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The Efficient Synthesis of Azaamino Acids

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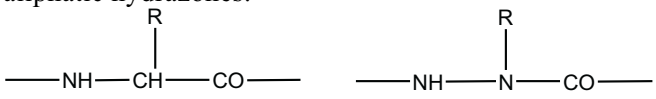
An efficient method of synthesis of N-t-butoxycarbonyl-azaamino acid ethyl esters has been described. This method consisted of three stages including: hydrazone formation, its reduction and acylation with ethyl chloroformate. The second step – reduction of the hydrazones to the appropriate hydrazides – was the most challenging. Some reducing agents have been tested, though $NaBH_3CN$ was found to lead to the final products with the highest yields in relatively short time and even to allow the selective reduction of C–N bond in the presence of nitro group.

Key words: azaamino acids, peptidomimetics, selective reduction

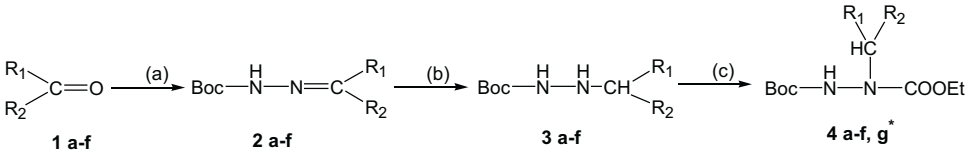
Azaamino acids are amino acid analogs, in which the α-CH group is replaced by a nitrogen atom (Scheme 1) [1]. Incorporation of the azaamino acid residue into a peptide chain leads to changes in local conformation of the modified peptide and to a higher acidity of the amino group attached to the α-nitrogen. Both effects mentioned affect the biological activity of the natural substances analogs modified with azaamino acid residue. In last years there have many publications appeared, which describe the application of azapeptides as analogs of various hormones and as enzyme inhibitors [2–7]. Also, many methods of azapeptides' synthesis have been described [1]. Most of them are based on the reaction between hydrazine or its derivatives with "carbonyl donor". The azapeptide chain can also be elongated by means of the traditional methods of peptide synthesis. In many syntheses the starting points are hydrazides, which enable the receiving the simple as well as more complex amino acids azaanalogs and their incorporation into the peptide chain. They can be obtained by hydrazinolysis of peptide esters or by the reduction of hydrazones formed in the reaction of amino acid hydrazides with the appropriate aldehydes or ketones. This step proved to be the most challenging.

In this paper we describe an effective method of synthesis of N-t-butoxycarbonyl-azaamino acid ethyl esters consisting of three stages (Scheme 2), with the application of $NaBH_3CN$ as the most convenient reducing agent for reduction of both aromatic and aliphatic hydrazones.



amino acid **azaamino acid**

Scheme 1. The comparison of the amino acid and azaamino acid structures.



(a) $BocNHNH_2$,
(b) $HCOO^-NH_4^+$, Pd/C, MeOH, (method A);
$NaBH_4$, THF/EtOH (2:1), (method B)
$NaBH_3CN$, TsOH, THF, (method C);
(c) ClCOOEt,
*obtained in the reaction of $BocNHNH_2$ with ClCOOEt

1–4	R_1	R_2	1–4	R_1	R_2
a	H	C_6H_5	**e**	CH_3	C_2H_5
b	H	C_6H_4OH	**f**	H	$C_6H_4(4\text{-}NO_2)$
c	H	$C_6H_4OCH_3$	**g**	$-CHR_1R_2 = -H$	
d	CH_3	CH_3			

Scheme 2. Synthesis of azaamino acid derivatives.

