New Building Blocks for Peptide Analogues Synthesis: Reduced Diaza-β^3-Peptides

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Abstract. Reduction of *N*-protected aza-β^3-amino esters and oxidation of the alcohols afford aza-β^3-amino aldehydes. Condensation of unprotected aza-β^3-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.

Keywords.

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 nonhydrolysable peptides are reduced peptides.[2] Peptide nucleic acid (PNA), which is a DNA analogues (the phosphodiester backbone has been replaced by a pseudopeptide backbone), is composed of a backbone built up from aminoethylglycine units or reduced dipeptide backbone (Fig 1), that makes them very stable in biological fluids.[3]

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] In our knowledge analogues of PNAs have not been described yet except constrained PNAs.[9]

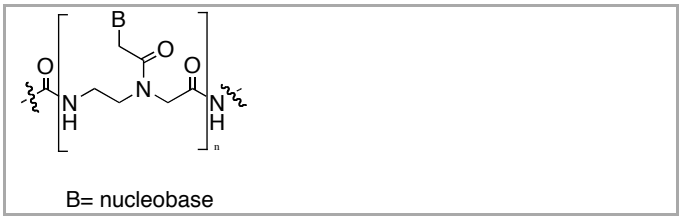


Figure 1 Structure of PNA

The aim of the work described here was to synthesize new building blocks that we could call hydrazino ethylglycine units or reduced diaza-β^3-peptide. These new analogues could be integrated in a peptide in construction or coupled on themselves to give aza analogues of PNA (Fig 2). This nitrogen-enriched backbone could permit to introduce various side chains allowing cell membranes penetrating.

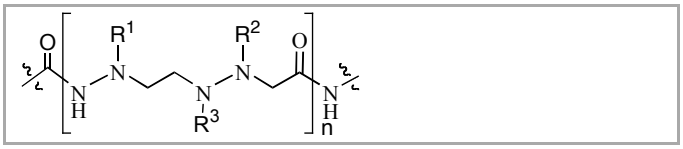
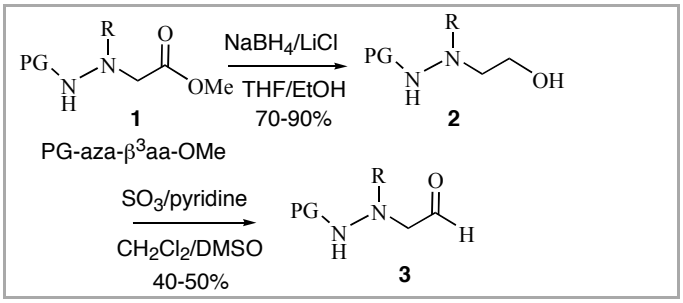


Figure 2 Aza analogues of PNA or reduced aza-β^3 peptides

In a previous paper[10] we have shown that N^{β}-Boc protected aza-β^3-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^{β}-Boc-aza-β^3-amino aldehydes (scheme1). 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^{β}-Fmoc-aza-β^3-amino aldehydes.

