New building blocks or dendritic pseudopeptides for metal chelating

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Abstract
Dendritic oligopeptides have been reported as useful building blocks for many interactions. Starting from hydrazine, we described an approach to create new dendritic pseudopeptides linked with biological systems, such as cell membrane, as chelate metal, Ni²⁺-nitrilotriacetic acid moieties which could target histidine rich peptides or proteins. Depending on the nature of these new chemical recognition units, they could be integrated into a peptide by coupling in C or N-termini.

Keywords: Aza-β３-amino acids, Dendritic pseudopeptides, Aza-β３-peptides, Aza-NTA

Background
Unnatural amino acids constitute attractive targets for drug design. Disposing of a wide variety of unnatural amino acids allows the modulation of physical and chemical properties of the resulting peptide depending on the selected side chains (Gentilucci et al. 2010). The aza-β３-amino acids represent an exciting type of analogs of β３-amino acids in which the CHβ is replaced by a nitrogen stereocenter conferring a better flexibility to the pseudopeptide due to the side chain borne on a chiral nitrogen atom with non-fixed configuration (Busnel et al. 2005). Moreover, the backbone modification makes these molecules more stable towards proteolytic degradation (Dali et al. 2007; Laurencin et al. 2012).

Transition metals chelated by nitrilotriacetic acid (NTA) have been successfully applied for purification (Hochuli et al. 1987; Ueda et al. 2003) and detection of oligohistidine-tagged proteins (Hart et al. 2003; Lata et al. 2005), as well as for immobilization on surfaces (Sigal et al. 1996; Gershon and Khilko 1995; Schmid et al. 1997; Xu et al. 2004; Schmitt et al. 2000). The hexahistidine tag provides binding sites for three NTA moieties, indeed, multiple NTA moieties into single entities increase the affinity adaptors for oligohistidine-tagged proteins (Lata et al. 2005).

Herein we aimed to design new amino acid analogues or building blocks that can be incorporated into any polypeptide by solid-phase peptide synthesis. Potential applications of these metal-chelating units will be as metal sensors for synthetic receptors that interact specifically with histidine-tagged peptides.

Results and discussion
As part of our research program we develop new peptide analogues with potentially useful biological properties. For this purpose, we have developed synthetic strategy for aza-β３-aspartic acid (Busnel and Baudy-Floc’h 2007; Abbour and Baudy-Floc’h 2013). We observed that during this process a double substitution of benzyl carbamate 1 occurred to afford Z-aza-β³-Asp(Ot-Bu)-Ot-Bu 4 in 19 % yield. By using tert-butyl bromoacetate (3 eq) 2 and N,N-Diisopropyl ethylamine (DIPEA) (2 eq) 3 was obtained in 80 % yield (Scheme 1). The hydrogenolysis of 3 over 10 % Pd/C gave our precursor 4. A nucleophilic substitution of 4 by tert-butyl bromoacetate (1 eq) in the presence of N,N-Diisopropyl ethylamine (DIPEA) (1 eq) afforded the expected building block 5 with one azanitritriacetic acid which could be coupled in C-termini (Scheme 1) with 20 % yield, we observed the formation of a secondary product 5'. To increase the yield of compound 5, we tried different solvents and different bases. The yield of 5 with acetonitrile/DIPEA or NEt３ was 18 %, with Toluene/potassium carbonate K₂CO₃ in suspension 20 %, and with μWaves (150 W, 90 °C, 45 min) 5 %.

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Reductive amination of trisubstituted hydrazine 5 with glyoxyllic acid in the presence of NaBH₄CN led to the tetrasubstituted hydrazine 6 as a new building block with one aza-NTA, which could be coupled in N-termini.

To create more flexibility to the aza-NTA, we first prepared the substituted aza-β³-glutamic ester 9. Compound 8 was obtained by nucleophilic substitution of methyl 3-bromopropanoate 7 and benzyl carbazate 1 in the presence of DIPEA with only 17% yield. The same reaction without solvent realized under microwaves activation provided 8 with 35% yield. Then a second nucleophilic substitution of tert-butyl bromoacetate 2 with compound 8 and DIPEA led to Z-aza-β³Glu(OMe)-Or-Bu 9 with 96% yield after stirring at 80°C for 5 days. Then hydrolysis of 9 over 10% Pd/C gave the monomer H-azaβ³Glu(OMe)-Or-Bu 10. Nucleophilic substitution with two equivalents of tert-butyl bromoacetate 2, H-azaβ³Glu(OMe)-Or-Bu 10 and DIPEA gave 11 (94% yield). Methyl ester of 11 could be saponified (Pascal and Sol 1998) by sodium hydroxide in MeOH in the presence of CaCl₂ afford ing the expected aza-NTA 12, which could be coupled in N-termini of a peptide (Scheme 2).

