FULL PAPER

Split-*Ugi* Reaction with Chiral Compounds: Synthesis of Piperazine- and Bispidine-Based Peptidomimetics

by **Mattia Stucchi*** and **Giordano Lesma**

Dipartimento di Chimica, Università degli Studi di Milano, via Golgi 19, IT-20133 Milan (phone: +39-02-50314077, e-mail: mattia.stucchi@unimi.it)

A simple, one-step, stereoconservative synthesis of diamine-based peptidomimetics is described, by split-*Ugi* multicomponent reaction, involving chiral N-protected amino acids and α-substituted isocyanoacetate. In particular, piperazine and bispidine (3,7-diazabicyclo[3.3.1]nonane) are exploited as diamine components, bispidine being the first example of a sterically demanding bicyclic system employed in a split-*Ugi* reaction.

Keywords: Split-*Ugi* reaction, Multicomponent reaction, Peptidomimetics, Chiral α-substituted isocyanoacetates, Bispidine.

Introduction

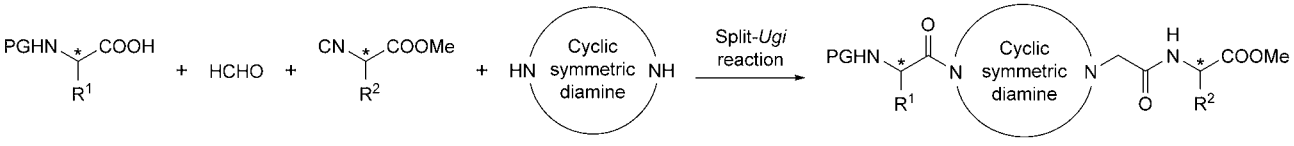
The split-*Ugi* reaction is an isocyanide-based multicomponent reaction (IMCR), developed by *Giovenzana et al.* in 2006 [1]. It resembles the *Ugi* four-component reaction (U-4CR) [2], but instead of a primary amine, a symmetric secondary diamine is used, allowing to generate a molecular scaffold in which one N-atom is acylated and the other one is alkylated in one step without the necessity of protecting groups. The key step of the mechanism is the final 'remote' *Mumm* rearrangement, which occurs thanks to the second N-atom, achieving in this way the observed regiochemical desymmetrization of the diamine core in only one step.

Although different cyclic and linear symmetrical secondary diamines proved to be suitable substrates [1][3], no examples are reported for sterically demanding, more constrained, and rigid bicyclic systems. Furthermore, to the best of our knowledge, no efforts have been made for the introduction of enantiopure chiral compounds, although they have been extensively employed in the related U-4CR [4], in particular, for the synthesis of peptidomimetics [5]. In principle, by simply using N-protected natural amino acids and enantiopure α-substituted isocyanoacetates and relying on the split-*Ugi* reaction, diamine-based peptidomimetics could be accessible. By choosing the appropriate conformationally constrained cyclic symmetric secondary diamine, it would be possible to induce a well-defined secondary structure on the resulting peptidomimetic (*Scheme 1*), opening the way to possible modulation of specific protein–protein interactions (PPIs) [6].

PPIs determine the biological role of the relative proteins, and only in the last decade have begun to gain attention as viable targets for therapeutic intervention [7]. Only a few studies have been published on the application of diamine-based conformationally constrained peptidomimetics for regulating PPIs [8 – 10]. We believe that such a unexplored class of potential PPI modulators is virtually accessible *via* the split-*Ugi* reaction in a straightforward manner. Therefore, relying on our previous experience with the use of chiral compounds in the U-4CR [11], and our recent effort in expanding the scope of the split-*Ugi* protocol [12], we decided to develop a synthetic route for obtaining diamine-based peptidomimetics in a one-step process. We chose the symmetric secondary diamines among those reported to induce a certain conformation or to increase the activity of the related peptidomimetic.

