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Hydrazino-Aza and *N*-Azapeptoids with Therapeutic Potential as Anticancer Agents

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Abstract—The ubiquitin-proteasome-mediated degradation pathway plays an important role in regulating protein turnover in eucaryotic cells and, consequently, regulates both cell proliferation and cell death. The proteasome influences many cellular regulatory signals and is thus a potential target for pharmacological agents. The study of proteasome function has led to the identification of several natural and synthetic compounds that can act as tumor cell growth inhibitors. In this study, we have developed a series of hydrazino-aza and *N*-azapeptoids, analogues of Ac-Leucyl-Leucyl-Norleucinal (ALLN) a non-specific peptidyl aldehyde inhibitor of the proteasome. These peptide analogues share a common backbone and bear different C- and N-terminal functions. Their antiproliferative activity on murine leukemia L1210 cells is reported here.

In cells, protein degradation is a key pathway for the destruction of abnormal or damaged proteins as well as for the elimination of proteins whose presence is no longer required.[1] Among the various cell proteases, the proteasome, a multicatalytic macromolecular complex, is specifically required for the specific degradation of ubiquitinated proteins. In particular, the proteasome ensures the elimination of numerous proteins that play critical roles in cell functions all along cell cycle progression.[2–4] The proteasome is relevant in human cancer because the cell cycle, tumor growth and survival are governed by a large repertoire of intracellular proteins that are regulated by the ubiquitin-mediated proteasome pathway. For this reason, the target degradation of key regulatory proteins is an essential element of cell cycle control.[5] Malignant cells are more sensitive to the loss of proteasome activities and studies comparing normal and malignant cells have shown that proteasome inhibition sensitizes malignant cells to apoptosis.[6] Consequently, proteasome inhibition has become a new and potentially significant strategy in cancer therapy.[7,8] In the development of new antitumor agents, one proteasome inhibitor, the dipeptide boronic acid analogue PS-341, that leads to an early stage of apoptosis and overcomes drug resistance in human myeloma cells in vitro may offer a promising new approach for treating cancer.[9]

Peptidyl aldehyde inhibitors, such as ALLN (Ac-Leucyl-Leucyl-Norleucinal) or MG 132 (*Z*-Leucyl-Leucyl-Leucinal) (Scheme 1), were first synthesized to improve the different enzymatic activities of the proteasome.[10] But these inhibitors are not specific for the proteasome and they also inhibit thiol proteases such as cathepsin B and calpains.[11] Furthermore, the substituent adjacent to the aldehyde is not configurationally stable, due to the acidity of the α-proton. Thus far, peptide inhibitors modified at the C-terminal position have been described. These include peptide trifluoromethyl ketones, chloromethyl ketones,[12] (often exploited as serine proteases), vinyl sulfones,[13] α, β-epoxy ketones,[14] α-keto aldehydes,[15] α-keto amides[16] and boronates.[17] Most of

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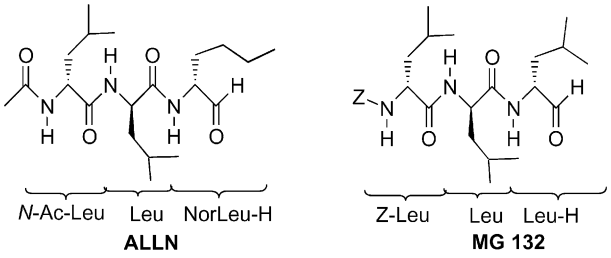
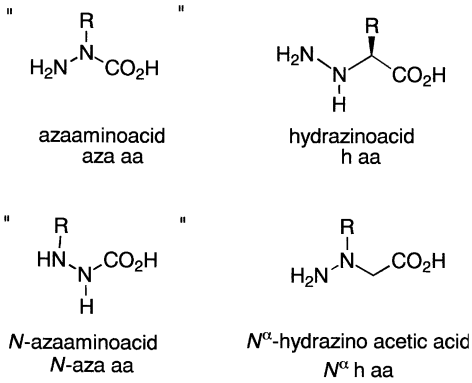


Scheme 1. Peptide aldehyde inhibitors of proteasome.

these inhibitors are too reactive, unstable, nonspecific or membrane impermeable. However, PS-341, a boronate peptide inhibitor has emerged and is actually in clinical trials for cancer treatment.[12,18]

The development of oligomeric peptidomimetics is currently the focus of increasing attention. It represents an interesting strategy to design analogues of structurally and physiologically important targets, as it avoids stereochemical constraints and contribute to enhanced proteolytic resistance. Some significant results have already been obtained following this route in particular with the most widely studied azapeptides,[19] peptoids,[20] retropeptoids,[21] ureapeptoids,[22] amino oxypeptoids,[23] β-peptoids,[24] in which side chains are linked to nitrogen atoms.

Our main focus is the synthesis of what we call hydrazino-azapeptoids, a new class of peptoid analogues.[25] These modified peptides, which have no chiral center, are built with N^{α}-substituted hydrazino acetic acid monomers (N^{α}h aa) and a C-terminal aza amino acid unit (aza aa) or an *N*-substituted azaglycine (*N*-aza aa) monomer (Scheme 2). These 'hybrid' pseudopeptides have nitrogen-enriched peptidic backbones, with nitrogen atoms carrying various side chains mimicking both proteinogenic or nonproteinogenic amino acids (Scheme 3). Here we investigated the in vitro anti-proliferative activity of different synthetic hydrazino-azapeptoids and *N*-azapeptoids, on murine leukemia L1210 cells.



Scheme 2. Adopted symbolism for amino acid analogues.

Results and Discussion

Synthesis of hydrazino-azapeptoids 5 and 6

We set out to prepare a series of hybrid pseudopeptides as new analogues of Ac-Leu-Leu-Norleucinal (ALLN) or *Z*-Leu-Leu-Leucinal (MG 132): the hydrazino-azapeptoids and the hydrazino *N*-aza peptoids with modified C-terminal position (Scheme 4).

The synthesis of hydrazino-aza and *N*-azapeptoids can be accomplished using a two-step procedure to form the hydrazinopeptoids backbones (Scheme 3), which were then deprotected and reacted with suitable nucleophiles (Scheme 4). In a first step, *N*,*N*-substituted carbazates **1** or *N*,*N*′-substituted carbazates **2**,[19a] were treated with bromoacetyl bromide and pyridine in dichloromethane at 0 °C to afford bromo acetylated hydrazines **3** or **4** in 35–75% yield. Nucleophilic substitution of compounds **3** or **4** by *N*,*N*′-substituted carbazates **2** affords the hydrazino-aza **5** or *N*-azapeptoids **6** with correct chains to mimic known proteasome inhibitors.

Hybrid pseudopeptoids **5** or **6** were then deprotected under standard conditions using piperidine in ether (PG = Fmoc) or hydrochloric acid in ether (PG = Boc). Different electrophilic groups, to give reversible or irreversible interaction with the active site of the proteasome, were then introduced in the C-terminal position. By nucleophilic substitution on the nitrogen atom of **7** or **8** target inhibitors **9** or **10** were obtained: respectively, a trifluoroketone by reaction of trifluoroacetic anhydride, a ketoester by reaction of ethyl oxalyl chloride. Formylation of **5** occurred using pentafluorophenylformate generated in situ by coupling formic acid with pentafluorophenol in the presence of DCC.[26]

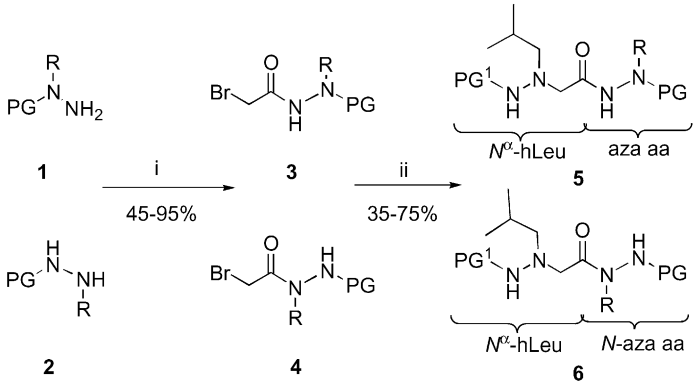
As dipeptidyl boronic acids are new potent proteasome inhibitors,[9,12,17,18] we synthesized analogues **11** with a boronic acid in C-terminal position by condensation of substituted hydrazine **8** with *ortho*-, *meta*- or *para*-formyl benzene boronic acid.

