Submonomer Solution Synthesis of Hydrazinoazapeptoids, a New Class of Pseudopeptides

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The development of oligomeric peptidomimetics is actually the focus of increasing attention. This arises from the very low bioavailability of peptides, which seriously limited their therapeutic applications. Azapeptides, peptoids, and ureapeptides belong to a new conceptual class of peptidomimetic in which the side chains are carried by nitrogen atoms. Moreover, the potentiality to automate the synthesis of such oligomers by iterative procedures in the solid phase enables the creation of libraries useful for lead-finding in drug discovery.

Our present results come within that general framework. More precisely, we are interested in the synthesis of new kinds of pseudopeptides that we termed hydrazinoazapeptoids by analogy with peptoids. These “hybrid” peptidomimetics combine a C-terminal azaamino acid unit (aza) or an N-substituted azaglycine (Naza) with N-linked substituted hydrazinoglycine (N^h)(Figure 1).

Although the chemistry of azaamino derivatives has been widely explored and is well documented, that of hydrazino acids has been the object of much less attention. Recently, some progress has been made in their preparation, although through laborious methods, in particular concerning the synthesis of optically enriched compounds. Nevertheless, only a limited number of side chains seem to be compatible with the chemical methods involved at that time. Moreover, due to the presence of the additional N^h, difficulties sometimes occurred during the preparation of hydrazino acids; and their utilization in pseudopeptides design for which the coupling with amino acids is not always regioselective. This should be the reason why commercially available hydrazinoids are N^h protected.

Results and Discussion

In this paper, following our previous research on hydrazinoazapeptoids, we describe a conceptually approach that circumvents these problems by shifting the side chain from the C^α to the adjacent N^h position, generating in this way N^h-substituted hydrazinoglycine. It can be pointed out that one natural compound, the antibiotic negamycin, incorporates such a building block. Our synthetic strategy relies on a sequential process using submonomer methodology. By comparison with the synthesis of peptoids, in our case the displacement of the bromine atom by a monosubstituted alkyl or aralkyl hydrazine can afford two regioisomers in variable proportions. Indeed, it is well-known that the nucleophilicities of the two nitrogens of monosubstituted hydrazines are balanced by both steric and electronic effects of the substituent. As our first goal was the synthesis of N^h-substituted hydrazinoglycine, only one regioisomer was desired. Thus, we had to engage in most cases N^h-protected hydrazines to avoid alkylation on this atom (step B, Scheme 1).

The reactions are easily followed by 1H NMR, looking for total extinction of the bromomethylene signal. After completion, the crude reaction mixture contains only the expected compound and the remaining hydrazine, which are conveniently separated by chromatography on silica gel. The pseudopeptoids so obtained are orthogonal protected and can thereby be further elongated on the C or N terminal after selective deprotection (Scheme 1).

Considering the models that we have tested, and the fact that numerous Boc or Z-protected alkyl and aralkyl hydrazine are commercially available, we set out to apply our methodology in the case of azapeptoids (Scheme 1).

Figure 1. Adopted symbolism for amino acid analogues.

![Chemical structure](https://example.com/structure.png)
Hydrazines are synthetically available, it seems consistent to postulate that a large variety of side chains can be introduced, mimicking both proteogenic or non-proteogenic amino acids (Scheme 1).

We have also prepared the pseudopeptide following the same principle. N,N' Disubstituted benzylcarbazate was easily prepared according to the literature and reacted with bromohydrazide (Scheme 2). This last example further illustrates the great steric tolerance of the substitution step. By this method, it is possible to introduce a methyl substituent on the N\(^\beta\) nitrogen that will increase the chemical stability toward peptidases when a peptidic or pseudopeptidic linkage is created on this position.

The repetition of steps A and B leads to superior homologues in a similar way, as illustrated by the synthesis of the three pseudotripeptides (Scheme 3).

### Notes


As bromohydrazides, which are our building blocks, are simply obtained by reacting a carbazate with bromoacetyl bromide, chemical variations can also be introduced on this part of our pseudopeptides. The synthesis of both pseudopeptides (Scheme 1) clearly shows that the relative positions of the two side chains can be easily modulated in this way. The compounds prepared are new pseudopeptidic variations of the Phe-Leu terminal dipeptide from leucine-enkephaline.

### Conclusion

These preliminary results demonstrate that the manipulation of the flexible chemistry of hydrazine constitutes a simple way to combine special kinds of azaamino acids and hydrazino acids where the side chains are introduced in unusual positions. Hybrid pseudopeptides with nitrogen-enriched peptidic backbones are obtained. These atoms can carry various side chains, allowing the iterative construction of elaborated peptidomimetics. In considering the related peptoids, the present compounds can be regarded as hydrazinoazapeptoids. In addition to the synthesis of oligomeric derivatives, such building
blocks can be integrated in peptidic fragments or combined with other amino acids' analogues, increasing the diversity of peptidomimetics.