In particular, bispidine (3,7-diazabicyclo[3.3.1]nonane; **2b**) was reported to act as secondary structure nucleator by *Haridas* and co-workers [13]. Indeed, when both N-atoms of the bispidine core are alkylated, an open-turn

Scheme 1. Synthetic approach toward diamine-based peptidomimetics by means of split-*Ugi* reaction. PG, protecting group.



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is observed, while when both N-atoms are acylated, a *β*-sheet conformation is induced. A helical conformation can be obtained if one N-atom is alkylated and the other one acylated [8]. Helix is one of the most common peptide secondary structures and a major recognition motif of PPIs, currently a compelling therapeutic target for small molecules-based drug discovery [14].

On the other side, targeting the specific PPI VLA-4-mediated MOLT-4 adhesion, *Dutta* and co-workers reported cyclic peptides containing the Ile-Leu-Asp-Val sequence, suitable as lead compounds for the development of new treatments for several inflammatory and autoimmune diseases. Among these cyclic peptides, the piperazine-based ones show promising results both *in vitro* and *in vivo*, indicating the presence of the piperazine moiety as crucial for their activity [9].

By employing chiral compounds, such as N-protected L-Ala and methyl (2*S*)-2-isocyanopropanoate, in combination with formaldehyde, we demonstrated the configurational stability of the chiral isocyanoacetate in the split-*Ugi* reaction. Using piperazine (**2a**) or, alternatively, bispidine (**2b**) as diamine substrate, we achieved the synthesis of relevant diamine-based peptidomimetics.

Results and Discussion

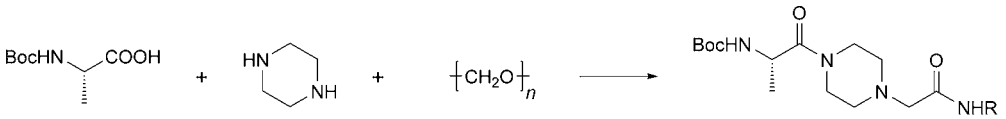
Firstly, we evaluated the compatibility of a N-protected amino acid under split-*Ugi* reaction conditions by using commercially available *N*-Boc L-alanine as carboxylic acid in combination with the well-studied piperazine (**2a**) as secondary diamine. Among the different carbonyl sources, we selected the simplest not prochiral one, formaldehyde, in its easy-to-handle polymeric form paraformaldehyde.

Despite the expected configurational instability of chiral α-substituted isocyanoacetates, *Carney and co-workers* [15] reported that a precondensation time of 2 h between the amine and the C=O moiety, followed by the sequential addition of carboxylic acid and isocyanoacetate, would allow to preserve the optical purity of the isocyanoacetate in U-4CR. Therefore, we the adapted their procedure to the split-*Ugi* protocol by adding *N*-Boc L-alanine (**1a**) from the beginning, in order to quench the basicity of the second N-atom of piperazine (**2a**), and by increasing the temperature, in order to depolymerize paraformaldehyde. The subsequent addition of isocyanides **3a** – **3d**, at room temperature, smoothly afforded the desired split-*Ugi* adducts **4a** – **4d** (*Table 1*).

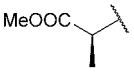
The steric hindrance of the isocyanide moiety did not affect the reaction kinetics, with bulky isocyanides **3a** and **3b** affording the desired products in moderate to good yields. By using commercially available methyl isocyanoacetate (**3c**), the first piperazine-based peptidomimetic **4c** was synthesized in good yields, bearing L-Ala and Gly amino acids. As expected, we did not observe any appreciable loss of optical purity using methyl (2*S*)-2-isocyanopropanoate (**3d**), prepared as pure enantiomer by a two-step protocol starting from commercially available L-alanine methyl ester hydrochloride [16]. Indeed, the piperazine-based peptidomimetic **4d** was smoothly obtained in good yield and as single diastereoisomer, as observed by ^{1}H- and ^{13}C-NMR.