Biological study

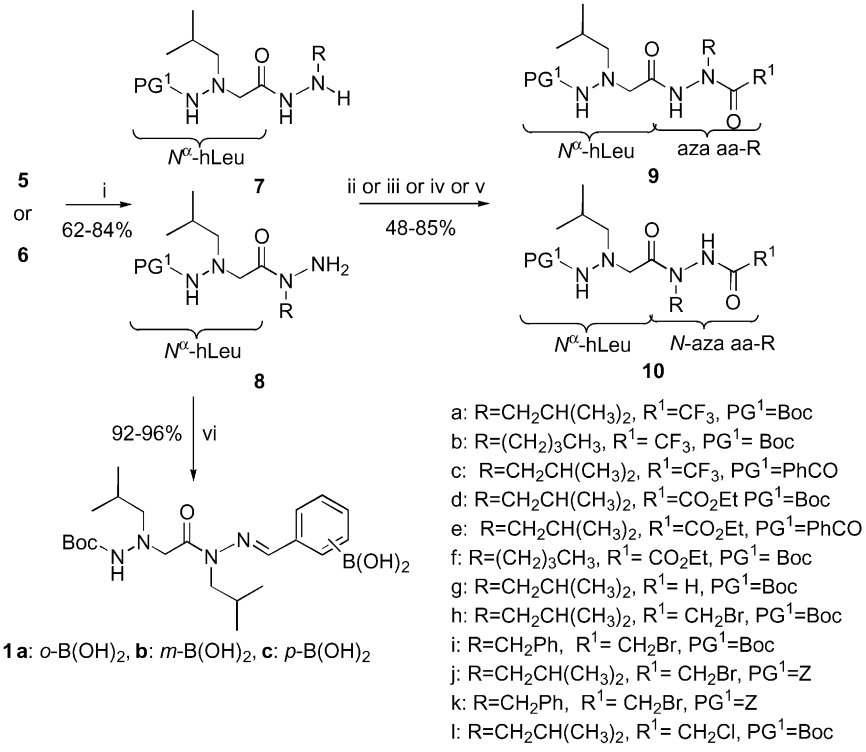
Effect of the different hydrazino-azapeptoids on the growth of the L1210. We examined the effect of the different compounds on the growth of L1210 leukemia cells. IC_{50}s after 24 or 48 h culture are reported in Table 1.

Among the synthesized hydrazino-*N*-azapeptoids, Boc-N^{α}hLeu-*N*-aza-Leu-CF_3 **10a,** PhCO-N^{α}hLeu-*N*-aza-Leu-CO_2Et **10e** and Boc-N^{α}hLeu-*N*-azaLeu-H **10g** had no effect on L1210 cell growth after 24 h culture. The compounds Boc-N^{α}hLeu-*N*-azaLeu-*o*-$C_6H_4B(OH)_2$ **11a** and Boc-N^{α}hLeu-*N*-azaLeu-*p*-$C_6H_4B(OH)_2$ **11c** bearing a C-terminus function *ortho* or *para* benzene boronic acid had a moderate anti-proliferative activity with IC_{50} of 18.5 and 10.5 μM, respectively, after 48 h.

Boc-N^{α}hLeu-*N*-azaLeu-CH_2Br **10 h,** Boc-N^{α}hLeu-*N*-azaPhe-CH_2Br **10i,** *Z*-N^{α}hLeu-*N*-azaPhe-CH_2Br **10k** and Boc-N^{α}hLeu-*N*-azaLeu-CH_2Cl **10l** were the more potent inhibitors of L1210 cell growth with IC_{50}s lower than 2 μM. It should be noted that the most potent



Scheme 3. Reagents and conditions: (i) $BrCH_2COBr$, C_5H_5N/CH_2Cl_2, 0 °C; (ii) $PG^1NHNHCH_2CH(CH_3)_2$, $CHCl_3$, Δ, 24 h.



Scheme 4. Reagent and conditions: (i) PG^1 = Fmoc/piperidine, Et_2O, 15 h (62–84%), PG^1 = Boc/HCl/ Et_2O, $NaHCO_3$ (62%), $PG^1 = Z$: H_2 (1 atm), 5% Pd/C, 2-propanol, 18 h (84%); (ii) $(CF_3CO)_2O$, TEA, Et_2O, rt, 6 h (65–82%); (iii) $ClCOCO_2Et$, TEA, Et_2O, rt, 6 h (48–56%); (iv) HCO_2H, C_6F_5OH, DCC, $CHCl_3$, rt, 4 h (85%); (v) $BrCOCH_2Br$ or $ClCOCH_2Cl$, pyridine, Et_2O, 0 °C, 5 h (57–60%); (vi) $HCOC_6H_4B(OH)_2$, Et_2O, rt, 1 h (92–96%).

compounds bear a bromoacetyl functionality at the C terminus position. Those bromoacetyl analogues are slightly more potent than ALLN.

Some of our new pseudopeptides are potent inhibitors of cell proliferation. These 'hybrid' peptidomimetics are C-modified hydrazino *N*-azapeptoid based compounds bearing diverse electrophilic functional groups such as boronic acid **11a**, **11c**, chloromethylcarbonyl **10l**. Although they have been synthesized to mimic ALLN (Ac-Leu-Leu-Norleu-H) or MG132 (*Z*-Leu-Leu-Leu-H), the ability of these compounds to inhibit the proteolytic activity of the proteasome has not been evaluated yet. Whether the observed anti-proliferative effect in L1210 cells is related or not to proteasome inhibition remains to be analyzed. The potency of the present analogues yet remains low compared to that of Bortezomib, a potent proteasome inhibitor which have

Table 1. IC_{50} of some of the synthesized inhibitors

Analogues	IC_{50} (μM)	
	24 h	48 h
ALLN	3.04±0.01	3.33±0.10
10a	>80	
10e	>80	
10g	>80	
10h	1.21±0.10	0.73±0.15
10i	2.18±0.76	1.33±0.58
10k	0.70±0.15	—
10l	8.10±0.80	3.81±0.38
11a	52.0±18.0	18.5±1.84
11c	17.6±0.6	10.5±2.4

entered clinical trials.[5] Nevertheless, the present study allowed the identification of appealing leads. Pharmacomodulations of those analogues are in progress in order to enhance their antiproliferative activity.

Experimental

General methods

NMR spectra were run at 200, 300 (^{1}H) or 75.5 MHz (^{13}C). HR-MS were obtained from the 'Centre Régional de Mesures Physiques de l'Ouest', using MS/MS Mass spectrometer ZAB Spec TOF. Elemental analyses were performed by the analytical laboratory (CNRS, Lyon). Thin-layer chromatography was performed on silica gel 60 F_{254} plates from Merck. Melting points were taken with a LEICA system Kofler.

Boc, Fmoc or *Z*-protected alkyl or aralkyl hydrazines **1** and **2** were prepared according to literature procedures by reduction of Boc or *Z*-protected hydrazones, derived from the condensation of Boc or *Z*-carbazate with either aldehyde or ketone.[19c,25]

$BrCH_2CO$-azaLeu-OFm [3a, $R=CH_2CH(CH_3)_2$, PG = Fmoc]. To a stirred and cooled (0 °C) solution of *N*-*i*-butyl-*N*-Fmoc hydrazine **1a** (3.1 g, 10 mmol) in CH_2Cl_2 (10 mL) and pyridine (0.95 g, 12 mmol), was added a solution of bromoacetyl bromide **2** (2.5 g, 12 mmol) in CH_2Cl_2 (10 mL). The resulting mixture was stirred over a period of 5 h, and washed three times with water (50 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated in vacuo to give the crude product **3a** (1.94 g, 45%) which was considered sufficiently pure for the following reactions. Mp 133 °C. ^{1}H NMR ($CDCl_3$, δ): 0.83 (br s, 6H), 1.73 (br s, 1H), 2.81 (br s, 2H), 3.26 (br s, 2H), 4.22 (br t, 1H), 4.55 (br d, 2H), 7.25–7.77 (m, 8H), 8.28 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 19.8, 26.2, 26.7, 47.1, 56.8, 67.5, 119.9, 124.7, 127.1, 127.7, 141.3, 143.5, 155.9, 164.8. Anal. calcd for $C_{21}H_{23}BrN_2O_3$: C, 58.47; H,5.34; Br, 18.56; N, 6.50. Found: C, 58.52; H, 5.50; Br, 17.98; N, 6.64.

