# New Building Blocks for Peptide Analogue Synthesis: Reduced Diaza-β³-Dipeptides

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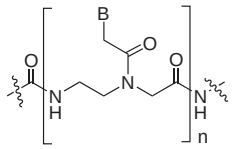
**Abstract:** Reduction of N-protected aza-β³-amino esters and oxidation of the alcohols afford aza-β³-amino aldehydes. Condensation of unprotected aza-β³-amino esters to the resultant aldehydes leads to hydrazones, which have been converted by reduction to useful synthetic building blocks for solid-phase synthesis of new peptidomimetics.

**Key words:** aza-β³ amino aldehydes, pseudopeptides, reductive amination, reduced aza-β³-peptides, PNA aza analogues

Unnatural oligomers are synthetic compounds designed to mimic peptides and others biopolymers and increased their bioavailability.[1] Within the past decade, these compounds have emerged as important research targets in drug discovery as well as conformational analysis. Research on solid-phase syntheses of oligomers with a defined sequence is proliferating as a consequence. Well-designed oligomers can be formed on a support by repeating the same types of coupling reactions. They can have chemically diverse side chains, but they need to have unproteolytically labile amide bonds. One class of non-hydrolysable peptides are reduced peptides.[2] Peptide nucleic acid (PNA), which is a DNA analogue (the phosphodiester backbone has been replaced by a pseudopeptide backbone), is composed of a backbone built up from *N*-aminoethyl glycine units or reduced dipeptide backbone (Figure 1), that makes them very stable in biological fluids.[3]




B = nucleobase

**Figure 1** Structure of PNA

In spite of its resistance to cellular enzymes such as nucleases and proteases, the major limitations confounding its application are poor solubility in aqueous media, inefficient cellular uptake, due to the uncharged backbone and ambiguity in orientational selectivity of binding.[4] Several attempts were done to overcome these problems such as introduction of chirality to PNA by linking chiral and functionalized amino acids,[5] peptides[6] and oligonucleotides[7] to the PNA or by using chiral amino acids in the backbone itself.[8] To our knowledge, analogues of PNAs have not been described yet except constrained PNAs.[9]

The aim of the work described here was to synthesize new building blocks that we could call *N*-hydrazinoethyl hydrazinoglycine units or reduced diaza-β³-dipeptide. These new analogs could be integrated in a peptide in construction or coupled on themselves to give aza analogues of PNA (Figure 2). This nitrogen-enriched backbone could permit to introduce various side chains allowing cell membrane penetration.

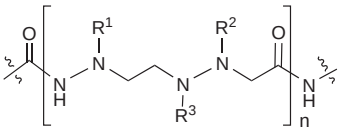
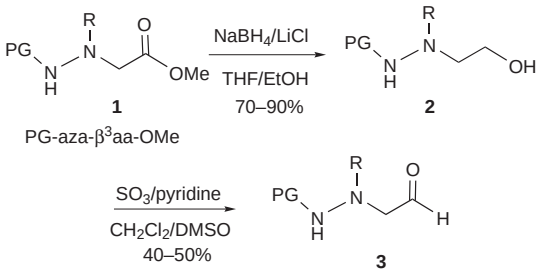



**Figure 2** Aza analogues of PNA or reduced aza-β³-peptides

In a previous paper[10] we have shown that $N^{\beta}$-Boc-protected aza-β³-amino esters could be reduced into the corresponding alcohols by $NaBH_4$/LiCl in THF–EtOH which then have been oxidized by the $SO_3$–pyridine complex in a $CH_2Cl_2$–DMSO mixture to give $N^{\beta}$-Boc-aza-β³-amino aldehydes (Scheme 1). With the aim to enable the synthesis of hybrids peptides as well as analogues of PNA using solid-phase synthesis, we undertook to prepare $N^{\beta}$-Fmoc-aza-β³-amino aldehydes.