To obtain a new ligand with an amine function, which could be coupled on C-termini peptide we choose to work on ornithine analogue. The 1-amino-3,3-diethoxypropane precursor 13 was first N-protected with a benzyl group by reaction with benzylchloroformate under the presence of sodium hydroxide to afford benzyl 3,3-diethoxypropylcarbamate 14 with excellent yield (99%). The acetal 14 was then treated with acetic acid and water (2/1) to give benzyl 2-formylethylcarbamate 15. The condensation of 15 with our precursor 4 led to the hydrazone 16. Reduction with sodium cyanoborohydride (NaBH₄CN) gave the hydrazine 17. Nucleophilic substitution of tert-butyl bromoacetate by hydrazine 17 afforded substituted aza-NTA 18. Hydrolysis of 18 under 10% Pd/C, gave a new ligand aza-NTA 19, bearing a long amino chain with more flexibility (Scheme 3).

Our goal was to get multimeric aza-NTA in order to increase the affinity to histidine tag proteins. Thus we built the dendritic pseudopeptides starting from our two building blocks 18 and 19. Deprotection of acid functions of 18 with TFA afforded 20. Then dendritic pseudopeptides or Z-aza-tris-NTA-tBu 21 were synthesized via standard EDCI coupling of one equivalent of the C-deprotected intermediate 18 with three equivalent of the N-deprotected one 19. We showed that it is possible to deprotect 21 either on C-ter to give Z-aza-tris-NTA-OH 22, or on N-ter to lead to H-aza tris-NTA-tBu 23. NMR and HMRS mass spectrometry were used to verify the structure and purity of the amphiphilic dendritic peptides (Scheme 4).

**Conclusion**

In summary, depending on the nature of our new chemical recognition units, these could be introduced by coupling in a peptide in C or N-termini as well as on peptidic chain. These new Ψ-NTA could open new ways to control protein–protein interactions, to design peptide-based interaction pairs or to generate switchable protein functions. Moreover it would be interesting to look at the self-assembly of our new dendritic pseudopeptides.

**Methods**

1H and 13C NMR spectra were recorded at 200 or 300 MHz and 75.5 MHz. 1H chemical shifts are reported in δ values in ppm relative to CHCl₃ (7.24 ppm) as internal standard and 13C chemical shifts are reported in ppm relative to CDCl₃ (77.0 ppm). Multiplicities in 1H NMR are reported as (br) broad, (s) singlet, (d) doublet, (t) triplet, (q) quartet, and (m) multiplet. The analytical laboratory from the Centre Régional de Mesures Physiques
de l’Ouest performed electrospray mass spectrometry (HRMS, ESI) studies using MS/MS Mass spectrometer ZAB Spec TOF. Thin layer chromatography was performed on silica gel 60 F254 plates (Merck). Flash chromatography was performed on SP silica gel 60 (230–600) mesh ASTM. DCM was distilled from CaH2 under nitrogen.

**Nucleophilic substitution procedure**

A mixture of hydrazine (4 mmol), DIPEA (1.1 g, 8 mmol) and tert-butyl bromoacetate 2 (1.87 g, 12 mmol) in toluene (20 mL) was stirred at 80 °C for 4 days. The solid was filtered and the filtrate was evaporated. The residue was purified by flash column chromatography on silica gel with DCM/EtOAc (9/1).

**Compound 3.**

Yield: 88 %.

$^{1}$H NMR (200 MHz, CDCl3): δ = 1.49 (s, 18H, t-Bu), 3.73 (s, 4H, N-CH$_2$), 5.15 (s, 2H, CH$_2$), 7.31 (m, 5H, C$_6$H$_5$).

$^{13}$C NMR (75 MHz, CDCl3): δ = 28.1, 53.3, 66.9, 81.7, 128.1, 128.2, 128.5, 136.1, 156.8, 170.6.

HRMS (ESI): m/z [M+Na]$^+$ calcld for C$_{20}$H$_{30}$N$_2$O$_6$Na: 417.2002; found 417.2002.

**Compound 5.**

Yield: 20 %.

$^{1}$H NMR (200 MHz, CDCl3): δ = 1.49 (s, 27H, t-Bu), 3.61 (s, 4H, N-CH$_2$), 3.63 (s, 2H, N-CH$_2$).

$^{13}$C NMR (75 MHz, CDCl3): δ = 27.5, 56.0, 62.5, 63.5, 80.2, 173.9.