As far as we know, bicyclic symmetric secondary diamines were never reported as substrates in the split-*Ugi* reaction and, in particular, the constrained bispidine (**2b**) was supposed to be a challenging and less reactive substrate in comparison to piperazine (**2a**). Firstly, we applied to **2b** the same reaction conditions as described above, by selecting benzoic acid (**1b**) and 1-(isocyanomethyl)-4-methoxybenzene (**3e**) as non-chiral compounds. In this case, the desired split-*Ugi* adduct **5a** was isolated in low yield, while 1,3-diazaadamantane (1,3-diazatricyclo [3.3.1.1^{3,7}]decane; **6**) proved to be the major product, as observed by the ^{1}H-NMR of the crude reaction mixture (*Scheme 2*). Due to prolonged reaction time, we hypothesized 1,3-diazaadamantane (**6**) to be an unreactive byproduct, and not a suitable electrophilic intermediate. To demonstrate its role, we synthesized **6** by treating bispidine (**2b**) with paraformaldehyde under acidic anhy-

Table 1. One-step synthesis of piperazine-based peptidomimetics **4a** – **4d** *via* split-*Ugi* reaction employing chiral compounds[a])

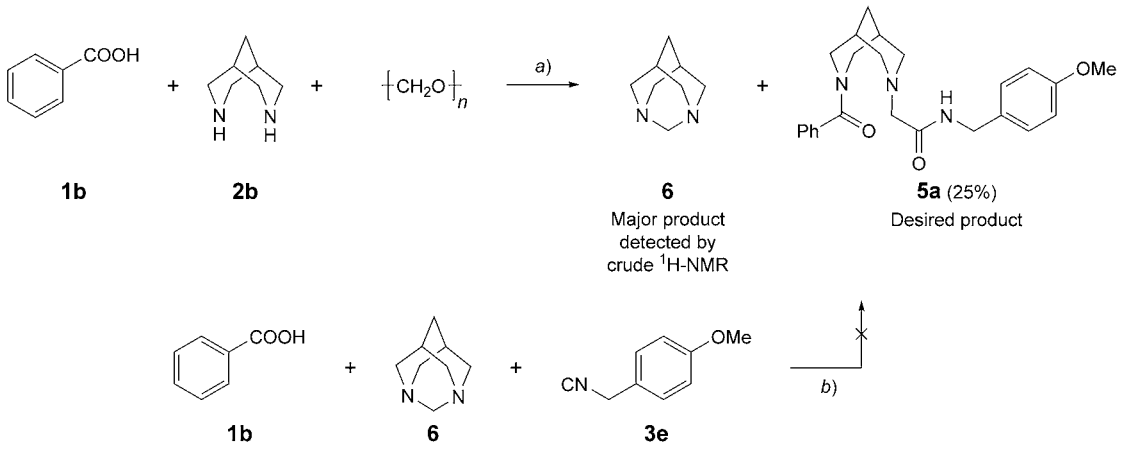


1a **2a** **4**

Entry		R	Product	Yield [%][b])
1	**3a**	^{t}Bu	**4a**	51
2	**3b**	$Me_3CC(Me)_2CH_2$–	**4b**	72
3	**3c**	$MeOOCCH_2$–	**4c**	79
4	**3d**		**4d**	66

[a]) Reagents and conditions: 1. **1a** (0.8 mmol), **2a** (0.87 mmol), paraformaldehyde (0.95 mmol), MeOH (2 ml), reflux, 2 h; 2. R–N≡C (**3**; 0.95 mmol), r.t., 24 h. Boc, ^{t}BuOC=O. [b]) Yield of isolated product.

Scheme 2. Split-*Ugi* reaction involving bispidine (**2b**) with the formation of the desired product **5a** in combination with 1,3-diazaadamantane (**6**).