$BrCH_2CO$-*N*-azaLeu-OFm [4a, $R=CH_2CH(CH_3)_2$, PG = Fmoc]. The title compound was synthesized from *N*-*i*-butyl-*N*′-Fmoc hydrazine **2a** (3.1 g, 10 mmol) following the general procedure for **3a** (4.1 g, 95%). Mp 167 °C. ^{1}H NMR ($CDCl_3$, δ): 0.89 (d, $J=6.7$ Hz, 6H), 1.81 (m, 1H), 3.59 (br s, 2H), 3.93 (br s, 2H), 4.26 (t, $J=7$ Hz, 1H), 4.74 (br d, 2H), 6.84 (s, 1H), 7.32–7.89 (m, 8H). ^{13}C NMR ($CDCl_3$, δ): 19.9, 26.1, 26.2, 47.4, 54.9, 66.9, 120.2, 125.1, 127.1, 128.2, 141.4, 143.1, 154.5, 169.3. Anal. calcd for $C_{21}H_{23}BrN_2O_3$: C, 58.47; H, 5.34; Br, 18.56; N, 6.50. Found: C, 56.13; H, 4.93; Br, 19.44; N, 6.89.

$BrCH_2CO$-*N*-azaNorleu-OFm [4b, $R=(CH_2)_3CH_3$, PG = Fmoc]. The title compound was synthesized from *N*-*n*-butyl-*N*′-Fmoc hydrazine **2b** (3.1 g, 10 mmol) following the general procedure for **3a**. **4b** (2.3 g, 52%). Mp 155 °C. ^{1}H NMR ($CDCl_3$, δ): 0.87 (t, $J=7.0$ Hz, 3H), 1.24 (br, 2H), 1.36 (br, 2H), 1.79 (s, 2H), 3.57 (s, 2H), 3.60 (br s, 2H), 4.20 (t, $J=6.5$ Hz, 1H), 4.67 (br s, 2H), 7.25–7.40 (m, 8H). ^{13}C NMR ($CDCl_3$, δ): 13.7, 19.7, 26.3, 28.2, 47.3, 47.7, 66.9, 120.1, 124.6, 127.1, 127.9, 141.5, 143, 154.7, 168.8. Anal. for $C_{21}H_{23}BrN_2O_3$: C, 58.47; H, 5.34; Br, 18.56; N, 6.50. Found: C, 58.34; H, 5.44; Br, 18.20; N, 6.64.

$BrCH_2CO$-*N*-azaLeu-O*t*-Bu [4d, $R=CH_2CH(CH_3)_2$, PG = Boc]. The title compound was synthesized from *N*-*i*-butyl-*N*′-Boc hydrazine **2d** (1.9 g, 10 mmol) following the general procedure for **3a**: (2.4 g, 78%). Mp 96 °C. ^{1}H NMR ($CDCl_3$, δ): 0.98 (d, $J=7.0$ Hz, 6H), 1.55 (s, 9H), 1.99 (m, 1H), 2.86–4.22 (br, 2×2H), 6.80 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 20.4, 26.6.1, 26.9, 28.6, 55.7, 82.9, 154.3, 169.9. Anal. calcd for $C_{11}H_{21}BrN_2O_3$: C,.42.73; H, 6.85; Br, 25.84; N, 9.08. Found: C, 42.53; H, 6.86; Br, 25,92; N, 9.06.

$BrCH_2CO$-*N*-azaPhe-O*t*-Bu [4e, $R=CH_2C_6H_5$, PG = Boc]. The title compound was synthesized from *N*-benzyl-*N*′-Boc hydrazine **2e** (2.2 g, 10 mmol) following the general procedure for **3a** (2.4 g, 68%). Mp 76 °C. ^{1}H NMR ($CDCl_3$, δ): 1.35 (s, 9H), 3.87 (s, 2H), 4.14–5.20 (br s, 2H), 6.73 (s, 1H), 7.17–7.27 (m, 5H). ^{13}C NMR ($CDCl_3$, δ): 27.1, 28.5, 51.3, 83.1, 128.7, 129.5, 129.7, 135.1, 154.3, 169.5. Anal. calcd for $C_{14}H_{19}BrN_2O_3$: C,.49.11; H, 5.60; Br, 23.07; N, 8.19. Found: C, 48.98; H, 5.62; Br, 22.93; N, 8.12.

$BrCH_2CO$-*N*-azaPhe-OBz (4f, $R=CH_2C_6H_5$, PG = *Z*). The title compound was synthesized from *N*-benzyl-*N*′-*Z* hydrazine **2f** (2.1 g, 10 mmol) following the general procedure for **3a** (2.5 g, 66%). Mp 76 °C. ^{1}H NMR ($CDCl_3$, δ): 3.98 (s, 2H), 4.15–5.40 (br s, 2H), 5.19 (s, 2H), 6.79 (s, 1H), 7.34–7.40 (m, 10H). ^{13}C NMR ($CDCl_3$, δ): 26.7, 51.2, 68.7, 128.6, 128.7, 128.8, 129.1, 129.3, 129.4, 134.8, 135.5, 155.2, 169.4. Anal. calcd for $C_{17}H_{17}BrN_2O_3$: C,.54.11; H, 3.56; Br, 21.22; N, 7.43. Found: C, 54.56; H, 4.67; Br, 20.45; N, 7.54.

Boc-N^{α}hLeu-azaLeu-OFm [5a, $R=CH_2CH(CH_3)_2$, PG = Fmoc, PG^1 = Boc]. To a stirred solution of *N*-*i*-butyl-*N*′-Boc hydrazine **2** (4.7 g, 25 mmol) in chloroform (10 mL) was added slowly the α-bromohydrazide **3a** (4.3 g, 10 mmol) in chloroform (5 mL). The reaction mixture was refluxed over a period of 24 h, and after cooling washed three times with water (50 mL), once with HCl 2 N (50 mL), once with $NaHCO_3$ 1 N (50 mL)

and once with water (50 mL). The organic phase was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by silica gel chromatography (hexane–EtOAc as eluent 1:1) to afford **5a** (3.9 g, 73%); oil; ^{1}H NMR ($CDCl_3$, δ): 0.87 (d, J = 6.5 Hz, 12H), 1.33 (s, 9H), 1.57 (m, 1H), 1.76 (m, 1H), 2.44 (d, J = 6.5 Hz, 2H), 3.30 (br, 2H), 3.34 (s, 2H), 4.15 (t, J = 6.7 Hz, 1H), 4.32 (d, J = 6.7 Hz, 2H), 5.59 (s, 1H), 7.20–7.75 (m, 8H), 10.02 (s, 1H) two rotameric forms are observed in proportion 90/10, only data of the major compound are reported. ^{13}C NMR ($CDCl_3$, δ): 18.3, 19.6, 25.3, 26.0, 28.6, 47.2, 59.2, 61.5, 62.1, 66.8, 82.1, 124.9, 127.1, 127.6, 141.4, 142.4, 154.8, 156.8, 171.3. Anal. calcd for $C_{30}H_{42}N_4O_5$: C,.66.88; H, 7.86; N, 10.41. Found: C, 66.89; H, 7.78; N, 10.38.