**Experimental Section**

NMR spectra were run at 200, 300 (1H), or 75.5 MHz (13C). HR-MS were obtained from the Centre Régional de Mesures Physiques de l'Ouest, using an MS/MS mass spectrometer ZAB Spec TOF. Infrared spectra were recorded on an FT-IR spectrometer as suspensions in KBr. Elemental analyses were performed by the analytical laboratory, CNRS (Lyon). Boc- or Z-protected aldehydes, alkyl halides 2a and 2c were prepared according to literature procedures by reduction of Boc- or Z-protected hydrazones, derived from the reaction of Boc- or Z-carboxylate with either aldehyde or ketone.11 Carbomethoxylation of 2a with MoCl and deprotection by HCl afforded 2b.

**Step A.** To a stirred and cooled (0 °C) solution of N-protected hydrazine (30 mmol, 3 equiv) in methylene chloride (20 mL) and pyridine (30 mmol, 3 equiv) was added the bromo acrylonitrile (10 mmol, 1 equiv) in methylene chloride (5 mL). The reaction mixture was stirred over a period of 6 h and washed three times with water (50 mL). The organic phase was dried over Na2SO4. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography.

BrCH2CO-ozaGly-OtBu (3): R1 = H, R = OtBu): yield 94% mp 77 °C; IR (KBr) 3500–3100, 1723, 1906, 1671 cm−1; 1H NMR (CDCl3) δ 1.54 (s, 9H), 3.97 (s, 2H), 7.03 (s br, 1H), 8.75 (s br, 1H); 13C NMR (CDCl3) δ 30.1; H, R 140 Hz), δ 20.90 (q, H, R 125 Hz), δ 27.07 (d, J = 17.5 Hz), δ 3.75 (s, 3H), δ 3.88 (s, 2H), 7.09 (s br, 1H); 13C NMR (CDCl3) δ 19.9 (J = 126 Hz), 26.4 (t, J = 154 Hz), 26.9 (d, J = 126 Hz), 53.6 (J = 143 Hz), 57.6 (d, J = 140 Hz), 155 (s), 163 (s); Anal. Calcd for C10H12N2O2Br: C, 35.95; H, 5.62; N, 10.49; Br, 25.96. Found: C, 35.96; H, 5.56; N, 10.47; Br, 25.87.

BrCH2CO-ozaGly-OBN (3): R1 = H, R = OCH3Ph): yield 80% mp 98 °C; IR (KBr) 3330, 3250, 1720, 1660 cm−1; 1H NMR (CDCl3) δ 3.90 (s, 2H), 5.19 (s, 2H), 6.88 (s br, 1H), 7.37 (s, 5H), 8.25 (s, 1H). Anal. Calcd for C24H19N3OBr: C, 72.83; H, 4.83; N, 5.70; Br, 9.45. Found: C, 72.89; H, 4.87; N, 5.66; Br, 9.41.

BrCH2CO-aazaGly-OtBu (3): R1 = CH2,CH(CH3)2, R = OCH3Ph): yield 99% oil; IR (KBr) 3250, 1650 cm−1; 1H NMR (CDCl3) δ 0.93 (d, J = 6.5 Hz), 1.9 (m, J = 3.5 Hz), 2.76 (s, 2H), 3.50 (s, 1H), 3.57 (d, J = 12.8 Hz), 4.19 (m, J = 12.8 Hz), 5.67 (t, J = 12.8 Hz), 6.51 (s, 2H), 8.15 (s, 1H); 13C NMR (CDCl3) δ 140 Hz), δ 126 Hz), 26.4 (t, J = 154 Hz), 26.9 (d, J = 126 Hz), 53.6 (J = 143 Hz), 53.7 (d, J = 140 Hz), 155 (s), 163 (s); Anal. Calcd for C24H19N3OBr: C, 35.95; H, 5.62; N, 10.49; Br, 25.96. Found: C, 35.91; H, 5.52; N, 10.50; Br, 25.78.