**Scheme 1** Synthesis of aza-β³-amino alcohols **2** and aldehydes **3**

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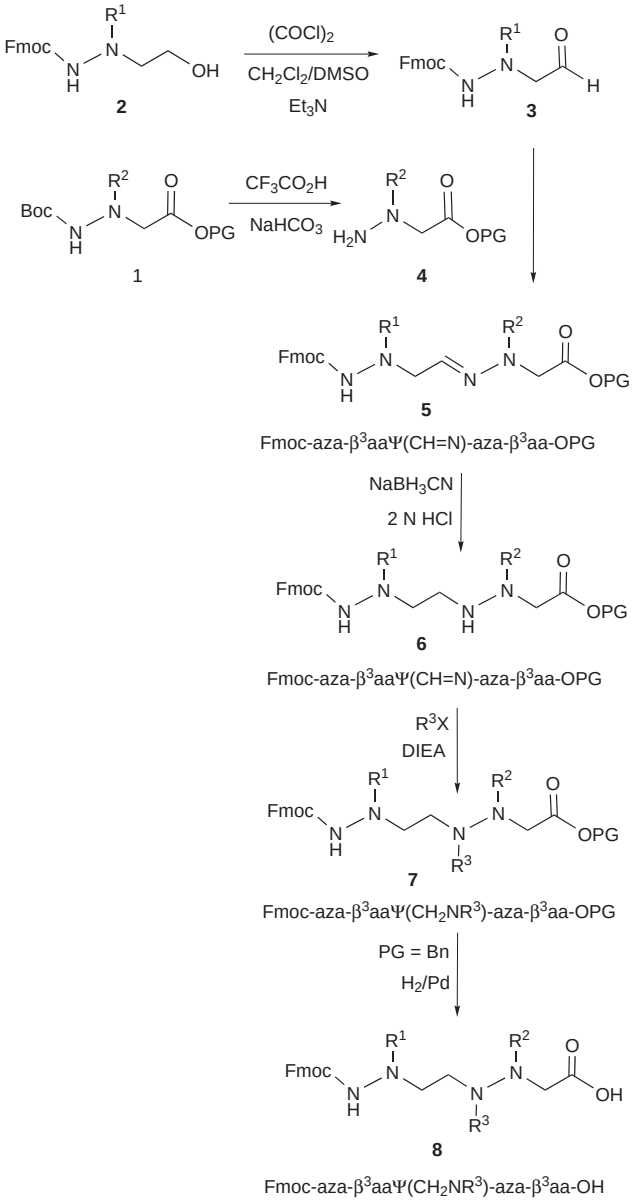
In our initial investigations, we applied the conditions employed in Scheme 1, so the synthesis of $N^{\beta}$-Fmoc-aza-$\beta^3$-amino alcohols **2** by reduction of the corresponding Fmoc-aza-$\beta^3$-amino ester **1** using $NaBH_4$/LiCl proceeds with 90% yield, but no reaction occurred during the oxidation of $N^{\beta}$-Fmoc-aza-$\beta^3$-amino alcohol **2** with the $SO_3$–pyridine complex, the starting alcohol was recovered. We examined alternative methods to generate the aldehyde, Swern oxidation was the best way to get the $N^{\beta}$-Fmoc-aza-$\beta^3$-amino aldehydes **3**.[11] Fmoc-aza-$\beta^3$-amino alcohol **2** was added to oxalyl chloride in DMSO–$CH_2Cl_2$ under nitrogen atmosphere at –78 °C for 15 minutes, addition of $Et_3N$ and stirring at room temperature for 45 minutes afforded Fmoc-aza-$\beta^3$-amino aldehyde **3** (PG = Fmoc). While **3** could be isolated pure by chromatography in 40% yield, due to its instability, it was used directly in the next step following the removal of solvent.

With the aldehydes in hand, we next turned our attention to their reductive amination to get Fmoc-aza-$\beta^3$-aa-$\psi(CH_2NH)$-aza-$\beta^3$-aa-OPG **6**. Crude Fmoc-aza-$\beta^3$-amino aldehyde **3** was mixed with *H*-aza-$\beta^3$-amino ester **4** in $CH_2Cl_2$ to give, after 12 hours, hydrazone **5** in an overall yield from the alcohol of 50%.[12] Reduction of the hydrazone **5** with $NaBH_3CN$ and 2 N HCl led to the expected Fmoc-aza-$\beta^3$-aa-$\psi(CH_2NH)$-aza-$\beta^3$-aa-OPG **6** (Scheme 2 and Table 1).[13]