Boc-N^{α}hLeu-*N*-azaLeu-OFm [6a, R = $CH_2CH(CH_3)_2$, PG = Fmoc, PG^1 = Boc]. The title compound was synthesized from α-bromohydrazide **4a** (4.3 g, 10 mmol) following the general procedure for **5a**. (3.7 g, 68%); oil; ^{1}H NMR ($CDCl_3$, δ): 0.83 (d, J = 6.7 Hz, 6H), 0.87 (d, J = 6.7 Hz, 6H), 1.34 (s, 9H), 1.59 (m, 1H), 1.78 (m, 1H), 2.37 (d, J = 6.7 Hz, 2H), 3.32 (s, 2H), 3.43 (br, 2H), 4.14 (t, J = 6.5 Hz, 1H), 4.43 (d, J = 6.5 Hz, 2H), 6.01 (s, 1H), 7.15–7.73 (m, 8H), 8.87 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 19.6, 20.7, 25.4, 28.6, 47.2, 60.9, 62.2, 64.4, 82.1, 119.9, 124.9, 127.2, 127.6, 141.4, 143.9, 156.6, 156.8, 169.9. Anal. calcd for $C_{30}H_{42}N_4O_5$: C,.66.88; H, 7.86; N, 10.41. Found: C, 66.79; H, 7.69; N, 10.31.

Boc-N^{α}hLeu-*N*-azaNorleu-OFm [6b, R = $(CH_2)_3CH_3$, PG = Fmoc, PG^1 = Boc]. The title compound was synthesized from α-bromohydrazide **4b** (4.3 g, 10 mmol) following the general procedure for **5a**. (4.2 g, 78%). oil. ^{1}H NMR ($CDCl_3$, δ): 0.83 (d, J = 6.3 Hz, 6H), 0.88 (t, J = 6.8 Hz, 3H), 1.34 (s, 9H), 1.10–1.49 (m, 4H), 1.62 (m, 1H), 2.33 (d, J = 6.3 Hz, 2H), 3.37 (br, 2H), 3.44 (s, 2H), 4.14 (t, J = 6.5 Hz, 1H), 4.43 (d, J = 6.5 Hz, 2H), 5.85 (s, 1H), 7.20–7.74 (m, 8H), 8.96 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 13.7, 19.6, 21.3, 25.3, 28.5, 30.5, 47.2, 49.4, 55.4, 62.1, 64.3, 82.1, 119.9, 124.9, 127.2, 127.6, 141.4, 143.9, 156.3, 156.8, 169.2. Anal. calcd for $C_{30}H_{42}N_4O_5$: C, 66.88; H, 7.86; N, 10.41. Found: C, 66.76; H, 7.82; N, 10.39.

PhCO-N^{α}hLeu-*N*-azaLeu-OFm [6c, R = $CH_2CH(CH_3)_2$, PG = Fmoc, PG^1 = PhCO]. The title compound was synthesized from α-bromohydrazide **4c** (4.3 g, 10 mmol) and *N*-*i*-butyl-*N*′-COPh hydrazine **1f** (1.9 g, 10 mmol) following the general procedure for **5a**. (3.4 g, 62%); oil. ^{1}H NMR ($CDCl_3$, δ): 0.77 (d, J = 6.7 Hz, 6H), 0.86 (d, J = 6.7 Hz, 6H), 1.65 (m, 1H), 1.81 (m, 1H), 2.57 (d, J = 6.7 Hz, 2H), 3.28 (d, J = 6.2 Hz, 2H), 3.61 (br s, 2H), 4.11 (t, J = 7 Hz, 1H), 4.42 (d, J = 7 Hz, 2H), 7.19–7.75 (m, 13H), 8.43 (s, 1H), 9.10 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 17.7, 20.8, 24.7, 26.4, 47.2, 54.7, 60.9, 61.6, 64.3, 119.9, 124.9, 127.2, 127.6, 128.3, 128.6, 133.4, 141.4, 144.0, 156.5, 168.9, 170.1. Anal. calcd for $C_{32}H_{38}N_4O_4$: C, 70.81; H, 7.06; N, 10.33. Found: C, 71.01; H, 7.04; N, 10.32.

Z-N^{α}hLeu-*N*-azaLeu-O*t*-Bu [6d, R = $CH_2CH(CH_3)_2$, PG = Boc, PG^1 = *Z*]. The title compound was synthesized from α-bromohydrazide **4d** (3.1 g, 10 mmol) and *N*-*i*-butyl-*N*′-*Z* hydrazine (2.2 g, 10 mmol) following the general procedure for **5a**. (2.9 g, 65%). Mp 90 °C. ^{1}H NMR ($CDCl_3$, δ): 0.82 (d, J = 6.5 Hz, 6H), 0.89 (d, J = 6.5 Hz, 6H), 1.44 (s, 9H), 1.62 (m, 1H), 1.77 (m, 1H), 2.53 (d, J = 6 Hz, 2H), 3.18–3.51 (br s, 2H), 3.38 (s, 2H), 5.04 (s, 2H), 7.35 (m, 5H), 8.44 (s, 1H), 9.37 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 20.3, 20.9, 26.2, 26.4, 28.2, 54.9, 60.1, 65.2, 65.8, 80.6, 128.0, 128.2, 128.7, 137.1, 154.6, 156.3, 171.2. Anal. for $C_{23}H_{38}N_4O_5$: C, 61.31; H, 8.50; N, 12.43. Found: C, 61.14; H, 8.56; N, 12.48.

Z-N^{α}hLeu-*N*-azaPhe-O*t*-Bu (6e, R = $CH_2C_6H_5$, PG = Boc, PG^1 = *Z*). The title compound was synthesized from α-bromohydrazide **4e** (3.5 g, 10 mmol) and *N*-*i*-butyl-*N*′-*Z* hydrazine (2.2 g, 10 mmol) following the general procedure for **5a**. (3.5 g, 72%). Mp 98 °C. ^{1}H NMR ($CDCl_3$, δ): 1.05 (d, J = 6 Hz, 6H), 1.52 (s, 9H), 1.82 (m, 1H), 2.72 (d, J = 5.75 Hz, 2H), 3.78 (s, 2H), 4.25–5.55 (br s, 2H), 5.09 (s, 2H), 6.94 (s, 1H), 7.43 (m, 10H), 7.64 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 21.1, 26.7, 28.5, 51.4, 60.5, 66.6, 67.2, 82.3, 128.3, 128.5, 128.9, 129.1, 129.7, 135.8, 136.6, 154.4, 156.6, 172.1. Anal. for $C_{26}H_{36}N_4O_5$: C, 64.46; H, 7.44; N, 11.57. Found: C, 64.20; H, 7.45; N, 11.63.

Boc-N^{α}hLeu-*N*-azaPhe-OBn (6f, R = $CH_2C_6H_5$, PG = *Z*, PG^1 = Boc). The title compound was synthesized from α-bromo hydrazide **4f** (3.8 g, 10 mmol) and *N*-*i*-butyl-*N*′-Boc hydrazine (1.9 g, 10 mmol) following the general procedure for **5a**. (4.2 g, 69%). oil. ^{1}H RMN ($CDCl_3$, δ): 0.87 (d, J = 6.5 Hz, 6H), 1.40 (s, 9H), 1.65 (m, 1H), 2.47 (d, J = 6.75 Hz, 2H), 3.58 (s, 2H), 4.77 (br s, 2H), 5.15 (s, 2H), 6.08 (s, 1H), 7.36 (m, 10H), 8.39 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 19.6, 25.3, 28.6, 49.9, 55.3, 62.1, 65.3, 82.1, 127.3, 124.9, 128.5, 128.6, 128.9, 134.6, 137.3, 155.8, 156.8, 169.7. Anal. for $C_{26}H_{36}N_4O_5$: C, 64.46; H, 7.44; N, 11.57. Found: C, 64.50; H, 7.35; N, 11.53.