HRMS (ESI): m/z [M+H]$^+$ calcld for C$_{18}$H$_{35}$N$_2$O$_6$: 375.2495; found 375.2495.

**Compound Z-Aza-β3Glu(OtBu)-OMe 9.**

Yield: 94 %.

$^{1}$H NMR (200 MHz, CDCl3): δ = 1.67 (s, 9H, t-Bu), 2.54 (m, 2H, CH$_2$), 3.22 (m, 2H, N-CH$_3$), 3.62 (m, 5H, CH$_3$ + N-CH$_2$), 5.12 (s, 2H, CH$_2$), 7.40 (m, 5H, C$_6$H$_5$).

$^{13}$C NMR (75 MHz, CDCl3): δ = 26.6, 31.2, 41.7, 48.6, 60.3, 66.4, 128.6, 128.7, 128.9, 129.0, 172.4, 173.4, 173.8.

HRMS (ESI): m/z [M+Na]$^+$ calcld for C$_{18}$H$_{26}$N$_2$O$_6$Na: 390.1688; found 389.1694.

**Compound 11.**
Yield: 99 %.

$^1$H NMR (200 MHz, CDCl$_3$): $\delta = 1.42$ (s, 9H, t-Bu), 2.47 (m, 2H, CH$_2$), 3.01 (m, 2H, N-CH$_2$), 3.41 (s, 4H, N-CH$_2$), 3.51 (s, 2H, N-CH$_2$), 3.64 (s, 3H, CH$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta =$ 28.6, 33.2, 52.1, 52.4, 57.4, 80.1, 169.6, 173.2.

HRMS (ESI): m/z [M + H]$^+$ calcld for C$_{22}$H$_{41}$N$_2$O$_8$: 461.2863; found 461.2856.

Compound 18.

Yield: 50 %.

$^1$H NMR (200 MHz, CDCl$_3$): $\delta =$ 1.47 (s br, 27H, t-Bu), 1.77 (m, 2H, CH$_2$), 2.75 (m, 2H, CH$_2$), 3.38 (m, 2H, N-CH$_2$), 3.48 (s, 2H, N-CH$_2$), 3.61 (s, 4H, N-CH$_2$), 5.15 (s, 2H, CH$_2$), 7.31 (m, 5H, C$_6$H$_5$).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta =$ 24.2, 28.6, 33.2, 52.1, 56.4, 57.4, 66.7, 80.1, 127.2, 127.5, 128.4, 135.8, 157.8, 169.6.

HRMS (ESI): m/z [M + H]$^+$ calcld for C$_{29}$H$_{48}$N$_3$O$_8$: 566.3441; found 566.3221.

Compound 8.

A mixture of Z-carbazate 1 (2 g, 12 mmol), methyl 3- bromopropanoate 7 (2 g, 12 mmol), DIPEA (1.56 g, 12 mmol), NaI (1.2 g, 12 mmol) in toluene (20 mL) was stirred at 80 °C for 7 days. The solid was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with DCM/EtOAc (9/1) to afford 8.

Yield: 0.5 g (17 %).

The same reaction was realized without solvent by microwave activation (SYNTHEWAVE 402: 150 W, 45 min, 90 °C) to get 8.

Yield: 1.1 g (35 %).

$^1$H NMR (200 MHz, CDCl$_3$): $\delta =$ 2.55 (t, 2H, CH$_2$), 3.21 (t, 2H, N-CH$_2$), 3.72 (s, 3H, CH$_3$), 5.19 (s, 2H, CH$_2$), 7.40 (s, 5H, C$_6$H$_5$).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta =$ 31.2, 38.8, 41.5, 47.8, 128.6, 128.7, 128.8, 128.9, 129.0, 134.6, 172.5, 173.9.

HRMS (ESI): m/z [M + H]$^+$ calcld for C$_{12}$H$_{16}$N$_2$O$_4$: 252.1110; found 252.1111.

Scheme 4 Synthesis of multimeric aza-NTA or dendritic pseudopeptides
layer was dried over anhydrous Na₂SO₄ and concentrated to give a crude foam, which was triturated in Et₂O to give 6, which was purified by chromatography on silica gel (DCM/MeOH: 9/1).

Yield: 1.8 g (81 %).

¹H NMR (200 MHz, CDCl₃): δ = 1.50 (s, 27H, t-Bu), 2.55 (m, 2H, N-CH₂), 3.64 (s, 2H, CH₂) and 3.66 (s, 6H, N-CH₃).

¹³C NMR (75 MHz, CDCl₃): δ = 26.5, 56.8, 61.0, 63.5, 63.9, 79.8, 174.9, 180.9.