RESULTS AND DISCUSSION

The method of synthesis of azaamino acid derivatives consisted of three stages (Scheme 2). The syntheses of hydrazones **2** (path **a** on Scheme 2) and N-Boc-azaamino acid ethyl esters **4** (path **c** on Scheme 2) were leading unproblematic to the appropriate products with high yields (66÷85%). The most synthetic difficulties we have faced during the reduction of hydrazones **2**, leading to hydrazides **3** (path **b** on Scheme 2). At first we have used the proposed by Dutta and Morley method of catalytic hydrogenation of hydrazones [8]. Unfortunately, this method required prolonged times and in some cases we had to use more severe reaction conditions (for example reduction of N-Boc-N′-2-butanone hydrazone proceeded at higher temperature (50°C over 30% palladium-carbon). As it has been stated, care must also be taken to avoid over-reduction. In the case of benzaldehyde hydrazone, hydrogenation longer than 20 min. led to hydrogenolysis of the benzyl C–N bond and formation of Boc-$NHNH_2$ instead of the expected product **3a**.

Because of these difficulties we have checked other reduction procedures. Ammonium formate acts as *in situ* hydrogen donor and it has been applied in many organic reactions, for instance in the reduction of azides, nitro and cyano groups and to the dehalogenation reactions [9–11]. The reduction of N-Boc-N′-benzaldehyde and N-Boc-N′-4-hydroxybenzaldehyde hydrazones proceeded in a short time (**3a**, **3b**), but with low yields (25–27%). Unfortunately we have also encountered the difficulties connected with the reduction of aliphatic hydrazones (*e.g.* hydrazones of N-Boc-

N′-2-butanone and N-Boc-N′-acetone), which were probably of steric origin. We failed to reduce those compound by ammonium formate.

According to Grehn and Ragnarsson [12], the aliphatic hydrazones can be readily reduced to hydrazides with $NaBH_4$. By this reducing agent we have tried to obtain N-Boc-N′-*sec*-butyl hydrazide. The yield of this reaction was rather high (65%) but considering the extended time (120 h) we have decided to use $NaBH_3CN$ in the next attempts. This method proved to be the most advantageous among those used so far, not only for reduction of aromatic hydrazones as it was previously described [12,13], but also for some aliphatic analogs. The reactions proceeded with high yields and in most cases (except the reduction of N-Boc-N′-4-methoxybenzaldehyde hydrazone in 30% yield), in relatively short time (4.5–7 h). Moreover, the application of $NaBH_3CN$ enables the selective reduction of C–N bond in the presence of nitro group. Analysis by mass-spectrometry revealed the presence of nitro group in the product of reduction of N-Boc-N′-4-nitrobenzaldehyde hydrazone. Our experiments led us to conclude that application of $NaBH_3CN$ is a very convenient method of reduction of both aromatic and aliphatic hydrazones, allowing the subsequent formation of azaamino acid derivatives.

EXPERIMENTAL

All solvents and chemicals used were of analytical purity, assessed by thin layer chromatography (TLC), ^{1}H-NMR (Varian Mercury 400 MHz), IR spectra (Bruker spectrometer IFS 66) and mass spectrometry (VG Mass Lab).

TLC was carried out by Merck aluminium sheets precoated with silica gel 60, F_{254}, with the following solvent systems (v/v): A; $CHCl_3$:MeOH (9:1); B; $CHCl_3$:MeOH:AcOH (85:10:5); C; $CHCl_3$:MeOH (15:1); D; CH_2Cl_2:MeOH (20:1); E; $CHCl_3$:EtOH (95:5); F; CH_2Cl_2:Et_2O (1:1); G; AcOEt:hexane (1:1); H; AcOEt: light petroleum (1:2); I; AcOEt:light petroleum (1:4); J; CH_2Cl_2:MeOH (10:1); K; CH_2Cl_2:acetone:AcOH (40:10:0.5). Components were detected by UV inspection and/or using ninhydrin or cerium sulfate – sodium molybdate as visualizing agents.

General method for synthesis of hydrazones 2a–f (N-t-butoxycarbonyl-N′-alkyl- or arylalkyl hydrazones) [8]. The appropriate aldehyde or ketone was added at room temperature over 5–10 min. to a solution of t-butoxycarbonyl-hydrazide (Boc-hydrazide) (1 mol. eq.) in tetrahydrofuran or toluene. After 0.5–12 h time, the mixture was evaporated and the residue was recrystallized. The following compounds were obtained:

N-t-butoxycarbonyl-N′-benzaldehyde hydrazone (2a). The product was recrystallized (MeOH). Yield: 66%. M.p.: 186°C (185°C [8]).

