosynthesis of *N*-Cbz-L-BMAA is proposed in Scheme 1. In order to do this, we use direct addition of amines to open lactones formed under Mitsonubu conditions (Scheme 2). The synthesis of this molecule is very important for further toxicological investigation and for the production of analytical standards.

Some derivatives and labeled analogues can also be used for the elucidation of its mechanism of action, which is not yet completely understood.

General methods for the synthesis of β-substituted amino acids are relatively difficult because of stereochemical




***N*-Cbz-*L*-BMAA** ***N*-Cbz-*L*-serine**

**Scheme 1.** Retrosynthetic analyses of *N*-Cbz-L-BMAA.

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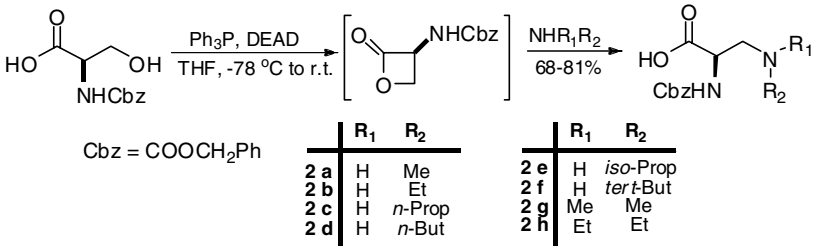


**Scheme 2.** Synthesis of *N*-Cbz-L-BMAA and derivatives.

control, poor yields or the necessity of numerous steps. For instance, to produce the mono-methylated amine *N*-Boc-L-BMAA as an intermediate for the total synthesis of target drugs, Gopin et al.[12] utilized DAP (Boc-L-2,4-diaminopropionic acid) as starting material in a three steps synthetic procedure with low overall yields.

Several alternative procedures for the synthesis of modified amino acids have been proposed in the last years. By using L-serine, Chhabra et al.[13] reported one efficient method via oxazolidine formation, a complex synthetic process leading to N-protected amino acids, but without stereoselective control, after oxidation by Jones reagent. In another method, the transformation of L-asparagine using iodobenzene diacetate produced via Hoffmann rearrangement[14] the product *N*-Cbz-3-dimethylamino-L-alanine. However, the use of costly reagents and the complex alkylation of primary amines, which may lead to isomer formation due to the nonselective alkylation, make this method complicated.

The use of β-lactones derived from serine in organic synthesis is functional and widely employed, mainly in the production of modified amino acids used as intermediate in synthesis. Venderas et al. confirmed the application of lactones to produce modified amino acids.[11,15] More recently, Schneider et al.[16] also applied this procedure, through a lactone intermediate, to obtain selenium and tellurium substituted amino acids. Nevertheless, the formation of this lactone depends on controlled conditions, which can complicate the execution of this procedure. Another difficulty with the traditional Mitsunobo conditions is the purification, since the product polarity and that of diethyl hydrazodicarboxylate (formed from DEAD) are almost the same. These problems can be solved by a simple procedure; make this reaction a one-pot process by adding to the same flask the nucleophilic agent after the formation of the lactone intermediate. With this one-pot procedure, it is possible to directly produce the desired compound that is easier to isolate and purify afterwards. In addition, Arnol et al. had already used ammonia to open these lactones[15] and described the use of powerful nucleophiles such as methoxide and ammonia to react with carbonyl groups. However, by simply changing solvent composition, it is possible to invert the proportion of the formation of amide/amine (in the case of ammonia). Other authors had also discussed and applied different strategies to obtain only amino acids,[11] where trimethylsilylamines were employed as nucleophilic agents, well known to be weaker nucleophiles.

In our hands, bubbling gaseous alkylamine derivatives through the reaction mixture leads only to the amine derivatives **2a**–**h** under one-pot conditions.[17] The experimental conditions utilized were similar to those of Ratemi and Vederas[11] (THF as a solvent and low temperature for the addition of amine, 0 °C).