Boc-N^{α}hLeu-NH-NHi-Bu (7a, R = $CH_2CH(CH_3)_2$, PG^1 = Boc). The hydrazino-azapeptoid **5a** (5.4 g, 10 mmol) was dissolved in ether (5 mL). A solution of piperidine (1.7 g, 20 mmol) in ether (3 mL) was added dropwise and the mixture was allowed to stir for 15 h. Evaporation of the solvent and trituration of the crude product with ethanol afforded a white precipitate of dibenzofulvene–piperidine adduct. After filtration of this secondary product, evaporation of the solvent and addition of ether gave the desired product **7a** (1.95 g, 62%). ^{1}H NMR ($CDCl_3$, δ): 0.86 (d, J = 6.7 Hz, 6H), 0.89 (d, J = 7 Hz, 6H), 1.36 (s, 9H), 1.67 (m, 2×1H), 2.43 (d, J = 6.7 Hz, 2H), 2.56 (d, J = 7 Hz, 2H), 3.32 (s, 2H), 5.54 (s, 1H), 9.31 (s, 1H). Anal. calcd for $C_{16}H_{34}N_4O_3$: C,.58.14; H, 10.38; N, 16.96. Found: C, 58.35; H, 10.32; N, 16.85.

Boc-N^{α}hLeu-N*i*-Bu-NH_2 [8a, R = $CH_2CH(CH_3)_2$, PG^1 = Boc]. The title compound **8a** was synthesized from **6a** (5.4 g, 10 mmol) following the general procedure for **7a**. (2.7 g, 84%); mp 104 °C. ^{1}H NMR ($CDCl_3$, δ): 0.93 (d, J = 6.7 Hz, 6H), 0.97 (d, J = 6.7 Hz, 6H), 1.45 (s, 9H), 1.76 (m, 1H), 2.04 (m, 1H), 2.64 (d, J = 6.7 Hz,

2H), 3.35 (d, $J=6.7$ Hz, 2H), 3.93 (d, $J=6.2$ Hz, 2H), 4.03 (s, 2H), 6.69 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 20.3, 21.1, 26.1, 27.1, 28.7, 57.1, 58.1, 65.8, 79.9, 155.8, 173.2 two rotameric forms are observed in proportion 85/15, only data of the major compound are reported. Anal. calcd for $C_{15}H_{32}N_4O_3$: C, 56.96; H, 10.13; N, 17.72. Found: C, 56.73; H, 10.19; N, 17.73.

Boc-N^{α}hLeu-*Nn*-Bu-NH_2 [8b, R = $(CH_2)_3CH_3$, PG^1 = Boc]. The title compound was synthesized from **6d** (5.4 g, 10 mmol) following the general procedure for **7a** (2.5 g, 78%); mp 105 °C. ^{1}H NMR ($CDCl_3$, δ): 0.93 (d, $J=6.7$ Hz, 6H), 0.94 (t, $J=7$ Hz, 3H), 1.34 (m, 2H), 1.43 (s, 9H), 1.57 (m, 2H), 1.73 (m, 1H), 2.61 (d, $J=6.7$ Hz, 2H), 3.50 (t, $J=7$ Hz, 2H), 3.88 (br s, 2H), 4.31 (br s, 2H), 6.79 (br s, 1H). ^{13}C NMR ($CDCl_3$, δ): 14.1, 20.1, 21.1, 26.9, 28.6, 30.2, 49.3, 57.9, 59.6, 65.9, 79.1, 155.8, 173.2, two rotameric forms are observed in proportion 90/10, only data of the major compound are reported. Anal. calcd for $C_{15}H_{32}N_4O_3$: C, 56.96; H, 10.13; N, 17.72. Found: C, 56.77; H, 9.99; N, 17.57.

PhCO-N^{α}hLeu- *Ni*-Bu-NH_2 [8c, R = $CH_2CH(CH_3)_2$, PG^1 = PhCO]. The title compound **8c** was synthesized from **6e** (5.4 g, 10 mmol) following the general procedure for **7a.** (3.5 g, 84%); oil. ^{1}H NMR ($CDCl_3$, δ): 0.91 (d, $J=6.7$ Hz, 6H), 0.98 (d, $J=6.5$ Hz, 6H), 1.81 (m, 1H), 2.02 (m, 1H), 2.81 (d, $J=6.7$ Hz, 2H), 3.34 (d, $J=6.5$ Hz, 2H), 3.91 (s, 2H), 4.12 (s, 2H), 7.345–7.83 (m, 5H), 8.79 (s, 1H) two rotameric forms are observed in proportion 85/15, only data of the major compound are reported. Anal. calcd for $C_{18}H_{30}N_4O_2$: C, 64.64; H, 9.04; N, 16.75. Found: C, 64.54; H, 8.89; N, 16.89.

Z-N^{α}hLeu-*Ni*-Bu-NH_2 (8d, R = $CH_2CH(CH_3)_2$, PG^1 = Z]. A solution of hydrazino-azapeptoid **6d** (4.5 g, 10 mmol) in ether (10 mL) was cooled to 0 °C. A saturated solution of HCl in ether (20 mL) was added dropwise, and the solution was allowed to warm to room temperature. A white precipitate was formed, which was collected by filtration. The residue was dissolved in $NaHCO_3$ 1 N (20 mL) and extracted with ether (3×20 mL). The organic layers was dried under $Na_2S_2O_4$ and evaporated to give product **8d** as an oil (2.2 g, 62%). ^{1}H NMR ($CDCl_3$, δ): 0.93 (d, $J=7.5$ Hz, 6H), 0.97 (d, $J=6.75$ Hz, 6H), 1.77 (m, 1H), 2.02 (m, 1H), 2.70 (d, $J=6.75$ Hz, 2H), 3.33 (d, $J=7.5$ Hz, 2H), 3.85–4.13 (br s, 2H), 4.01 (s, 2H), 5.13 (s, 2H), 7.26 (s, 1H), 7.36 (s, 5H). ^{13}C NMR ($CDCl_3$, δ): 20.6, 21.2, 26.7, 28.6, 56.1, 63.2, 67.2, 128.3, 128.6, 128.9, 136.5, 154.7, 157.1. Two rotameric forms are observed in proportion 95/5; only data of the major compound are reported. Anal. calcd for $C_{18}H_{29}N_4O_3$: C, 61.87; H, 8.36; N, 16.03. Found: C, 61.75; H, 8.32; N, 15.95.

Z-N^{α}hLeu-*N*Bz- NH_2 (8e, R = $CH_2C_6H_5$, PG^1 = Z). The title compound **8e** was synthesized from **6e** (4.9 g, 10 mmol) following the general procedure for **8d.** (2.5 g, 64%); oil. ^{1}H NMR ($CDCl_3$, δ): 0.96 (d, $J=6.5$ Hz, 6H), 1.77 (m, 1H), 2.50 (s, 1H), 2.75 (d, $J=7$ Hz, 2H), 3.73 (s, 2H), 4.02 (s, 2H), 4.66 (s, 2H), 5.11 (s, 2H), 7.26 (s, 1H), 7.35 (m, 2×5H). Anal. calcd for $C_{21}H_{27}N_4O_3$: C,.65.78; H, 7.10; N, 14.61. Found: C, 65.56; H, 7.05; N, 14.52.

Boc-N^{α}hLeu-*N*Bz-NH_2 (8f, R = $CH_2C_6H_5$, PG^1 = Boc). To a solution of **6f** (5.4 g, 10 mmol) in 2-propanol (50 mL) was added Pd/C 10% (60 mg per mmol, 6 mg). After 18 h of stirring under H_2 atmosphere, the solution was filtered on Celite and the solvent was evaporated to afford **8f** (2.9 g, 84%). Mp 90 °C. ^{1}H NMR ($CDCl_3$, δ): 1.01 (d, $J=6.5$ Hz, 6H), 1.52 (s, 9H), 1.82 (m, 1H), 2.76 (d, $J=6.5$ Hz, 2H), 3.86 (s, 2H), 4.05 (s, 2H), 4.77 (s, 2H), 6.98 (s, 1H), 7.41 (m, 5H). ^{13}C NMR ($CDCl_3$, δ): 21.1, 27.1, 28.8, 53.2, 59.0, 65.8, 79.8, 127.3, 128.4, 129.0, 129.3, 135.9, 155.7, 173.1. Anal. calcd for $C_{18}H_{30}N_4O_3$: C, 61.69; H, 8.63; N, 15.99. Found: C, 61.16; H, 8.66; N, 15.71.