N-t-butoxycarbonyl-N′-4-hydroxybenzaldehyde hydrazone (2b). The final product was recrystallized (THF/cyclohexane). Yield: 82%. M.p.: 167÷168°C (182°C [8]).

N-t-butoxycarbonyl-N′-4-methoxybenzaldehyde hydrazone (2c). The crude product was recrystallized (THF/cyclohexane). Yield: 85%. M.p.: 137÷138°C.

N-t-butoxycarbonyl-N′-acetone hydrazone (2d). The product was recrystallized (THF/cyclohexane). Yield: 77%. M.p.: 100÷103°C (104÷105°C [8]).

N-t-butoxycarbonyl-N′-2-butanone hydrazone (2e). The crude product was recrystallized (cyclohexane). Yield: 77%. M.p.: 74÷76°C (78°C [8]).

N-t-butoxycarbonyl-N′-4-nitrobenzaldehyde hydrazone (2f). The final product was recrystallized (THF/cyclohexane). Yield: 81%. M.p.: 171÷172°C (168°C [13]).

General method for synthesis of hydrazides 3a–f (N-t-butoxycarbonyl-N′-alkyl- or arylalkyl hydrazides).

- ***Reduction according to method A***. To a solution of the appropriate hydrazone **2** in MeOH, 5% Pd/C (0.05 g per mmol of hydrazone) was added, followed by a solution of the ammonium formate (4 mol. eq.). When TLC indicated completion of the reaction, the catalyst was filtered, and the filtrate was concentrated under reduced pressure. The solution of the residue in ethyl acetate was washed successively with brine, saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and evaporated under reduced pressure to give the appropriate hydrazide.
- ***Reduction according to method B*** [12]. The appropriate hydrazone **2** was dissolved in THF/EtOH (2:1) under argon and the resulting clear solution was treated with $NaBH_4$ (2.5 mol. eq.) in small portions with vigorous stirring. The reaction was left to proceed under stirring with monitoring by TLC. After 48 h the additional portion of $NaBH_4$ (1 mol. eq.) was added. After the subsequent 72 h the reaction mixture was treated with 2M AcOH and after 5 min. the clear solution was partitioned between Et_2O and 1M Na_2CO_3. The aqueous phase was further extracted with Et_2O. The combined extracts were washed twice with brine and dried ($MgSO_4$). After removal of the solvent the appropriate product was obtained.
- ***Reduction according to method C*** [13]. The appropriate hydrazone **2** in dry THF was cautiously treated with small portions of $NaBH_3CN$ (2 mol. eq.) with rapid stirring under argon. A few grains of bromocresol green were introduced and to the resulting blue solution, a solution of p-toluenesulfonic acid (1 mol. eq.) in THF was added dropwise; any new addition was performed after indicator toning. After the colour persisted for over 30 min, the mixture was diluted with ethyl acetate and the suspension extracted with brine, saturated $NaHCO_3$ and brine. The organic phase was dried with $MgSO_4$ and the solvents were evaporated under reduce pressure giving the appropriate product.

The following compounds were obtained:

N-t-butoxycarbonyl-N′-benzyl hydrazide (3a). The product was obtained according to method A. Time of reduction: 2h. Recrystallization (AcOEt/hexane) yielded pure **3a** in 27%. M.p.: 180÷183°C (b.p.: 136÷140°C (2.3 mmHg) [8]).

N-t-butoxycarbonyl-N′-4-hydroxybenzyl hydrazide (3b). The final product was obtained by method A of reduction (1.5 h) and purified to homogeneity by crystallization (THF/cyclohexane). Yield: 25%. M.p.: 164°C (162÷164°C [8]).

N-t-butoxycarbonyl-N′-4-methoxybenzyl hydrazide (3c). The product was synthesized by method C. Reduction (5 h) followed by recrystallization (THF/cyclohexane) yielded pure **3c** (30%). M.p.: 129÷130°C.

N-t-butoxycarbonyl-N′-*iso*-propyl hydrazide (3d). Method of reduction: C. Time: 4.5 h. The product was left as oil. Yield: 99%.