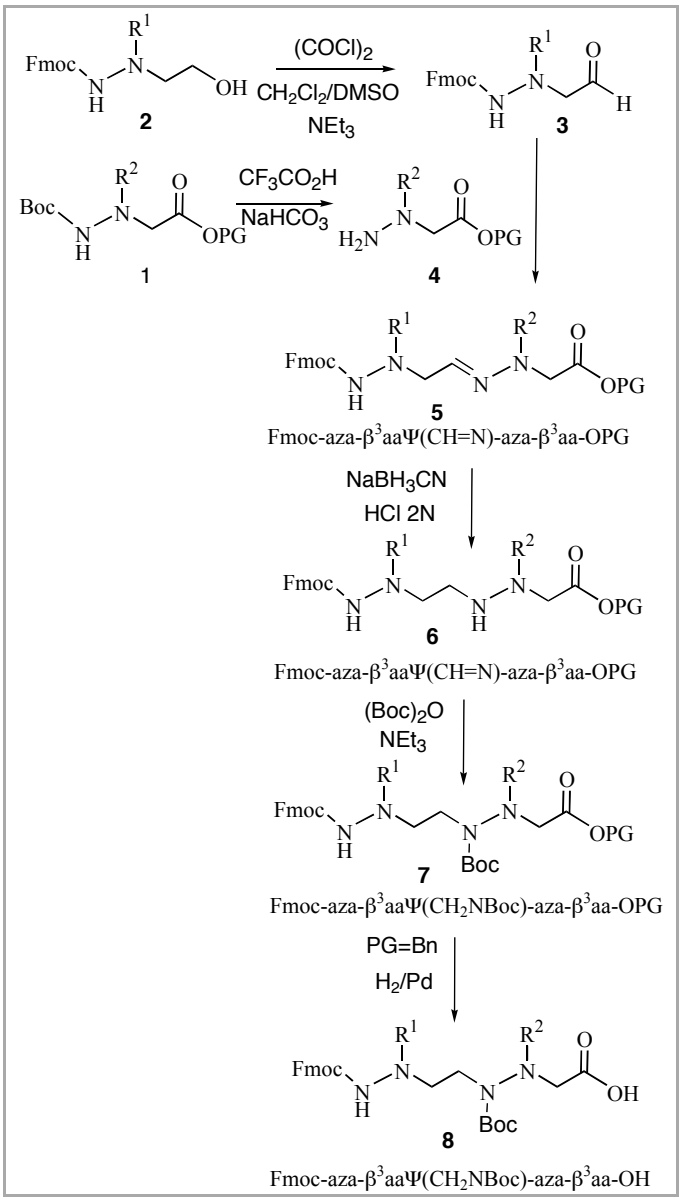


Scheme 1 Synthesis of aza-β^3 amino alcohols **2** and aldehydes **3**

In our initial investigations, we applied the conditions employed in Scheme 1, so the synthesis of N^{β}-Fmoc-aza-β^3-amino alcohols **2** by reduction of the corresponding Fmoc-aza-β^3-amino ester **3** using $NaBH_4$/LiCl proceeds with 90% yield, but no reaction occurred during the oxidation of N^{β}-Fmoc-aza-β^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^{β}-Fmoc-aza-β^3-amino aldehydes **3**.[11] Fmoc-aza-β^3-amino alcohol **2** was added to oxalyl chloride in DMSO/CH_2Cl_2 under nitrogen atmosphere at -78°C for 15 min, addition of NEt_3 and stirring at room temperature for 45 min afforded Fmoc-aza-β^3-amino aldehyde **3** (PG=Fmoc). While **3** could be isolated pure by chromatography in 40% yield, due to its unstability,

it was used directly in the next step following removal of solvent.

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



Scheme 2. Synthesis of Reduced Diaza-β^3 Peptides.

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] 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-β^3-Gly-OH, due to the formation of polymeric products during activation of N^α unprotected hydrazino acids,[15] so it was necessary to protect the monomer in N^α-position. Coupling the protected monomer Fmoc-aza-β^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 NH-CH_2 of the reduced dimer **6,** we realized the acylation of **6** with $(Boc)_2O$ in the presence of NEt_3. Fmoc-aza-β^3-aa-ψ(CH_2NBoc)-aza-β^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.

Table 1 Yields of reduced diaza-β^3 peptides **6**, **7**, **8**.

6a	Fmoc-aza-β^3-Leu-ψ(CH_2NH)-aza-β^3-Ala-OBn	$CH_2CH(CH_3)_2$	CH_3	96
6b	Fmoc-aza-β^3-Leu-ψ(CH_2NH)-aza-β^3-Tyr(OCH_2OEt)-OBn	$CH_2CH(CH_3)_2$	$CH_2C_6H_4OCH_2OEt$	72
7a	Fmoc-aza-β^3-Leu-ψ(CH_2NBoc)-aza-β^3-Ala-OBn	$CH_2CH(CH_3)_2$	CH_3	66
7b	Fmoc-aza-β^3-Leu-ψ(CH_2NBoc)-aza-β^3-Tyr(OCH_2OEt)-OBn	$CH_2CH(CH_3)_2$	$CH_2C_6H_4OCH_2OEt$	41
8a	Fmoc-aza-β^3-Leu-ψ(CH_2NBoc)-aza-β^3-Ala-OH	$CH_2CH(CH_3)_2$	CH_3	72
8b	Fmoc-aza-β^3-Leu-ψ(CH_2NBoc)-aza-β^3-Tyr(OCH_2OEt)-OH	$CH_2CH(CH_3)_2$	$CH_2C_6H_4OCH_2OEt$	75