Boc-N^{α}hLeu-azaLeu-CF_3 [9a, R = $CH_2CH(CH_3)_2$, PG^1 = Boc, R^1 = CF_3]. To a stirred and cooled solution (0 °C) of **7a** (1.6 g, 5 mmol) in ether (5 mL) and triethylamine (0.6 g, 5.5 mmol) was added dropwise trifluoroacetic anhydride (1.2 g, 5.5 mmol) in 5 mL of ether. The mixture was allowed to stir for 6 h. The reaction mixture was washed three times with water (30 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated in vacuo to give the crude product **9a** which precipitate slowly in ether: (0.7 g, 65%). Mp 105 °C. ^{1}H NMR ($CDCl_3$, δ): 0.87 (d, $J=7$ Hz, 12H), 1.37 (s, 9H), 1.57 (m, 1H), 1.88 (m, 1H), 2.50 (br, 2H), 3.07–3.86 (br, 2H), 3.42 (s, 2H), 5.81 (s, 1H), 10.70 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 20.1, 20.7, 26.1, 26.9, 28.4, 56.2, 62.3, 69.1, 81.8, 119.3, 157.3, 158.5, 170.1. Anal. calcd for $C_{17}H_{31}F_3N_4O_4$: C, 49.52; H, 7.52; F, 13.84; N, 13.59. Found: C, 49.77; H, 7.80; F, 13.42; N, 13.88.

Boc-N^{α}hLeu-*N*-azaLeu-CF_3 [10a, R = $CH_2CH(CH_3)_2$, PG^1 = Boc, R^1 = CF_3]. The title compound was synthesized from **8a** (1.6 g, 5 mmol) following the general procedure for **9a**. (2.7 g, 65%). Mp 110 °C. ^{1}H NMR ($CDCl_3$, δ): 0.84 (d, $J=6.7$ Hz, 6H), 0.88 (d, $J=6.7$ Hz, 6H), 1.35 (s, 9H), 1.67 (m, 1H), 1.77 (m, 1H), 2.31 (d, $J=6.7$ Hz, 2H), 3.33 (d, $J=6.7$ Hz, 2H), 3.35 (s, 2H), 5.39 (s, 1H), 11.22 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 20.8, 21, 26.3, 27.4, 28.5, 55.8, 64.3, 67.9, 81.9, 119.8, 156.1, 157.4, 169.2. Anal. calcd for $C_{17}H_{31}F_3N_4O_3$: C, 49.51; H, 7.52; F, 13.83; N, 13.59. Found: C, 49.30; H, 7.83; F, 13.51; N, 13.86.

Boc-N^{α}hLeu-*N*-azaNorleu-CF_3 [10b, R = $(CH_2)_3CH_3$, PG^1 = Boc, R^1 = CF_3]. The title compound was synthesized from **8b** (1.6 g, 5 mmol) following the general procedure for **9a** (3.3 g, 81%). Mp 90–95 °C. ^{1}H NMR ($CDCl_3$, δ): 0.80 (d, $J=6.7$ Hz, 6H), 0.86 (t, $J=7$ Hz, 3H), 1.24 (m, 2H), 1.35 (s, 9H), 1.45 (m, 2H), 1.65 (m, 1H), 2.32 (d, $J=7$ Hz, 2H), 3.34 (s, 2H), 3.49 (br, 2H), 5.73 (s, 1H), 11.32 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 14.1, 20.3, 20.9, 26.3, 28.5, 29.3, 48.2, 64.1, 67.7, 81.6, 113.2, 156.2, 157.3, 168.5. Anal. calcd for $C_{17}H_{31}F_3N_4O_4$: C, 49.52; H, 7.52; F, 13.84; N, 13.59. Found: C, 49.68; H, 7.68; F, 13.77; N, 13.55.

PhCO-N^{α}hLeu-*N*-azaLeu-CF_3 [10c, R = $CH_2CH(CH_3)_2$, PG^1 = PhCO, R^1 = CF_3]. The title compound was synthesized from **8c** (2.1 g, 5 mmol) following the general procedure for **9a** (4.2 g, 82%). Mp 144 °C. ^{1}H NMR ($CDCl_3$, δ): 0.87 (d, $J=6.5$ Hz, 6H), 0.99 (d, $J=6.5$ Hz,

6H), 1.68 (m, 1H), 1.86 (m, 1H), 2.54 (d, $J=6.5$ Hz, 2H), 3.44 (s large, 2H), 3.51 (s, 2H), 7.46–7.76 (m, 5H), 7.89 (s, 1H), 11.90 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 20.8, 21.1, 26.5, 27.3, 55.9, 63.7, 67.5, 127.8, 129.1, 132.1, 132.9, 155.9, 156.7, 168.8, 169.8. Anal. calcd for $C_{19}H_{27}F_3N_4O_3$: C, 54.81; H, 6.49; F, 13.46; N, 13.70. Found: C, 55.15; H, 6.53; F, 13.28; N, 13.61.

Boc-N^{α}hLeu-azaLeu-CO_2Et [9d, R $CH_2CH(CH_3)_2$, PG^1 = Boc, R^1 = CO_2Et]. To a stirred and cooled solution (0 °C) of **7a** (1.6 g, 5 mmol) in ether (5 mL) and triethylamine (0.6 g, 5.5 mmol) was added dropwise ethyl oxalyl chloride (0.75 g, 5.5 mmol) in 5 mL of ether. The mixture was allowed to stir for 6 h. The reaction mixture was washed three times with water (30 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated in vacuo to give the crude product **9d**, which slowly precipitates in ether (2.3 g, 56%). Mp 147 °C. ^{1}H NMR ($CDCl_3$, δ): 0.86 (d, $J=6.5$ Hz, 6H), 0.90 (d, $J=6.5$ Hz, 6H), 1.24 (t, $J=7.2$ Hz, 3H), 1.38 (s, 9H), 1.57 (m, 1H), 1.85 (m, 1H), 2.47 (d, $J=7$ Hz, 2H), 3.38 (s, 2H), 3.40 (d, $J=6.7$ Hz, 2H), 4.18 (q, $J=7.2$ Hz, 2H), 5.75 (s, 1H), 10.40 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 14.3, 20.2, 20.8, 26.3, 26.8, 28.5, 54.6, 62.2, 62.7, 68.8, 81.7, 157.1, 162.5, 163.6, 169.8. Anal. calcd for $C_{19}H_{36}N_4O_6$: C, 54.81; H, 8.65; N, 13.46. Found: C, 54.26; H, 8.49; N, 13.04.

Boc-N^{α}hLeu-*N*-azaLeu-CO_2Et [10d, R = $CH_2CH(CH_3)_2$, PG^1 = Boc, R^1 = CO_2Et]. The title compound was synthesized from **8a** (1.6 g, 10 mmol) following the general procedure for **9d** (2.24 g, 54%). Mp 100–105 °C. ^{1}H NMR ($CDCl_3$, δ): 0.99 (d, $J=6.5$ Hz, 12H), 1.49 (t, $J=7$ Hz, 3H), 1.51 (s, 9H), 1.78 (m, 1H), 1.94 (m, 1H), 2.49 (d, $J=7$ Hz, 2H), 3.48 (s, 2H), 3.54 (d, $J=7$ Hz, 2H), 4.45 (q, $J=7$ Hz, 2H), 5.91 (s, 1H), 11.06 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 13.7, 18.8, 19.5, 25.7, 28.5, 54.1, 60.3, 62.1, 63.2, 82.1, 149.1, 156.8, 162.4. Anal. calcd for $C_{19}H_{36}N_4O_6$: C, 54.81; H, 8.65; N, 13.46. Found: C, 54.85; H, 8.58; N, 13.31.

PhCO-N^{α}hLeu-*N*-azaLeu-CO_2Et [10e, R = $CH_2CH(CH_3)_2$, PG^1 = PhCO, R^1 = CO_2Et]. The title compound was synthesized from **8c** (2.1 g, 10 mmol) following the general procedure for **9d.** (2.85 g, 55%). Mp 105 °C. ^{1}H NMR ($CDCl_3$, δ): 0.77 (d, $J=6.7$ Hz, 6H), 0.86 (d, $J=6.7$ Hz, 6H), 1.34 (t, $J=7.2$ Hz, 3H), 1.70 (m, 1H), 1.76 (m, 1H), 2.47 (d, $J=7$ Hz, 2H), 3.35 (d, $J=7$ Hz, 2H), 3.49 (s, 2H), 4.31 (q, $J=7.2$ Hz, 2H), 7.33–7.67 (m, 5H), 7.83 (s, 1H), 11.16 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 13.7, 18.7, 19.5, 25.6, 28.5, 54.1, 60.3, 62.1, 63.2, 82.1, 149.1, 156.9, 162.5, 168.4. Anal. calcd for $C_{21}H_{32}N_4O_5$: C, 60.00; H, 7.62; N, 13.33. Found: C, 59.69; H, 7.69; N, 12.96.