N-t-butoxycarbonyl-N′-*sec*-butyl hydrazide (3e). Method of reduction: B. Time: 120 h. The product was left as oil. Yield: 65%.

N-t-butoxycarbonyl-N′-4-nitrobenzyl hydrazide (3f). The final product was obtained by method C of reduction (6h) and crystallization was used for final purification (light petroleum). M.p.: 97÷98°C. Yield: 80%.

General method for synthesis of N-t-butoxycarbonyl-azaamino acid ethyl esters (4a–f, g) [14]. The appropriate hydrazide **3** (or $BocNHNH_2$; g) in ethyl acetate containing triethylamine (1 mol. eq.) was treated at 0°C with a solution of ethyl chloroformate (1.05 mol. eq.) in AcOEt. The mixture was stirred and when TLC indicated completion of the reaction, the mixture was washed with water; 0.1 M Na_2CO_3, 1 M $KHSO_4$ and water, dried ($MgSO_4$) and evaporated.

N-t-butoxycarbonyl-azaphenylalanine ethyl ester (4a). The crude product was recrystallized (hexane). Yield: 54%. M.p.: 64÷66°C (61÷63°C [14]). IR (KBr), υ (cm^{-1}): 3307 (ν_{N-H}), 1712 ($\nu_{C=O}$, br.). ^{1}H-NMR ($CDCl_3$, TMS), δ (ppm): 1.29 (t, J = 6.8 Hz, 3H, C$\underline{H}_3$ (Et)); 1.44 (s, 9H, (C$\underline{H}_3$)$_3$ (Boc)); 4.22 (q, J = 6.8 Hz, 2H, C$\underline{H}_2$ (Et)); 4.68 (s, 2H, C$^{\beta}\underline{H}_2$); 6.24 (s, 1H, N$\underline{H}$); 7.26–7.36 (m, 5H, $C_6\underline{H}_5$). MS (FAB): 295 $(M+H)^+$.

N-t-butoxycarbonyl-azatyrosine ethyl ester (4b). The final product was recrystallized (Et_2O/hexane). Yield: 50%. M.p.: 108÷109°C. IR (KBr), υ (cm^{-1}): 3311 (ν_{N-H}), 1714 ($\nu_{C=O}$, br.). ^{1}H-NMR ($CDCl_3$, TMS), δ (ppm): 1.28 (t, J = 6.8 Hz, 3H, C$\underline{H}_3$ (Et)); 1.44 (s, 9H, (C$\underline{H}_3$)$_3$ (Boc)); 4.21 (q, J = 6.8 Hz, 2H, C$\underline{H}_2$ (Et)); 4.60 (s, 2H, C$^{\beta}\underline{H}_2$); 6.79 (d, J=8.4 Hz, 2H, $C_6\underline{H}_4$); 7.17 (d, J = 8.0 Hz, 2H, $C_6\underline{H}_4$). MS (FAB): 311 $(M+H)^+$.

N-t-butoxycarbonyl-(O-methyl)-azatyrosine ethyl ester (4c). The final product was left as oil. Yield: 74%. IR (film), υ (cm^{-1}): 3306 (ν_{N-H}), 1712 ($\nu_{C=O}$, br.). ^{1}H-NMR ($CDCl_3$, TMS), δ (ppm): 1.29 (t, *J* = 6.4 Hz, 3H, C$\underline{H}_3$ (Et)); 1.44 (s, 9H, (C$\underline{H}_3$)$_3$ (Boc)); 3.80 (s, 3H, C$\underline{H}_3$ (Me)); 4.21 (q, *J* = 6.4 Hz, 2H, C$\underline{H}_2$ (Et)); 4.61 (s, 2H, C$^{\beta}\underline{H}_2$); 6.86 (d, *J* = 7.6 Hz, 2H, C$_6\underline{H}_4$); 7.21 (d, *J* = 7.6 Hz, 2H, C$_6\underline{H}_4$). MS (FAB): 325 $(M+H)^+$.