BrCH2CO-Nazaleu-Ome (5): R1 = CH2,CH(CH3)2, R = OCH2Ph): yield 54% mp 168 °C; IR (KBr) 3360, 3197, 1712, 1665 cm−1; 1H NMR (CDCl3) δ 0.93 (d, J = 6.7 Hz), 1.95 (m, 1H), 3.41 (br, 2H), 3.79 (s, 3H), 3.89 (s, 2H), 8.55 (s br, 1H); 13C NMR (CDCl3) δ 20.3 (J = 126 Hz), 26.9 (d, J = 154 Hz), 28.1 (t, J = 131 Hz), 53.2 (J = 148 Hz), 55.7 (t, J = 140 Hz), 156.6 (s). Anal. Calcd for C25H21N3O3Br: C, 35.95; H, 5.62; N, 10.49; Br, 25.96. Found: C, 35.91; H, 5.52; N, 10.50; Br, 25.78.

BrCH2CO-Nazaleu-NH2 (5): R1 = CH2,CH(CH3)2, R = NH2): yield 54% mp 168 °C; IR (KBr) 3367, 3191, 1712, 1665 cm−1; 1H NMR (DMSO-d6) δ 0.85 (br, 6H), 1.88 (m, 1H), 2.82 (dd, 1H, J = 10.5, 6.8 Hz), 3.69 (dd, 1H, J = 10.5, 6.8 Hz), 3.91 (d, 1H, J = 12.8 Hz), 4.21 (d, 1H, J = 12.8 Hz), 6.21 (s, 2H), 8.58 (s, 1H); Anal. Calcd for C25H21N3O3: C, 33.33; H, 5.56; N, 16.67; Br, 31.75. Found: C, 33.34; H, 5.65; N, 16.92; Br, 31.44.

**Step B.** To a stirred solution of N-protected hydrazine (20 mmol, 3 equiv) in chloroform (12 mL) was added slowly the α-bromo hydrazide (10 mmol, 1 equiv) in chloroform (5 mL). The reaction mixture was refluxed over a period of 12 h and, after cooling, washed once with NaHCO3 1 N (30 mL) and twice with water (30 mL). The organic phase was dried over Na2SO4. The solvent was removed under reduced pressure, and the crude product was purified by silica gel chromatography (hexane–EtOAc as eluent 1:1).

**Notes**


(12) These values were obtained at 55 °C (78 °C for 7) as the room temperature spectrum is poorly resolved because of partial coalescence of most of the signals.
Boc-N\textsuperscript{+}hPCF\textsubscript{3}Phe-azaGly-OBn (4j): yield 78%; mp 136 °C; IR (KBr) 3291, 3174, 1744, 1702, 1685 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\) 0.96 (d, 6H, \(J = 6.6\) Hz), 0.97 (d, 6H, \(J = 6.7\) Hz), 1.47 (s, 9H), 1.82 (m, 1H), 2.01 (m, 1H), 2.53 (d br, 2H, \(J = 6.7\) Hz), 3.47 (br, 2H), 3.58 (br, 2H), 5.37 (s br, 2H), 6.24 (s br, 1H), 8.69 (s br, 1H); HR-MS FAB m/z for C\textsubscript{20}H\textsubscript{31}N\textsubscript{4}O\textsubscript{5} calcd 409.2463, obsd 409.2463. Anal. Calcd: C, 58.82; H, 7.84; N, 13.73. Found: C, 58.57; H, 7.93; N, 13.77.

Boc-N\textsuperscript{+}hdiPhenylmethyl)Ala-azaGly-OBn (4l): yield 70%; mp 139 °C; IR (KBr) 3300, 1758, 1702, 1669 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\) 1.38 (s, 9H), 2.21 (d, 2H, \(J = 4.8\) Hz), 2.54 (s, 3H), 3.21 (s, 2H), 5.10 (s, 2H), 6.28 (s br, 1H), 7.16 (m, 5H), 9.45 (s br, 1H); HR-MS FAB m/z for C\textsubscript{27}H\textsubscript{38}N\textsubscript{6}O\textsubscript{6} calcd 543.2930, obsd 543.2931. Anal. Calcd: C, 53.27; H, 6.62; N, 18.54. Found: C, 53.44; H, 6.24; N, 18.06.

Boc-N\textsuperscript{+}hPhe-N\textsubscript{a}Lys(Boc)-azaGly-OBn (4k): yield 40%; mp 110 °C; IR (KBr) 3342, 3328, 3291, 1640 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\) 1.45 (s, 9H), 2.30 (s, 3H), 3.00 (s, 2H), 3.58 (s, 2H), 4.13 (s, 2H), 7.40 (s, 5H), 8.80 (s br, 1H), 9.25 (s br, 1H), 9.80 (s br, 1H); HR-MS FAB m/z for C\textsubscript{37}H\textsubscript{48}N\textsubscript{12}O\textsubscript{6} calcd 625.2961, obsd 625.2961. Anal. Calcd: C, 59.78; H, 6.90; N, 12.63.

Notes

(13) The title compound is present in two rotameric forms in solution; the data of only the major compound are reported.