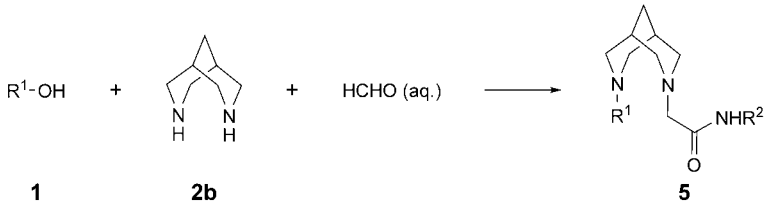
a) 1. **1b** (0.8 mmol), **2b** (0.87 mmol), paraformaldehyde (0.95 mmol), MeOH (2 ml), reflux, 2 h; 2. **3e** (0.95 mmol), r.t., 24 h. b) **1b** (0.8 mmol), **6** (0.87 mmol), **3e** (0.95 mmol), MeOH, r.t., 24 h.

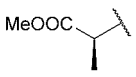
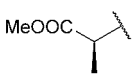
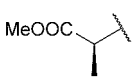
drous conditions at high temperature [17]. After mixing it with benzoic acid (**1a**) and 1-(isocyanomethyl)-4-methoxybenzene (**3e**), we could recover only starting materials, even after prolonged reaction times (*Scheme 2*). Therefore, we concluded that 1,3-diazaadamantane (**6**) is an unreactive byproduct, whose formation is facilitated by the high temperature of the previously optimized conditions.

At room temperature, by replacing paraformaldehyde with a highly reactive carbonyl source, such as aqueous formaldehyde, product yields increase drastically (*Table 2*), avoiding the formation of 1,3-diazaadamantane (**6**). This result can be explained by taking into account the double effect of a lower temperature and of a little amount of H_2O, which reduce the kinetic of the intramolecular formation of **6**, favoring the formation of the thermodynamic split-*Ugi* product. Under these milder conditions, complete conversion was achieved, although the desired split-*Ugi* adducts were isolated in moderate to good yields, due to the formation of typical U-4CR byproducts, like the *Passerini* adduct and the formamide derived from hydration of the isocyanide.

The reaction proceeded smoothly with carboxylic acids **1b** and **1c**, allowing the formation of desired products **5a** and **5b** in good yields, without any relevant influence of electronic properties, steric hindrance, and acidity of the carboxylic acid (*Table 2*). Although benzyl isocyanide **3e** can be used with good results, aromatic iso-

Table 2. One-pot synthesis of bispidine-containing split-*Ugi* adducts **5a** and **5b**, and bispidine-based peptidomimetics **5c** – **5e**[a])



Entry		R^1		R^2	Product	Yield [%][b])
1	**1b**	Bz	**3e**	4-MeO–C_6H_4–CH_2–	**5a**	60
2	**1c**	Ac	**3e**	4-MeO–C_6H_4–CH_2–	**5b**	62
3	**1a**	Boc–L-Ala–	**3d**		**5c**	39
4	**1d**	Cbz–L-Ala–	**3d**		**5d**	38
5	**1e**	Fmoc–L-Ala–	**3d**		**5e**	40

[a]) Reagents and conditions: **1** (0.95 mmol), **2b** (0.8 mmol), aqueous HCHO (1.6 mmol), R^2–N≡C (**3**; 0.95 mmol), MeOH (1.6 ml), r.t., 24 h. Boc, ^{t}BuOC=O; Cbz, BnOC=O; Fmoc, (9*H*-fluoren-9-ylmethoxy)carbonyl. [b]) Yield of isolated product.

cyanides 1-bromo-4-isocyanobenzene and 1-isocyano-4-methoxybenzene afforded only complex mixtures, with no significant formation of the expected split-*Ugi* adducts. We also explored the use of differently N-protected L-alanines (**1a**, **1d**, and **1e**) in combination with the chiral α-isocyanoacetate **3d**, obtaining bispidine-based peptidomimetics **5c** – **5e** in moderate yields. ^{1}H- and ^{13}C-NMR spectra showed no evidence of loss of optical purity, and no N-deprotection was observed, even when the labile (9*H*-fluoren-9-ylmethoxy)carbonyl (Fmoc) functional group was employed (*Table 2*).