The $R^1$, $R^2$ groups, mimicking the peptidic chain, the nucleobase or favorizing the solubilization in biological medium, the cell membrane crossing, could be introduced on the starting compound following our previous method.[14] The $R^3$ group could be incorporated by nucleophilic substitution considering the good nucleophilicity of the unsubstituted nitrogen atom of reduced analogue **6**. Moreover, in a previous work we have shown that side reaction occurred during the coupling of the analogue Fmoc-aza-$\beta^3$-Gly-OH, due to the formation of polymeric products during activation of $N^{\alpha}$-unprotected hydrazino acids,[15] so it was necessary to protect the monomer in $N^{\alpha}$-position. Coupling the protected monomer Fmoc-aza-$\beta^3$-Gly(Boc)-OH has led to the expected peptide. To avoid side reactions during the coupling of our new building blocks and to control the possibility of substitution on the $CH_2NH$ of the reduced dimer **6**, we realized the acylation of **6** with $(Boc)_2O$ in the presence of DIEA. Fmoc-aza-$\beta^3$-aa-$\psi(CH_2NBoc)$-aza-$\beta^3$-aa-OBn **7** was obtained in 41–66% yield.[16] Finally, deprotection of the carboxylic group by catalytic hydrogenation affords the free acid **8**[17] which could be then coupled on solid-phase synthesis to afford hybrid peptides or PNA analogues.

In conclusion, we have shown that new building blocks corresponding to reduced diaza-$\beta^3$-dipeptides, Fmoc-aza-$\beta^3$-aa-$\psi(CH_2NH)$-aza-$\beta^3$-aa-OH, can be conveniently prepared by reductive amination of the corresponding Fmoc-aza-$\beta^3$-amino aldehydes. Moreover, substitution of the nitrogen atom ($CH_2NH$) was possible, this result offers the opportunity to introduce various side chains to control either the solubility or the cell membrane permeation. The syntheses of analogues bearing natural or non-standard nucleobases are under progress. These new dimers will facilitate investigation for the preparation of new oligomers or mixed peptides on solid-phase support. The solid-phase synthesis of oligomers and hybrid peptides are under investigations.




**Scheme 2** Synthesis of reduced diaza-$\beta^3$-dipeptides

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**Table 1** Yields of Reduced Diaza-β³-dipeptides **6–8**

| Reduced diaza-β³-dipeptides | | $R^1$ | $R^2$ | $R^3$ | Yield (%) |
|---|---|---|---|---|---|
| **6a** | Fmoc-aza-β³-Leu-ψ($CH_2NH$)-aza-β³-Ala-OBn | $CH_2CH(CH_3)_2$ | $CH_3$ | H | 96 |
| **6b** | Fmoc-aza-β³-Leu-ψ($CH_2NH$)-aza-β³-Tyr($OCH_2OEt$)-OBn | $CH_2CH(CH_3)_2$ | $CH_2C_6H_4OCH_2OEt$ | H | 72 |
| **7a** | Fmoc-aza-β³-Leu-ψ($CH_2NBoc$)-aza-β³-Ala-OBn | $CH_2CH(CH_3)_2$ | $CH_3$ | Boc | 66 |
| **7b** | Fmoc-aza-β³-Leu-ψ($CH_2NBoc$)-aza-β³-Tyr($OCH_2OEt$)-OBn | $CH_2CH(CH_3)_2$ | $CH_2C_6H_4OCH_2OEt$ | Boc | 41 |
| **8a** | Fmoc-aza-β³-Leu-ψ($CH_2NBoc$)-aza-β³-Ala-OH | $CH_2CH(CH_3)_2$ | $CH_3$ | Boc | 72 |
| **8b** | Fmoc-aza-β³-Leu-ψ($CH_2NBoc$)-aza-β³-Tyr($OCH_2OEt$)-OH | $CH_2CH(CH_3)_2$ | $CH_2C_6H_4OCH_2OEt$ | Boc | 75 |