The great advantage of the procedure developed here is the retention of the serine stereochemistry, which was solvent dependent in the work of Ratemi and Vederas.[11] Moreover, our strategy joined the functionality of a one-pot procedure for product formation with mild conditions, leading to goods yields, from 68% to 81%. In the case of **2a**, our method showed a 78% of yield whereas in a previous report, which uses two steps, it was 70%[11] for purified molecules. Our approach reduces common problems present in other methodologies (i.e., low stability) that use lactones from serine as intermediates.[11,15–17] This efficient method is appropriate for obtaining *N*-Cbz-L-BMAA, **2a**, and generates the cyanotoxin L-BMAA. The production of this compound is very important for toxicological assays as well as an analytical standard in water monitoring. The analogues of *N*-Cbz-L-BMAA, **2b**–**h**, produced by using different alkylating agents in gaseous form, can be prepared for assay in toxicological experiments. In conclusion, our method is more efficient than previous published routes that use Mitsunobu lactone intermediate.[11,15–17] In addition, these compounds can be easily used as intermediates in other procedures of total synthesis. All products were identified by $^{1}H$ and $^{13}C$ NMR and ESI-MSn.[18]

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17. *N-(Benzyloxycarbonyl)-β-alkylamine-L-alanine* (**2a**–**h**) *general procedure*: To a stirred solution of dried $Ph_3P$ (6.43 g, 24.5 mmol) in 100 mL of anhydrous THF at −78 °C was added distilled diethyl azodicarboxylate (2.70 mL, 3.58 g, 24.5 mmol) dropwise over 10 min. After 10 min, a solution of dried L-serine (5.84 g, 24.4 mmol) in 100 mL of THF was added dropwise over 15 min to the stirred slurry at −78 °C. The mixture was stirred for 20 min at −78 °C and then for 2.5 h at 20 °C.[14] Subsequently, dry amine $NHR_1R_2$ was bubbled (~0.3 mL/min) through this solution stirred at 0 °C for 30 min. Afterward, the solvent was removed by vacuum, and the residue was chromatographed on silica gel. The product was eluted after other compounds with MeOH. The product could be recrystallized with MeOH/$Et_2O$.