Conclusions

We reported an easier access to biologically relevant cyclic secondary diamine-based peptidomimetics by a split-*Ugi* reaction involving chiral compounds, such as N-protected amino acids and α-substituted isocyanoacetates. The regiochemical desymmetrization of the diamine is achieved in a one-step process, without expensive coupling agents and protection/deprotection steps. In particular, piperazine (**2a**) reacts smoothly, giving desired optical pure compounds in good yields. We also reported the first example of a split-*Ugi* reaction using a bicyclic secondary diamine, namely bispidine (**2b**), which reacts under mild conditions with moderate to good yields. In particular, bispidine-based peptidomimetics were synthesized as α-helix nucleators, bearing different N-protecting groups, which make them suitable for incorporation into peptide sequences. Further studies on different symmetric secondary diamines are underway, in order to expand the range of secondary peptidomimetic structure potentially achieved by this method.

Experimental Part

General

All commercial materials (*Sigma-Aldrich*, Milan, Italy) were used without further purification. All solvents were of reagent or HPLC grade. Compound **2b** was synthesized *via* a slightly modified reported procedure [18] and stored as perchlorate salt. All reactions were carried out under N_2 atmosphere unless otherwise noted. All reactions were monitored by thin-layer chromatography (TLC). TLC: precoated silica gel *60* F_{254} (SiO_2); visualized under UV light or by treatment with 1% aq. $KMnO_4$ soln. Flash column chromatography (FC): SiO_2 *60* (230 – 400 mesh). UV/VIS spectra: *JASCO V-650* spectrophotometer (*JASCO*, Cremella, Italy); λ_{max} (log ε) in nm. CD Spectra: *JASCO J-715* spectropolarimeter; λ_{max} (Δε) in nm. ^{1}H- and ^{13}C-NMR spectra: *Bruker AC 300*, (300 and 75 MHz, resp.) and *Bruker Avance I400* (400 and 100 MHz, resp.) spectrometers; δ in ppm rel. to residual solvent, *J* in Hz. ^{13}C-NMR spectra were recorded using the APT pulse sequence. HR-ESI-MS: *Waters Q-Tof Micro* instrument; in *m*/*z*.

Typical Synthesis Procedure for Piperazine-Peptidomimetics Exemplified with **4a**. To a soln. of piperazine (**2a**; 0.068 g, 0.793 mmol) in anhyd. MeOH (2 ml) under N_2 were added *N*-Boc-L-alanine (0.165 g, 0.872 mmol) and HCHO (0.029 g, 0.952 mmol). The soln. was heated under reflux and stirred for 2 h. After cooling to room temperature, ^{t}BuN≡C (**3a**; 0.108 ml, 0.952 mmol) was added and stirring was continued for 48 h. The solvent was evaporated *in vacuo* and the crude mixture was purified by FC (SiO_2; AcOEt) to afford pure product **4a**.

***tert*-Butyl [(2*S*)-1-{4-[2-(*tert*-Butylamino)-2-oxoethyl]piperazin-1-yl}-1-oxopropan-2-yl]carbamate (4a)**. Yield: 0.136 g (51%). White solid. $[\alpha]_D^{30}$ = +8.3 (*c* = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 6.87 (*s*, 1 H); 5.49 (*d*, *J* = 7.8, 1 H); 4.65 – 4.60 (*m*, 1 H); 3.70 – 3.45 (*m*, 4 H); 2.95 (*s*, 2 H); 2.64 – 2.49 (*m*, 4 H); 1.46 (*s*, 9 H); 1.39 (*s*, 9 H); 1.32 (*d*, *J* = 6.9, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.3; 168.4; 155.1; 79.6; 62.1; 53.3; 53.0; 50.7; 46.0; 45.4; 42.0; 28.8 (3 C); 28.4 (3 C); 19.38. HR-ESI-MS: 371.2661 ($[M + H]^+$, $C_{18}H_{35}N_4O_4^+$; calc. 371.2653).