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(12) To a solution of $CH_2Cl_2$ (20 mL, freshly distilled on $CaH_2$), under nitrogen atmosphere, was added oxalyl chloride freshly distilled (1.35 mL, 1.2 equiv). The solution was cooled at –78 °C, and DMSO (2.23 mL, 2.4 equiv, freshly distilled on KOH) was carefully added. The solution was stirred for 15 min and Fmoc-aza-β³-Leu alcohol (**2**, 4.63 g, 1 equiv) in $CH_2Cl_2$ (20 mL) was added dropwise, the mixture was stirred for 15 min at –78 °C. After adding $Et_3N$ (5.52 mL, 3 equiv, freshly distilled on $CaH_2$) the mixture was given up to r.t. during 45 min. Then, $CH_2Cl_2$ was added (50 mL) with a solution of $NaHCO_3$ (1 M, 20 mL). The organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated to give a crude oil suitable for the next step. Purification by chromatography on silica gel (EtOAc–PE 3:7) gave 40% yield of **3a** (1.84 g). $^1$H NMR ($CDCl_3$): δ = 0.95 (br, 6 H, $CH_3$), 1.69 (m, 1 H, CH), 2.67 (br, 2 H, $CH_2$), 3.72 (br, 2 H, $CH_2$), 4.23 (br t, 2 H, *J* = 6.3 Hz, CH), 4.53 (br d, 2 H, *J* = 6.3 Hz, $CH_2$), 6.39 (br, 1 H, NH), 7.31–7.83 (m, 8 H, $CH_{ar}$), 9.12 (s, 1 H, CHO) ppm. The crude oil was diluted in $CH_2Cl_2$ (50 mL) and aza-β³-Tyr-OBn (0.9 equiv) was added, the mixture was stirred overnight at r.t. on $Na_2SO_4$. After filtration, the solution was concentrated and purified by chromatography on silica gel (EtOAc–PE 3:7) to give 2.64 g (55%) of corresponding hydrazone (**5b**) as a colorless oil. $^1$H NMR ($CDCl_3$): δ = 0.93 (d, 6 H, *J* = 6.6 Hz, $CH_3$), 1.23 (t, 3 H, *J* = 7.0 Hz, $CH_3$), 1.72 (m, 1 H, CH), 2.47 (br, 2 H, $CH_2$), 3.50 (s, 2 H, $CH_2$), 3.73 (q, 2 H, *J* = 7.0 Hz, $CH_2$), 3.97 (s, 2 H, $CH_2$), 4.24 (t, 1 H, *J* = 6.9 Hz, CH), 4.36 (s, 2 H, $CH_2$), 4.42 (d, 2 H, *J* = 6.9 Hz, $CH_2$), 5.11 (s, 2 H, $CH_2$), 5.18 (s, 2 H, $CH_2$), 5.82 (br s, 1 H, NH), 6.66 (br, 1 H, CH), 6.93–7.80 (m, 17 H, $CH_{ar}$) ppm. $^{13}$C NMR ($CDCl_3$): δ = 170.08, 156.85, 155.81, 144.07, 141.38, 135.63, 131.66, 129.15, 128.61, 128.37, 127.73, 116.45, 127.73, 127.11, 125.28, 120.00, 93.14, 66.49, 65.20, 64.22, 60.44, 56.54, 54.61, 47.37, 26.37, 20.84, 15.21 ppm. HRMS (ESI): *m*/*z* calcd for $C_{40}H_{46}N_4O_6Na$ $[M + Na]^+$: 701.33151; found: 701.3331 (2 ppm).

(13) The hydrazone **5b** (2.64 g, 3.9 mmol) was dissolved in MeOH (20 mL). Sodium cyanoborohydride (0.62 g, 2.5 equiv) was added and pH was brought to 3 by slowly adding a solution of 2 N HCl. The mixture was stirred for 2 h, then the pH was adjusted to 1. After 10 min of stirring, the solution was neutralized with solid $NaHCO_3$, the mixture was filtrated, concentrated under vacum and the residue was taken up with EtOAc (50 mL) and washed with $H_2O$ and brine. The organic layer was dried over $Na_2SO_4$ and the solvent was removed to give crude oil which was purified by chromatography on silica gel (EtOAc–PE 3:7 and 5:5) to give 1.91 g (72%) of corresponding hydrazine **6b** as a colorless oil. $^1$H NMR ($CDCl_3$): δ = 0.93 (d, 6 H, *J* = 6.7 Hz, $CH_3$), 1.24 (t, 3 H, *J* = 7.0 Hz, $CH_3$), 1.66 (m, 1 H, CH), 2.43 (d, 2 H, *J* = 6.6 Hz, $CH_2$), 2.86 (br, 4 H, $CH_2$), 3.45 (s, 2 H, $CH_2$), 3.74 (q, 2 H, *J* = 7.0 Hz, $CH_2$), 3.90 (s, 2 H, $CH_2$), 4.20 (t, 1 H, *J* = 6.3 Hz, CH), 4.48 (d, 2 H, *J* = 6.3 Hz, $CH_2$), 5.16 (s, 2 H, $CH_2$), 5.21 (s, 2 H, $CH_2$), 5.75 (br s, 1 H, NH), 6.95–7.82 (m, 17 H, $CH_{ar}$) ppm. $^{13}$C NMR ($CDCl_3$): δ = 171.02,

156.78, 155.89, 143.98, 141.40, 135.96, 130.40, 128.55, 128.33, 128.24, 116.17, 127.66, 127.11, 125.04, 120.01, 93.14, 66.20, 66.03, 65.90, 64.16, 58.93, 56.52**,** 55.93, 47.48, 46.10, 26.30, 20.83, 15.21 ppm. HRMS (ESI): *m*/*z* calcd for $C_{40}H_{49}N_4O_6$ $[M + H]^+$: 681.36521; found: 681.3656 (1 ppm).