HRMS (ESI): m/z [M + H]^+ calcd for C₂₀H₃₇N₂O₈: 433.25499; found 433.2546.

Yield: 96 %.

¹H NMR (200 MHz, CDCl₃): δ = 1.40 (s, 9H, t-Bu), 2.74 (m, 2H, CH₂), 3.24 (m, 2H, N-CH₂), 3.50 (br, 2H, NH₂), 3.62 (s, 3H, CH₃), 4.25 (s, 2H, N-CH₂).

¹³C NMR (75 MHz, CDCl₃): δ = 26.9, 31.1, 50.3, 54.3, 65.3, 81.6, 169.4, 173.4.

HRMS (ESI): m/z [M + H]^+ calcd for C₁₉H₂₁N₂O₄: 233.15013; found 233.1498.

Compound Aza NTA 19.

Yield: 99 %.

¹H NMR (200 MHz, CDCl₃): δ = 1.50 (s br, 27H, t-Bu), 2.14 (m, 2H, CH₂), 2.73 (m, 2H, N-CH₂), 3.31 (m, 2H, N-CH₂), 3.40 (m, 2H, N-CH₂), 3.48 (br, 2H, NH₂), 3.53 (s, 4H, N-CH₂).

¹³C NMR (75 MHz, CDCl₃): δ = 27.1, 27.9, 38.1, 50.3, 54.3, 55.3, 81.6, 169.4.

HRMS (ESI): m/z [M + H]^+ calcd for C₁₂H₂₅N₂O₄: 432.30736; found 432.2978.

Compound 23.

Yield: 95 %.

¹H NMR (300 MHz, CDCl₃): δ = 1.53 (br, 81H, t-Bu), 1.75 (m, 8H, CH₂), 2.58-2.72 (m, 10H, N-CH₂), 3.41-3.58 (m, 30H, N-CH₂).

¹³C NMR (75 MHz, CDCl₃): δ = 26.8, 27.2, 37.3, 39.1, 51.4, 52.3, 57.5, 58.3, 169.7, 170.4.

HRMS (ESI): m/z [M + H]^+ calcd for C₁₇H₃₅N₁₂O₂₁: 1503.9865; found: 1503.9764 (1 ppm).

Compound 14.

A solution of 1-Amino-3,3-diethoxypropane 13 (2 g, 13.6 mmol) was added into a solution of NaOH (0.55 g, 13.6 mmol) in water (20 mL) and cooled at 0 °C. The solution of benzylchloride (2.32 g, 13.6 mmol) was added into a solution of CH₃CO₂H/H₂O (7 mL/3.5 mL), and stirred for 5 h. NaHCO₃ was added into the solution until basic pH. The product was washed with H₂O, the organic phase was dried and concentrated under vacuum to afford benzyl 3,3-diethoxypropyl carbamate 14.

Yield: 3.9 g (99 %).

¹H NMR (200 MHz, CDCl₃): δ = 1.24 (t, 6H, J = 7 Hz, OCH₂CH₂J), 1.85 (m, 2H, CH₂), 3.33 (m, 2H, CH₂), 3.53 (m, 4H, OCH₂CH₂J), 4.59 (t, 1H, J = 5.4 Hz, CH), 5.14 (s, 2H, CH₂), 7.39 (m, 5H, C₆H₅).

¹³C NMR (75 MHz, CDCl₃): δ = 16.5, 30.3, 32.8, 63.6, 66.9, 127.5, 127.7, 136.5, 157.1.

HRMS (ESI): m/z [M + H]^+ calcd for C₁₃H₂₃NO₄: 282.1834; found 282.1836.

Compound 15.

Benzyl 3,3-diethoxypropyl carbamate 14 (3.9 g, 13.6 mmol) was dissolved into a solution of CH₃CO₂H/H₂O (7 mL/3.5 mL) and stirred for 5 h. NaHCO₃ was added into the solution until basic pH. The product was extracted with Et₂O (20 mL × 2) and dried over Na₂SO₄. The solvent was removed under vacuum to afford benzyl (3-oxopropyl) carbamate 15, which was used immediately without purification.

Yield: 2.6 g (92 %).
1H NMR (200 MHz, CDCl3): δ = 2.78 (m, 2H, CH2), 3.53 (m, 2H, N-CH2), 5.13 (s, 2H, CH3), 7.39 (m, 5H, CH=CH2). 13C NMR (75 MHz, CDCl3): δ = 34.2, 40.8, 65.8, 127.6, 128.7, 137.6, 152.5, 193.9.