Boc-N^{α}hLeu-*N*-azaNorleu-CO_2Et [10f, R = $(CH_2)_3CH_3$, PG^1 = Boc, R^1 = CO_2Et]. The title compound was synthesized from **8a** (1.6 g, 10 mmol) following the general procedure for **9d** (2.28 g, 55%). Mp 105 °C; ^{1}H NMR ($CDCl_3$, δ): 0.83 (d, $J=6.7$ Hz, 6H), 0.85 (t, $J=7.2$ Hz, 3H), 1.17–1.41 (m, 4H), 1.30 (t, $J=7.2$ Hz, 3H), 1.32 (s, 9H), 1.61 (m, 1H), 2.30 (d, $J=6.7$ Hz, 2H), 3.34 (s, 2H), 3.46 (t, $J=7.2$ Hz, 2H), 4.24 (q, $J=7.2$ Hz, 2H), 5.93 (s, 1H), 11.01 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 14.1, 14.2, 20.3, 21.0, 26.4, 28.6, 29.3, 48.1, 63.1, 63.6, 67.4, 80.9, 155.7, 156.6, 159.5, 169.1. Anal. calcd for $C_{19}H_{36}N_4O_6$: C, 54.81; H, 8.65; N, 13.46. Found: C, 54.62; H, 8.81; N, 13.48.

Boc-N^{α}hLeu-*N*-azaLeu-H [10g, R = $CH_2CH(CH_3)_2$, PG^1 = Boc, R^1 = H]. To a stirred and cooled solution (0 °C) of pentafluorophenol (0.92 g, 5 mmol) in ether (5 mL) was added formic acid (0.28 g, 6 mmol) and DCC (1.03 g, 5 mmol). After 10 min, **8a** (0.8 g, 2.5 mmol) in solution of chloroform (5 mL) was added to the mixture and stirred at room temperature. After 4 h, the reaction mixture was diluted by chloroform (20 mL) and DIEA (0.58 g, 5 mmol) was added. The reaction mixture was washed with HCl 1 N (10 mL), $NaHCO_3$ 5% (10 mL) and water (10 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated in vacuo to give **10g** as a precipitate (2.92 g, 85%). Mp 124 °C. ^{1}H NMR ($CDCl_3$, δ): 0.94 (d, $J=6.5$ Hz, 6H), 0.98 (d, $J=6.5$ Hz, 6H), 1.46 (s, 9H), 1.75 (m, 1H), 1.94 (m, 1H), 2.47 (d, $J=6.5$ Hz, 2H), 3.48 (s, 2H), 3.50 (d, $J=6.5$ Hz, 2H), 5.69 (s, 1H), 8.12 (s, 1H), 10.34 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 20.7, 21.0, 26.5, 27.1, 28.7, 55.3, 63.8, 67.0, 81.4, 156.8, 159.8, 169.7. Anal. calcd for $C_{16}H_{32}N_4O_4$: C, 55.76; H, 9.36; N, 16.27. Found: C, 55.74; H, 9.47; N, 16.18.

Boc-N^{α}hLeu-*N*-azaLeu-CH_2Br [10h, R = $CH_2CH(CH_3)_2$, PG^1 = Boc, R^1 = CH_2Br]. To a stirred and cooled solution (0 °C) of **8a** (1.6 g, 5 mmol) in dichloromethane (10 mL) and pyridine (0.6 g, 6 mmol) was added dropwise bromo acetyl bromide (1.2 g, 6 mmol) in 5 mL of dichloromethane. The mixture was allowed to stir for 5 h. The reaction mixture was washed three times with water (50 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated in vacuo to give the crude product **10h** which precipitate slowly in ether (1.27 g, 58%). Mp 105 °C. ^{1}H NMR ($CDCl_3$, δ): 0.95 (d, $J=6.5$ Hz, 6H), 0.98 (d $J=6.5$ Hz, 6H), 1.48 (s, 9H), 1.75 (m, 1H), 1.91 (m, 1H), 2.50 (d, $J=7$ Hz, 2H), 3.46 (d, $J=7$ Hz, 2H), 3.49 (s, 2H), 3.89 (s, 2H), 5.79 (s, 1H), 10.39 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 18.9, 19.5, 19.7, 25.2, 26.1, 27.5, 55.0, 55.4, 60.8, 64.4, 78.8, 154.8, 171.1, 174.1. Anal. calcd for $C_{17}H_{33}N_4O_4Br$: C, 46.68; H, 7.60; N, 12.81; Br, 18.27. Found: C, 46.56; H, 7.52; N, 12.75; Br, 18.14.

Boc-N^{α}hLeu-*N*-azaPhe-CH_2Br (10i, R = $CH_2C_6H_5$, PG^1 = Boc, R^1 = CH_2Br). The title compound was synthesized from **8f** (3.5 g, 10 mmol) following the general procedure for **10h** (2.9 g, 62%); mp 135 °C; ^{1}H NMR ($CDCl_3$, δ): 0.81 (d, $J=6.5$ Hz, 6H), 1.44 (s, 9H), 1.65 (m, 1H), 2.50 (d, $J=6$ Hz, 2H), 3.52 (s, 2H), 3.85 (s, 2H), 4.85 (br s, 2H), 5.69 (s, 1H), 7.33 (m, 5H), 10.15 (s,1H). ^{13}C NMR ($CDCl_3$, δ): 19.5, 25.3, 28.4, 36.5, 54.1, 54.5, 62.1, 82.1, 125.7, 125.4, 127.3, 138.5, 156.6, 168.2, 170.5. Anal. calcd for $C_{20}H_{31}N_4O_4Br$: C, 50.96; H, 6.63; N, 11.89; Br, 16.95. Found: C, 50.69; H, 6.60; N, 11.82; Br, 16.79.

Z-N^{α}hLeu-*N*-azaLeu-CH_2Br [10j, R = $CH_2CH(CH_3)_2$, PG^1 = Z, R^1 = CH_2Br]. The title compound was synthesized from **8d** (3.5 g, 10 mmol) following the general

procedure for **10h** (3.3 g, 69%); oil; ^{1}H NMR ($CDCl_3$, δ): 0.85 (d, $J=7$ Hz, 2×6H), 1.65 (m, 1H), 1.79 (m, 1H), 2.52 (d, $J=7$ Hz, 2H), 2.63 (d, $J=6.75$ Hz, 2H), 3.64 (s, 2H), 4.59 (s, 2H), 5.12 (s, 2H), 6.58 (s, 1H), 7.45 (m, 5H), 10.24 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 18.7, 19.5, 25.3, 25.6, 36.5, 53.6, 60.3, 61.6, 120.3, 125.7, 128.8, 147.2, 151.2, 168.5, 171.5. Anal. calcd for $C_{20}H_{32}N_4O_4Br$: C, 50.85; H, 6.83; N, 11.86; Br, 16.91. Found: C, 50.75; H, 6.78; N, 11.78; Br, 16.82.

Z-N^{α}hLeu-*N*-azaPhe-CH_2Br (10k, $R=CH_2C_6H_5$, $PG^1=Z$, $R^1=CH_2Br$). The title compound was synthesized from **8e** (3.8 g, 10 mmol) following the general procedure for **10h** oil (3 g, 60%). ^{1}H NMR ($CDCl_3$, δ): 0.83 (d, $J=6.75$ Hz, 6H), 1.68 (m, 1H), 2.50 (d, $J=7$ Hz, 2H), 3.54 (s, 2H), 3.75 (s, 2H), 4.77 (s, 2H), 5.01 (s, 2H), 6.67 (s, 1H), 7.35 (m, 5H), 10.04 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 19.5, 25.3, 36.5, 53.7, 54.8, 61.6, 120.3, 125.4, 127.3, 128.8, 138.4, 147.1, 151.2, 168.2, 170.4. Anal. calcd for $C_{23}H_{29}N_4O_4Br$: C, 54.66; H, 5.78; N, 11.09; Br, 15.81. Found: C, 54.32; H, 5.65; N, 10.98; Br, 15.78.