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(16) To a solution of **6b** (1.69 g, 2.5 mmol) in dioxane (15 mL) was added successively DIPEA (33 mg, 0.1 equiv) and $Boc_2O$ (1.25 g, 2.3 equiv). The mixture was stirred during 24 h at 50 °C then added to a solution 1 M of $NaHSO_4$ (40 mL) and extracted twice with $Et_2O$. The organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated. The crude oil was purified by chromatography on silica gel (EtOAc–PE 1:9 and 2.5:7.5) to afford 0.79 g (41%) of **7b** as a colorless oil. $^1$H NMR ($CDCl_3$, 348 K): δ = 0.96 (d, 6 H, *J* = 5.8 Hz, $CH_3$), 1.11 (t, 3 H, *J* = 7.0 Hz, $CH_3$), 1.54 (s, 9 H, $CH_3$), 1.79 (m, 1 H, CH), 2.65 (br, 2 H, $CH_2$), 2.93 (br, 2 H, $CH_2$), 3.13 (br, 2 H, $CH_2$), 3.59 (q, 2 H, *J* = 7.0 Hz, $CH_2$), 4.18 (s, 2 H, $CH_2$), 4.25 (s, 2 H, $CH_2$), 4.38 (br, 1 H, CH), 4.53 (br, 2 H, $CH_2$), 5.06 (s, 2 H, $CH_2$), 5.10 (br s, 2 H, $CH_2$), 6.41 (br s, 1 H, NH), 7.12–7.70 (m, 17 H, $CH_{ar}$) ppm. $^{13}$C NMR ($CDCl_3$, 348 K): δ = 170.56, 157.50, 155.3, 144.41, 141.59, 136.25, 130.90, 128.42, 128.28, 128.05, 116.37, 127.56, 127.00, 125.14, 119.87, 95.33, 80.13, 66.19, 66.05, 64.87, 63.96, 58.62, 58.45, 54.18, 49.41, 47.79, 28.41, 26.41, 20.54, 14.88 ppm. HRMS (ESI): *m*/*z* calcd for $C_{45}H_{56}N_4O_8Na$ $[M + Na]^+$: 803.39959; found: 803.3992 (0 ppm).

(17) To a solution of **7b** (0.30 g, 0.39 mmol) in MeOH (10 mL) was added 10% Pd/C (20 mg). After purging 3 times with $H_2$, the resulting suspension was stirred for 30 min (end of reaction controlled by TLC). The catalyst was removed by filtration through Celite. The filtrate was evaporated and purified by chromatography on silica gel (EtOAc–$CH_2Cl_2$, 1:1 and $Et_2O$–MeOH, 40%) to afford 0.2 g (75%) of **8b** as a yellow oil. $^1$H NMR ($CDCl_3$, 353 K): δ = 1.01 (d, 6 H, *J* = 5.4 Hz, $CH_3$), 1.13 (t, 3 H, *J* = 6.8 Hz, $CH_3$), 1.50 (s, 9 H, $CH_3$), 1.81 (m, 1 H, CH), 2.56 (br, 2 H, $CH_2$), 2.98 (br, 2 H, $CH_2$), 3.26 (br, 2 H, $CH_2$), 3.60 (q, 2 H, *J* = 6.8 Hz, $CH_2$), 4.02 (br, 2 H, $CH_2$), 4.21 (s, 2 H, $CH_2$), 4.31 (br, 1 H, CH), 4.60 (br, 2 H, $CH_2$), 5.08 (s, 2 H, $CH_2$), 6.50 (br s, 1 H, NH), 7.12–7.75 (m, 12 H, $CH_{ar}$) ppm. $^{13}$C NMR ($CDCl_3$, 348 K): δ = 174.78, 157.54, 156.27, 144.39, 141.61, 131.28, 127.99, 127.70, 127.45, 116.38, 127.36, 127.07, 125.19, 119.86, 93.32, 80.84, 66.43, 63.94, 59.15, 58.36, 55.86, 47.83, 47.73, 28.33, 26.52, 20.62, 14.86 ppm. HRMS (ESI): *m*/*z* calcd for $C_{38}H_{50}N_4O_8Na$ $[M + Na]^+$: 713.35263; found: 713.3523 (0 ppm).