**Compound 16**

Benzyl (3-oxopropyl) carbamate 15 (2.6 g, 12.6 mmol) and (3.25 g, 12.6 mmol) were dissolved into DCM (30 mL). Na2SO4 was added to absorb the water and accelerated the reaction. The solution was stirred overnight at room temperature and filtrated to remove Na2SO4. The filtrate was concentrated and purified by chromatography over silica gel with PE/EtOAc (7/3) first and then (6/4) to give pure hydrazone 16.

Yield: 2 g (97%).

1H NMR (CDCl3): δ = 1.75 (m, 8H, CH2), 2.65 (m, 8H, N-CH2), 3.12-3.68 (m, 32H, N-C(CH3)2). 13C NMR (CDCl3): δ = 28.0, 32.6, 38.1, 56.6, 66.6, 79.4, 127.9, 128.1, 128.4, 154.9, 173.6.

**Compound 21**

A mixture of 18 (0.13 g, 0.30 mmol), 20 (0.43 g, 1 mmol), HOBt (0.18 g, 1.16 mmol), EDCI (0.23 g, 1.16 mmol), DIPEA (0.52 g, 4 mmol) in dry DCM (20 mL) was stirred at room temperature for 2 weeks. The solution was washed with 0.5 N HCl solution (10 mL), and then with H2O (20 mL), and brine (10 mL). The organic solution was dried over anhydrous Na2SO4 and evaporated under vacuum and purified by flash chromatography with DCM/EtOAc (9/1) to afford multimaric 21.

Yield: 0.11 g (21%).

1H NMR (300 MHz, CDCl3): δ = 1.45 (m, 8H, t-Bu), 1.77 (m, 8H, CH2), 2.75 (m, 8H, N-CH2), 3.12-3.68 (m, 32H, N-CH2), 5.09 (s, 2H, CH2), 7.33 (m, 5H, CH=CH2). 13C NMR (75 MHz, CDCl3): δ = 24.9, 28.7, 37.6, 52.1, 52.3, 53.6, 56.8, 59.1, 56.3, 66.6, 127.1, 127.7, 128.9, 136.0, 155.9, 170.8, 171.4.

HRMS (ESI): m/z [M + H]+ calcd for C44H68N12O23: 1133.4599; found: 1133.4567 (1 ppm).

**Cleavage of t-Bu protection**

2 mmol of protected compound were dissolved in the solution of DCM (5 mL)/TFA (5 mL), and stirred for 5 h. The solvent was removed under vacuum to get compounds 20 and 22.

**Compound 20**

Yield: 87%.

1H NMR (200 MHz, CDCl3): δ = 2.12 (m, 2H, CH3), 2.78 (m, 2H, N-CH2), 3.42 (m, 2H, N-CH2), 3.49 (s, 2H, N-CH2), 3.53 (s, 4H, N-CH2), 4.88 (s, 2H, CH2), 7.11 (m, 5H, CH=CH2).

**Compound 22**

Yield: 59%.

1H NMR (300 MHz, CDCl3): δ = 2.12 (m, 2H, CH3), 2.78 (m, 2H, N-CH2), 3.42 (m, 2H, N-CH2), 3.49 (s, 2H, N-CH2), 3.53 (s, 4H, N-CH2), 4.88 (s, 2H, CH2), 7.11 (m, 5H, CH=CH2).

13C NMR (75 MHz, CDCl3): δ = 24.4, 37.5, 51.2, 52.1, 57.9, 58.9, 66.8, 127.2, 128.9, 134.9, 156.9, 172.8.

HRMS (ESI): m/z [M + H]+ calcd for C17H24N3O8: 280.1564; found 280.1564.

**Abbreviations**

t-Bu: tert-buty1, CDCl3: chloroform, DCM: dichloromethane; DIPEA: N,N-diisopropylethylamine; EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; EtOAc: ethylacetate; Et2O: diethyl ether; HOBt: 1-hydroxy benzotriazole; MeOH: methanol; MW: microwaves; NaBH4CN: sodium cyanoborohydride; NaOH: sodium hydroxide; Na2SO4: sodium sulfate; PE: petroleum ether; rt: room temperature; TEA: triethyl amine; TFA: trifluoro acetic acid; THF: tetrahydrofuran; Z: benzzyloxy carbonyl.

**Authors’ contributions**

MR carried out all the synthesis and performed the analysis. IN have made substantial contributions to conception and performed some analysis. MFB conceived of the study, and participated in its design and coordination and have been involved in drafting the manuscript. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.
Ethical approval
This article does not contain any studies with human participants or animals performed by any of the authors.

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