18. *N-(Benzyloxycarbonyl)-β-methylamine-L-alanine* (**2a**): Compound **2a** was isolated with 78% of yield; mp 54 °C; $^1$H NMR (300 MHz, $CDCl_3$, ppm) $\delta$ 2.70 (s, 3H, $NCH_3$), 3.58 (dd, 1H, $J^1 = 6.25$, $J^2 = 4.65$), 4.00 (dd, 1H, $J^1 = 11.47$, $J^2 = 3.87$), 4.11 (dd, $J = 3.20$, 1H), 5.03 (s, 2H, $CH_2$Ph), 7.38–7.21 (m, 5H, Ph); $^{13}$C NMR (75 MHz, $CDCl_3$, ppm) $\delta$ 25.3, 54.3, 61.7, 66.4, 127.2 (2C), 127.4, 127.6 (2C), 134.9, 155.8, 170.6; ESI-MS 275 $[M+Na]^+$, 252.9 $[M+H]^+$, 208.9 $[M-COOH+H]^+$, 91.3 $[PhCH_2]^+$.
*N-(Benzyloxycarbonyl)-β-ethylamine-L-alanine* (**2b**): Compound **2b** was isolated with 76% of yield; mp 128 °C; $^1$H NMR (300 MHz, $CDCl_3$, ppm) $\delta$ 1.05 (t, 3H, $J = 7.08$, $NCH_2CH_3$), 3.21 (q, t, 2H, $J = 7.08$, $NCH_2CH_3$), 3.58 (dd, 1H, $J^1 = 6.31$, $J^2 = 4.56$), 4.05 (dd, 1H, $J^1 = 11.53$, $J^2 = 3.85$), 4.11 (dd, $J = 3.25$, 1H), 5.06 (s, 2H, $CH_2$Ph), 7.28 (m, 5H, Ph); $^{13}$C NMR (75 MHz, $CDCl_3$, ppm) $\delta$ 13.6, 22.5, 54.2, 61.8, 66.4, 127.1 (2C), 127.4, 127.6 (2C), 134.9, 155.8, 169.8; ESI-MS 289 $[M+Na]^+$, 266.9 $[M+H]^+$, 222.9 $[M-COOH+H]^+$, 91.3 $[PhCH_2]^+$.
*N-(Benzyloxycarbonyl)-β-n-propylamine-L-alanine* (**2c**): Compound **2c** was isolated with 81% of yield; mp 114 °C; $^1$H NMR (300 MHz, $CDCl_3$, ppm) $\delta$ 0.82 (t, 3H, $J = 7.38$, $NCH_2CH_2CH_3$), 1.23 (sex, 2H, $J = 7.68$, $NCH_2CH_2CH_3$), 3.14 (q, 2H, $J = 7.23$, $NCH_2CH_2CH_3$), 3.60 (dd, 1H, $J^1 = 6.31$, $J^2 = 4.56$), 4.05 (dd, 1H, $J^1 = 11.43$, $J^2 = 3.87$), 4.11 (dd, 1H, $J = 3.18$), 5.07 (s, 2H, $CH_2$Ph), 7.29 (m, 5H, Ph); $^{13}$C NMR (75 MHz, $CDCl_3$, ppm) $\delta$ 11.2, 22.6, 41.2, 55.1, 62.8, 67.4, 128.1 (2C), 128.4, 128.6 (2C), 135.9, 156.8, 170.9; ESI-MS 303 $[M+Na]^+$, 280.9 $[M+H]^+$, 263 $[M-OH+H]^+$, 236.9 $[M-COOH+H]^+$, 91.3 $[PhCH_2]^+$.
*N-(Benzyloxycarbonyl)-β-n-butylamine-L-alanine* (**2d**): Compound **2d** was isolated with 81% of yield; mp 86 °C; $^1$H NMR (300 MHz, $CDCl_3$, ppm) $\delta$ 0.82 (t, 3H, $J = 7.36$, $NCH_2CH_2CH_2CH_3$), 1.31 (sex, 2H, $J = 7.32$, $NCH_2CH_2CH_2CH_3$), 1.38 (qui, 2H, $J = 7.23$, $NCH_2CH_2CH_2CH_3$), 3.04 (sex, 2H, $J = 7.05$, $NCH_2CH_2CH_2CH_3$), 3.56 (dd, 1H, $J^1 = 6.33$, $J^2 = 4.50$), 3.98 (dd, 1H, $J^1 = 11.20$, $J^2 = 3.92$), 4.10 (dd, 1H, $J = 3.18$), 5.05 (s, 2H, $CH_2$Ph), 7.28 (m, 5H, Ph); $^{13}$C NMR (75 MHz, $CDCl_3$, ppm) $\delta$ 11.2, 20.1, 28.6, 41.4, 54.1, 62.6, 67.2, 128.4 (2C), 128.5, 128.6 (2C), 136.2, 156.9, 171.2; ESI-MS 317 $[M+Na]^+$, 294.9 $[M+H]^+$, 250.9 $[M-COOH+H]^+$, 91.3 $[PhCH_2]^+$.