***tert*-Butyl [(2*S*)-1-Oxo-1-(4-{2-oxo-2-[(2,2,3,3-tetramethylbutyl)amino]ethyl}piperazin-1-yl)propan-2-yl]carbamate (4b)**. Yield: 0.243 g (72%). White solid. $[\alpha]_D^{30}$ = +7.19 (*c* = 0.9, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.02 (*s*, 1 H); 5.49 (*d*, *J* = 7.5, 1 H); 4.62 (*m*, 1 H); 3.82 – 3.43 (*m*, 4 H); 2.94 (*s*, 2 H); 2.54 (*m*, 4 H); 1.72 (*s*, 2 H); 1.45 (*s*, 15 H); 1.31 (*d*, *J* = 6.8, 3 H); 1.04 (*s*, 9 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.9; 168.9; 155.7; 80.3; 63.1; 55.4; 54.1; 53.7; 53.4; 46.7; 46.0; 42.7 (2 C); 32.2 (3 C); 29.5 (2 C); 29.0 (3 C); 20.1. HR-ESI-MS: 427.3271 ($[M + H]^+$, $C_{22}H_{43}N_4O_4^+$; calc. 427.3279).

Methyl *N*-({4-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]piperazin-1-yl}acetyl)glycinate (4c). Yield: 0.242 g (79%). Yellowish oil. $[\alpha]_D^{30}$ = +5.2 (*c* = 0.3, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.54 (br. *m*, 1 H); 5.48 (*d*, *J* = 7.7, 1 H); 4.59 (*m*, 1 H); 4.08 (*d*, *J* = 5.7, 2 H); 3.75 (*s*, 3 H); 3.74 – 3.43 (*m*, 4 H); 3.09 (*s*, 2 H); 2.58 (*m*, 4 H); 1.42 (*s*, 9 H); 1.29 (*d*, *J* = 6.9, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.8; 170.6; 169.7; 155.5; 80.2; 60.8; 53.4; 53.2; 52.9; 46.4; 45.0; 41.2; 40.8; 28.8 (3 C); 19.6. HR-ESI-MS: 409.2071 ($[M + Na]^+$, $C_{17}H_{30}N_4NaO_6^+$; calc. 409.2058).

Methyl *N*-({4-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]piperazin-1-yl}acetyl)-L-alaninate (4d). Yield: 0.210 g (66%). Yellowish oil. $[\alpha]_D^{30}$ = +4.1 (*c* = 0.6, $CHCl_3$). ^{1}H-NMR (400 MHz, (D_6)DMSO, 80°): 7.57 (br. *m*, 1 H); 5.51 (*d*, *J* = 7.7, 1 H); 4.63 (*m*, 2 H); 3.77 (*s*, 3 H); 3.76 – 3.49 (*m*, 4 H); 3.13 (*d*, *J* = 16.2, 1 H); 3.03 (*d*, *J* = 16.2, 1 H); 2.78 – 2.47 (*m*, 4 H); 1.47 – 1.42 (*m*, 12 H); 1.31 (*d*, *J* = 6.9, 3 H). ^{13}C-NMR (100 MHz, (D_6)DMSO, 80°): 174.0; 171.9; 169.8; 155.7; 80.3; 61.9; 54.0; 53.6; 53.2; 48.1; 46.7; 46.0; 42.7; 29.0 (3 C); 20.0; 19.1. HR-ESI-MS: 423.2209 ($[M + Na]^+$, $C_{18}H_{32}N_4NaO_6^+$; calc. 423.2214).

Typical Synthesis Procedure for Bispidine-Peptidomimetics Exemplified with **5a**. To a soln. of bispidine (**2b**;

0.100 g, 0.792 mmol) in MeOH (1.6 ml) were added benzoic acid (0.116 g, 0.950 mmol) and aq. 37 wt% HCHO soln. (0.118 ml, 1.584 mmol). After 2 h, 1-isocyano-4-methoxybenzene (**3e**; 0.140 g, 0.950 mmol) was added and stirring was continued for 48 h. The solvent was evaporated *in vacuo* and the crude mixture was purified by FC (SiO_2; AcOEt) to afford the pure product **5a**.