In conclusion, we have shown that new building blocks corresponding to a reduced diaza-β^3-amino peptides, Fmoc-aza-β^3-aa-ψ(CH_2NH)-aza-β^3-aa-OH, can be conveniently prepared by reductive amination of the corresponding aza-β^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 will be reported soon.

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(12) To a solution of DCM (20 mL, freshly distilled on CaH_2), under nitrogen atmosphere, was added oxalyle chloride freshly distilled (1.35 mL, 1.2 eq). The solution was cooled at -78°C, DMSO (2.23 mL, 2.4 eq, freshly distilled on KOH) was carefully added. The solution was stirred for 15 min and Fmoc-aza-β^3-Leu alcohol **2** (4.63 g, 1 eq) in DCM (20 mL) was added dropwise, the mixture was stirred for 15 min at -78°C. After adding Et_3N (5.52 mL, 3 eq, freshly distilled on CaH_2) and mixture was given up to room temperature during 45 min. DCM was added (50 mL) with solution of $NaHCO_3$ 1M (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 (ethyl acetate/PE 3/7) gave 40% yield of **3a** (1.84g). ^{1}H NMR ($CDCl_3$) δ ppm: 0.95 (br, 6H, CH_3), 1.69 (m, 1H, CH), 2.67 (br, 2H, CH_2), 3.72 (br, 2H, CH_2), 4.23 (brt, 2H, *J* = 6.3 Hz, CH), 4.53 (brd, 2H, *J* = 6.3 Hz, CH_2), 6.39 (br, 1H, NH), 7.31-7.83 (m, 8H, CH_{ar}), 9.12 (s, 1H, CHO). The crude oil was diluted in DCM (50 mL) and aza-β^3-Tyr-OBn (0.9 eq) was added, the mixture was stirred overnight at room temperature on Na_2SO_4. After filtration, the solution was concentrated and purified by chromatography on silica gel (ethyl acetate/PE 3/7) to give 2.64 g (55 %) of corresponding hydrazone **(5b)** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ ppm : 0.93 (d, 6H, *J* = 6.6 Hz, CH_3), 1.23 (t, 3H, *J* = 7.0 Hz, CH_3), 1.72 (m, 1H, CH), 2.47 (br, 2H, CH_2), 3.50 (s, 2H, CH_2), 3.73 (q, 2H, *J* = 7.0 Hz, CH_2), 3.97 (s, 2H, CH_2), 4.24 (t, 1H, *J* = 6.9 Hz, CH), 4.36 (s, 2H, CH_2), 4.42 (d, 2H, *J* = 6.9 Hz, CH_2), 5.11 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 5.82 (brs, 1H, NH), 6.66 (br, 1H, CH), 6.93-7.80 (m, 17H, CH_{ar}).^{13}C NMR ($CDCl_3$) δ ppm : 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. HRMS (ESI) calcd for $[M + Na]^+$ ($C_{40}H_{46}N_4O_6Na$) 701.33151, found 701.3331 (2 ppm).