*N-(Benzyloxycarbonyl)-β-iso-propylamine-L-alanine* (**2e**): Compound **2e** was isolated with 71% of yield; mp 96 °C; $^1$H NMR (300 MHz, $CDCl_3$, ppm) $\delta$ 1.02 (d, 6H, $J = 6.63$, $NCH(CH_3)_2$), 4.00 (sep, 1H, $J = 6.84$, $NCH_2(CH_3)_2$), 3.57 (dd, 1H, $J^1 = 5.97$, $J^2 = 5.04$), 4.04 (dd, 1H, $J^1 = 11.43$, $J^2 = 3.87$), 4.06 (dd, 1H, $J = 3.51$), 5.21 (s, 2H, $CH_2$Ph), 7.28 (m, 5H, Ph); $^{13}$C NMR (75 MHz, $CDCl_3$, ppm) $\delta$ 22.5 (2C), 41.6, 55.4, 62.8, 67.2, 128.0 (2C), 128.2, 128.4 (2C), 136.1, 157.1, 170.0; ESI-Ms 303 $[M+Na]^+$, 280.9 $[M+H]^+$, 263 $[M-OH+H]^+$, 236.9 $[M-COOH+H]^+$, 91.3 $[PhCH_2]^+$.
*N-(Benzyloxycarbonyl)-β-tert-butylamine-L-alanine* (**2f**): Compound **2f** was isolated with 69% of yield; mp 104 °C; $^1$H NMR (300 MHz, $CDCl_3$, ppm) $\delta$ 1.20 (s, 9H, $NC(CH_3)_3$), 3.58 (dd, 1H, $J^1 = 6.01$, $J^2 = 5.04$), 4.11 (dd, 1H, $J^1 = 11.43$, $J^2 = 3.87$), 4.22 (dd, 1H, $J = 3.51$), 5.04 (s, 2H, $CH_2$Ph), 7.22 (m, 5H, Ph); $^{13}$C NMR (75 MHz, $CDCl_3$, ppm) $\delta$ 27.6 (3C), 51.9, 56.9, 66.7, 66.9, 128.0 (2C), 128.1, 128.5 (2C), 136.3, 156.4, 170.1; ESI-MS 317 $[M+Na]^+$, 295.1 $[M+H]^+$, 238.9 $[M-C(CH_3)_3+H]^+$, 91.3 $[PhCH_2]^+$.
*N-(Benzyloxycarbonyl)-β-dimethylamine-L-alanine* (**2g**): Compound **2g** was isolated with 68% of yield; mp 103 °C; $^1$H NMR (300 MHz, $CDCl_3$, ppm) $\delta$ 2.97 (s, 3H, $N(CH_3)_2$), 3.03 (s, 3H, $N(CH_3)_2$), 3.69 (m, 2H), 4.66 (dd, 1H, $J = 3.66$), 5.02 (s, 2H, $CH_2$Ph), 7.25 (m, 5H, Ph); $^{13}$C NMR (75 MHz, $CDCl_3$, ppm) $\delta$ 35.9, 37.3, 52.1, 63.9, 67.2, 128.0 (2C), 128.2, 128.5 (2C), 136.1, 156.4, 170.5; ESI-MS 289 $[M+Na]^+$, 266.9 $[M+H]^+$, 222.9 $[M-COOH+H]^+$, 91.3 $[PhCH_2]^+$.
*N-(Benzyloxycarbonyl)-β-diethylamine-L-alanine* (**2h**): Compound **2h** was isolated with 70% of yield; mp 92 °C; $^1$H NMR (300 MHz, $CDCl_3$, ppm) $\delta$ 1.02 (t, 3H, $J = 7.02$, $N(CH_2)_2(CH_3)_2$), 1.08 (t, 3H, $J = 7.12$, $N(CH_2)_2(CH_3)_2$), 2.41 (q, 2H, $J = 7.06$, $N(CH_2)_2(CH_3)_2$), 2.52 (q, 2H, $J = 7.04$, $N(CH_2)_2(CH_3)_2$), 3.72 (m, 2H), 4.68 (dd, 1H, $J = 3.68$), 5.00 (s, 2H, $CH_2$Ph), 7.27 (m, 5H, Ph); $^{13}$C NMR (75 MHz, $CDCl_3$, ppm) $\delta$ 13.3, 13.7, 40.9, 41.6, 53.1, 64.6, 67.1, 128.3 (2C), 128.4, 128.6 (2C), 136.4, 156.3, 171.5; ESI-Ms 317 $[M+Na]^+$, 294.9 $[M+H]^+$, 280.9 $[M-COOH+H]^+$, 91.3 $[PhCH_2]^+$.