2-(7-Benzoyl-3,7-diazabicyclo[3.3.1]nonan-3-yl)-*N*-(4-methoxybenzyl)acetamide (5a). Yield: 0.194 g (60%). Yellowish solid. ^{1}H-NMR (300 MHz, $CDCl_3$, rotameric mixture): 8.70 (*m*, 0.5 H); 8.33 (*m*, 0.5 H); 7.67 – 7.24 (*m*, 5 H); 7.17 (*d*, *J* = 8.7, 2 H); 6.87 – 6.71 (*m*, 2 H); 4.94 (br. *d*, *J* = 13.7, 1 H); 4.45 (br. *t*, *J* = 6.4, 1 H); 4.33 (br. *d*, *J* = 5.5, 2 H); 3.81 – 3.50 (*m*, 6 H); 3.40 (*m*, 1 H); 3.25 (br. *d*, *J* = 11.2, 1 H); 3.15 (*m*, 1 H); 2.99 (*d*, *J* = 12.0, 1 H); 2.65 (*m*, 1 H); 2.48 – 1.57 (*m*, 4 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.2; 169.6; 158.5; 136.6; 131.9; 129.8 (2 C); 129.5; 128.5 (2 C); 126.8 (2 C); 113.6 (2 C); 63.3; 58.7 (2 C); 55.2; 53.4; 46.0; 42.2; 32.2; 29.7; 28.8. HR-ESI-MS: 408.2286 ($[M + H]^+$, $C_{24}H_{30}N_3O_3^+$, calc. 408.2282).

2-(7-Acetyl-3,7-diazabicyclo[3.3.1]nonan-3-yl)-*N*-(4-methoxybenzyl)acetamide (5b). Yield: 0.164 g (62%). Yellowish solid. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.74 (br. *m*, 1 H); 7.36 (*d*, *J* = 8.2, 2 H); 6.86 (*d*, *J* = 8.2, 2 H); 4.75 (br. *d*, *J* = 12.9, 1 H); 4.47 (br. *dd*, *J* = 14.0, 6.7, 1 H); 4.29 (br. *dd*, *J* = 14.0, 5.2, 1 H); 3.86 (br. *d*, *J* = 11.7, 1 H); 3.80 (*s*, 3 H); 3.40 (br. *d*, *J* = 12.6, 1 H); 3.15 – 2.97 (br. *m*, 2 H); 2.93 (br. *d*, *J* = 9.5, 1 H); 2.83 (br. *d*, *J* = 13.1, 1 H); 2.70 (br. *d*, *J* = 15.8, 1 H); 2.55 (br. *d*, *J* = 8.5, 1 H); 2.20 (br. *d*, *J* = 9.5, 1 H); 2.05 – 1.85 (br. *m*, 5 H); 1.83 – 1.63 (br. *m*, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.0; 169.5; 158.6; 131.6; 129.5 (2 C); 113.7 (2 C); 61.6; 59.2; 58.7; 51.3; 45.8 (2 C); 42.2; 29.7; 28.8 (2 C); 22.1. HR-ESI-MS: 368.1954 ($[M + Na]^+$, $C_{19}H_{27}N_3NaO_3^+$, calc. 368.1945).