Boc-N^{α}hLeu-*N*-azaLeu-CH_2Cl [10l, $R=CH_2CH(CH_3)_2$, $PG^1=Boc$, $R^1=CH_2Cl$]. The title compound was synthesized from **8a** (3.2 g, 10 mmol) following the general procedure for **10h** (2.2 g, 57%); mp 135 °C; ^{1}H NMR ($CDCl_3$, δ): 0.95 (d, $J=6.5$ Hz, 6H), 0.98 (d, $J=6.5$ Hz, 6H), 1.47 (s, 9H), 1.75 (m, 1H), 1.88 (m, 1H), 2.48 (d, $J=7$ Hz, 2H), 3.48 (br, 2×2H), 4.10 (s, 2H), 5.63 (s, 1H), 10.34 (s, 1H). ^{13}C NMR ($CDCl_3$, δ): 18.8, 19.6, 25.3, 25.7, 28.5, 38.5, 54.2, 60.3, 62.1, 82.1, 156.8, 165.2, 168.5. Anal. calcd for $C_{17}H_{33}N_4O_4Cl$: C, 52.04; H, 8.41; N, 14.29; Cl, 8.92. Found: C, 51.95; H, 8.48; N, 14.12; Cl, 8.95.

Boc-N^{α}hLeu-*N*-azaLeu-CHPh-*o*-$B(OH)_2$ (11a). To a stirred solution of **8a** (1.8 g, 5 mmol) in ether (5 mL) was added dropwise 2-formylbenzene boronic acid (0.8 g, 5.5 mmol) in solution of ether (5 mL), a white precipitate appeared. After 1 h stirring, the precipitate was filtered and washed with ether. (2.4 g, 94%). Mp 159 °C. ^{1}H NMR (DMSO-d_6, δ): 0.79 (d, $J=6.75$ Hz, 6H), 0.82 (d, $J=6.75$ Hz, 6H), 1.29 (s, 9H), 1.56 (m, 1H), 1.97 (m, 1H), 2.55 (d, $J=6.75$ Hz, 2H), 3.68 (d, $J=6.75$ Hz, 2H), 4.06 (s, 2H), 7.24–7.51(m, 4H), 7.76 (d, $J=7.25$ Hz, 1H), 8.16 (s, 2H), 8.26 (s, 1H). ^{13}C NMR (DMSO-d_6, δ): 20.3, 20.9, 24.9, 26.5, 28.5, 46.9, 58.6, 64.7, 78.5, 126.1, 128.8, 129.3, 134.1, 136.7, 138.1, 142.5, 154.8, 171.7. ^{11}B NMR (DMSO-d_6/Et_2OBF_3, δ): 30 (br s). Anal. calcd for $C_{22}H_{37}N_4O_5$ B: C, 58.93; H, 8.32; N, 12.50; B, 2.41. Found: C, 58.69; H, 8.32; N, 12.50; B, 2.45.

Boc-N^{α}hLeu-*N*-azaLeu-CHPh-*m*-$B(OH)_2$ (11b). The title compound was synthesized from **8a** (1.8 g, 5 mmol) and 3-formylbenzene boronic acid (0.8 g, 5.5 mmol) following the general procedure for **11a**. (2.3 g, 92%). Mp 110 °C. ^{1}H NMR (DMSO-d_6, δ): 0.92 (d, $J=6.75$ Hz, 6H), 0.95 (d, $J=6.75$ Hz, 6H), 1.41 (s, 9H), 1.54 (m, 1H), 1.68 (m, 1H), 2.68 (d, $J=6.75$ Hz, 2H), 4.01 (s large, 2H), 4.13 (s, 2H), 7.45 (s large, 1H), 7.84 (s, 1H), 7.88 (s, 1H), 8.05 (s, 1H), 8.17 (s, 1H), 8.23 (s, 2H). ^{13}C NMR (DMSO-d_6, δ): 20.3, 20.9, 24.9, 26.5, 28.5, 46.8, 58.6, 64.8, 78.4, 128.6, 129.6, 129.9, 134.2, 135.9, 138.2, 141.5, 156.8, 171.8. ^{11}B NMR (DMSO-d_6/Et_2OBF_3, δ): 30 (br s). Anal. calcd for $C_{22}H_{37}N_4O_5$ B: C, 58.93; H, 8.32; N, 12.50; B, 2.41. Found: C, 58.69; H, 8.32; N, 12.50; B, 2.45.

Boc-N^{α}hLeu-*N*-azaLeu-CHPh-*p*-$B(OH)_2$ (11c). The title compound was synthesized from **8a** (1.8 g, 5 mmol) and 4-formylbenzene boronic acid (0.8 g, 5.5 mmol) following the general procedure for **11a**: (2.5 g, 96%). Mp 186 °C. ^{1}H NMR (DMSO-d_6, δ): 0.83 (d, $J=6.75$ Hz, 12H), 1.31 (s, 9H), 1.59 (m, 1H), 1.95 (m, 1H), 2.58 (d, $J=6.75$ Hz, 2H), 3.76 (d, $J=6.75$ Hz, 2H), 4.07 (s, 2H), 7.46 (s, 1H), 7.64–7.81 (m, 4H), 7.93 (s, 1H), 8.10 (s, 2H). ^{13}C NMR (DMSO-d_6, δ): 20.3, 20.9, 25.1, 26.5, 28.4, 46.5, 58.7, 64.8, 78.5, 126.3, 134.7, 136.1, 136.5, 140.5, 154.8, 171.6. ^{11}B NMR (DMSO-d_6/Et_2OBF_3, δ): 30 (br s). Anal. calcd for $C_{22}H_{37}N_4O_5$ B: C, 58.93; H, 8.32; N, 12.50; B, 2.41. Found: C, 58.69; H, 8.32; N, 12.50; B, 2.45.

Biological Assay

All reagents used in the biological assay were purchased from Merck, (Darmstadt, Germany) or Sigma (Chemical CO. (St Louis, MO). DFMO was obtained from ILEX Oncology (San Antonio, TX, USA). Compounds: The compounds were all dissolved in DMSO and stored at minus 80 °C as a stock solution 100 mM. For experimental procedure, the compounds were diluted from stock solution in filtered growth medium and used immediately. Data are given as means values of three experiments. Comparisons between means are made using the Student's *t*-test assuming significance at $p<0.01$.

Analysis of the cytotoxicity

Cell culture line. L1210 murine leukemia cells were grown in RPMI 1640 medium (Eurobio, Les Ulis, France) supplemented with 10% fetal calf serum, 2 mM glutamine penicillin (100 Units/mL), and streptomycin (50 μg/mL) (BioMerieux Marcy l'Etoile, France) (full medium). Cells were cultivated at 37 °C under a humidified 5% CO_2 atmosphere and maintained in exponential growth as described by Delcros et al.[27]

In vitro evaluation of cytotoxicity. The effect of the different hydrazino-aza and *N*-azapeptoides on cell growth was assayed in sterile 96 wells microtiter tissue plates (Becton Dickinson, Oxnard, CA, USA). The cells were seeded at 5×10^5 cells/mL of medium (100 μL per well). The different hydrazino-aza and *N*-azapeptoides (5 μL/per well) at the appropriate concentration (10 nM–1 mM) were added at the time of seeding. The incubations were performed at 37 °C during 24 and 48 h. After exposure to the compounds, cell growth was determined by measuring the formazan formation from 3-(4,5 - dimethylthiazol - 2yl) - 2,5 - diphenyltetrazolium (MTT) as previously described.[28] A Titertek Multiskan MCC/340 microplate reader (Labsystems, Cergy Pontoise, France) was used for absorbance measurements (540 nm).

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