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(14) The hydrazone **(5b)** (2.64 g, 3.9 mmol) was dissolved in MeOH (20 mL). Sodium cyanoborohydride (0.62 g, 2.5 eq) was added and pH was brought to 3 by slowly adding a solution of 2N HCl. The mixture was stirred for 2 h, then adjust pH 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 water 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 (ethyl acetate/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 ppm : 0.93 (d, 6H, *J* = 6.7 Hz, CH_3), 1.24 (t, 3H, *J* = 7.0 Hz, CH_3), 1.66 (m, 1H, CH), 2.43 (d, 2H, *J* = 6.6 Hz, CH_2), 2.86 (br, 4H, CH_2), 3.45 (s, 2H, CH_2), 3.74 (q, 2H, *J* = 7.0 Hz, CH_2), 3.90 (s, 2H, CH_2), 4.20 (t, 1H, *J* = 6.3 Hz, CH), 4.48 (d, 2H, *J* = 6.3 Hz, CH_2), 5.16 (s, 2H, CH_2), 5.21 (s, 2H, CH_2), 5.75 (brs, 1H, NH), 6.95-7.82 (m, 17H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ ppm : 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. HRMS (ESI) calcd for $[M + H]^+$ ($C_{40}H_{49}N_4O_6$) 681.36521, found 681.3656 (1 ppm).

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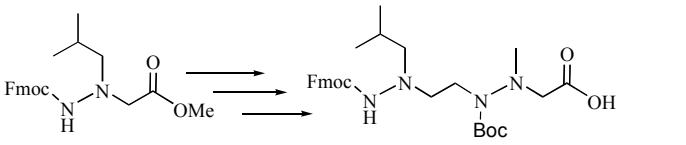
(16) To a solution of **(6b)** (1.69 g, 2.5 mmol) in dioxane (15 mL) was added successively DIPEA (33 mg, 0.1 eq) and Boc_2O (1.25 g, 2.3 eq). The mixture was stirred during 24 h at 50°C then added to a solution 1 M of $NaHSO_4$ (40 mL) and extracted 2 times with ether. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude oil was purified by chromatography on silica gel (ethyl acetate/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 ppm : 0.96 (d, 6H, *J* = 5.8 Hz, CH_3), 1.11 (t, 3H, *J* = 7.0 Hz, CH_3), 1.54 (s, 9H, CH_3), 1.79 (m, 1H, CH), 2.65 (br, 2H, CH_2), 2.93 (br, 2H, CH_2), 3.13 (br, 2H, CH_2), 3.59 (q, 2H, *J* = 7.0 Hz, CH_2), 4.18 (s, 2H, CH_2), 4.25 (s, 2H, CH_2), 4.38 (br, 1H, CH), 4.53 (br, 2H, CH_2), 5.06 (s, 2H, CH_2), 5.10 (brs, 2H, CH_2), 6.41 (brs, 1H, NH), 7.12-7.70 (m, 17H, CH_{ar}). ^{13}C NMR ($CDCl_3$, 348 K) δ ppm : 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, 93.33, 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. HRMS (ESI) calcd for $[M + Na]^+$ ($C_{45}H_{56}N_4O_8Na$) 803.39959, found 803.3992 (0 ppm).

(17)

(18) 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 hydrogen, 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 (ethyl acetate/DCM 1/1 and ether/MeOH 40 %) to afford 0.2 g (75 %) of **(8b)** as a yellow oil. ^{1}H NMR ($CDCl_3$, 353 K) δ ppm : 1.01 (d, 6H, *J* = 5.4 Hz, CH_3), 1.13 (t, 3H, *J* = 6.8 Hz, CH_3), 1.50 (s, 9H, CH_3), 1.81 (m, 1H, CH), 2.56 (br, 2H, CH_2), 2.98 (br, 2H, CH_2), 3.26 (br, 2H, CH_2), 3.60 (q, 2H, *J* = 6.8 Hz, CH_2), 4.02 (br, 2H, CH_2), 4.21 (s, 2H,

CH_2), 4.31 (br, 1H, CH), 4.60 (br, 2H, CH_2), 5.08 (s, 2H, CH_2), 6.50 (brs, 1H, NH), 7.12-7.75 (m, 12H, CH_{ar}). ^{13}C NMR ($CDCl_3$, 348 K) δ ppm : 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. HRMS (ESI) calcd for $[M+Na]^+$ ($C_{38}H_{50}N_4O_8Na$) 713.35263, found 713.3523 (0 ppm).



Fmoc-aza-β^{3}Leu-OMe Fmoc-aza-β^{3}Leu-(ΨCH$_2$NBoc)-aza-β^{3}Ala-OH