N-t-butoxycarbonyl-azavaline ethyl ester (4d). The product was left as oil. Yield: 94%. IR (film), υ (cm^{-1}): 3307 (ν_{N-H}), 1710 ($\nu_{C=O}$, br.). ^{1}H-NMR ($CDCl_3$, TMS), δ (ppm): 1.07 (d, *J* = 6.8 Hz, 3H, C$^{\gamma}\underline{H}_3$); 1.13 (d, *J* = 8.0 Hz, 3H, C$^{\gamma}\underline{H}_3$); 1.27 (t, *J* = 7.2 Hz, 3H, C$\underline{H}_3$ (Et)); 1.48 (s, 9H, (C$\underline{H}_3$)$_3$ (Boc)); 4.18 (q, *J* = 7.2 Hz, 2H, C$\underline{H}_2$ (Et)); 4.41 (m, 1H, C$^{\beta}\underline{H}$); 6.08 (s, 1H, N$\underline{H}$). MS (FAB): 247 $(M+H)^+$.

N-t-butoxycarbonyl-azaisoleucine ethyl ester (4e). The product was purified by column chromatography on silica gel (AcOEt:light petroleum 1:4) and was left as oil. Yield: 50%. IR (film), υ (cm^{-1}): 3303 (ν_{N-H}), 1709 ($\nu_{C=O}$, br.). ^{1}H-NMR ($CDCl_3$, TMS), δ (ppm): 0.84–0.89 (m, 3H, C$^{\delta}\underline{H}_3$); 1.08 (d, *J* = 6.8 Hz, 3H, C$^{\gamma}\underline{H}_3$); 1.21 (t, *J* = 6.8 Hz, 3H, C$\underline{H}_3$ (Et)); 1.31–1.37 (m, 2H, C$^{\gamma}\underline{H}_2$); 1.42 (s, 9H, (C$\underline{H}_3$)$_3$ (Boc)); 3.91 (q, *J* = 6.8 Hz, 2H, C$\underline{H}_2$ (Et)); 4.12–4.30 (m, 1H, C$^{\beta}\underline{H}$); 6.24 (s, 1H, N$\underline{H}$). MS (FAB): 261 $(M+H)^+$.

N-t-butoxycarbonyl-(4-nitro)-azaphenylalanine ethyl ester (4f). The final product was left as oil. Yield: 82%. IR (film), υ (cm^{-1}): 3313 (ν_{N-H}), 1714 ($\nu_{C=O}$, br.), 1346 ($\nu_{N=O}$). ^{1}H-NMR ($CDCl_3$, TMS), δ (ppm): 1.28 (t, *J* = 7.2 Hz, 3H, C$\underline{H}_3$ (Et)); 1.44 (s, 9H, (C$\underline{H}_3$)$_3$ (Boc)); 4.23 (q, *J* = 7.2 Hz, 2H, C$\underline{H}_2$ (Et)); 4.77 (s, 2H, C$^{\beta}\underline{H}_2$); 6.32 (s, 1H, N$\underline{H}$); 7.48 (d, *J* = 8.0 Hz, 2H, C$_6\underline{H}_4$); 8.20 (d, *J* = 8.8 Hz, 2H, C$_6\underline{H}_4$). MS (FAB): 340 $(M+H)^+$.

N-t-butoxycarbonyl-azaglycine ethyl ester (4g). The crude product was recrystallized (AcOEt/hexane). Yield: 55%. M.p.: 90÷93°C (92–93°C [14]). IR (KBr), υ (cm^{-1}): 3283 (ν_{N-H}), 1697 ($\nu_{C=O}$). ^{1}H-NMR ($CDCl_3$, TMS), δ (ppm): 1.28 (t, *J* = 6.8 Hz, 3H, C$\underline{H}_3$ (Et)); 1.49 (s, 9H, (C$\underline{H}_3$)$_3$ (Boc)); 4.22 (q, *J* = 6.8 Hz, 2H, C$\underline{H}_2$ (Et)); 6.25–6.40 (m, 2H, N$^{\alpha}\underline{H}$ i N$\underline{H}$). MS (FAB): 204 $(M+H)^+$.

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