Methyl *N*-({7-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl]-3,7-diazabicyclo[3.3.1]nonan-3-yl}acetyl)-L-alaninate (5c). Yield: 0.136 g (39%). White solid. $[\alpha]_D^{30} = -17.6$ (*c* = 0.95, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$, rotameric mixture): 7.75 (*m*, 0.5 H); 7.6 (br. *d*, *J* = 9.5, 1 H); 6.3 (*m*, 0.5 H); 5.11 – 4.73 (*m*, 2 H); 4.7 – 4.5 (*m*, 1 H); 4.4 (br. *d*, *J* = 10.3, 0.75 H); 4.20 – 4.07 (*m*, 0.25 H); 3.8 – 3.7 (*m*, 3 H); 3.5 – 3.3 (*m*, 1 H); 3.2 – 3.0 (*m*, 1 H); 3.0 – 2.8 (*m*, 1 H); 2.7 – 2.5 (*m*, 1 H); 2.5 – 2.35 (*m*, 1 H); 2.2 – 2.1 (*m*, 1 H); 2.0 – 1.9 (*m*, 2 H); 1.9 – 1.7 (*m*, 2 H); 1.6 – 1.3 (*m*, 15 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 174.5 – 173.2 (2 C); 171.2; 156.0; 80.1; 63.9 – 62.6; 59.0 – 58.3 (2 C); 53.0 – 52.6; 51.0 – 50.7; 48.4 – 47.8; 47.2 – 46.6; 45.8; 34.5 – 32.0; 29.5 – 28.7 (5 C); 19.2 – 17.5 (2 C). HR-ESI-MS: 463.2516 ($[M + Na]^+$, $C_{21}H_{36}N_4NaO_6^+$; calc. 463.2527).

Methyl *N*-[(7-{*N*-[(Benzyloxy)carbonyl]-L-alanyl}-3,7-diazabicyclo[3.3.1]nonan-3-yl)acetyl]-L-alaninate (5d). Yield: 0.143 g (38%). White solid. $[\alpha]_D^{30} = -10.2$ (*c* = 0.8, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$, rotameric mixture): 7.61 (br. *d*, *J* = 9.0, 1 H); 7.45 – 7.23 (*m*, 5 H); 6.78 (br. *d*, *J* = 9.3, 1 H); 5.20 – 4.98 (*m*, 2 H); 4.91 (*m*, 1 H); 4.87 – 4.75 (*m*, 2 H); 4.39 – 3.95 (*m*, 1 H); 3.75 – 3.72 (*m*, 3 H); 3.55 – 2.77 (*m*, 3 H); 2.82 – 2.45 (*m*, 2 H); 2.45 (*d*, *J* = 16.1, 1 H); 2.28 – 2.07 (*m*, 2 H); 2.07 – 1.60 (*m*, 4 H); 1.58 – 1.44 (*m*, 3 H); 1.36 – 1.29 (*m*, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 174.2 – 172.0 (2 C); 169.4; 155.9; 136.5; 128.5 – 128.1 (2 C); 66.6; 62.7; 59.0 – 58.3 (2 C); 52.4 – 52.1 (2 C); 50.3; 47.2; 46.2 – 46.0 (2 C); 45.8; 32.1; 28.9 (2 C); 18.7; 17.4. HR-ESI-MS: 475.2562 ($[M + H]^+$, $C_{24}H_{35}N_4O_6^+$; calc. 475.2551).

Methyl *N*-[(7-{*N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-L-alanyl}-3,7-diazabicyclo[3.3.1]nonan-3-yl)acetyl]-L-alaninate (5e). Yield: 0.178 g (40%). White solid. $[\alpha]_D^{30} = -6.8$ (*c* = 1.0, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$, rotameric mixture): 7.77 (br. *d*, *J* = 6.9, 2 H); 7.69 (br. *d*, *J* = 9.4, 1 H); 7.65 – 7.58 (*m*, 2 H); 7.41 (br. *d*, *J* = 7.3, 2 H); 7.37 – 7.29 (*m*, 2 H); 6.98 (br. *d*, *J* = 9.5, 1 H); 4.97 (*m*, 1 H); 4.92 – 4.78 (*m*, 1.5 H); 4.71 (*m*, 0.5 H); 4.54 – 4.30 (*m*, 2 H); 4.30 – 4.13 (*m*, 1.5 H); 4.13 – 3.93 (*m*, 0.5 H); 3.84 – 3.69 (*m*, 3 H); 3.58 – 3.35 (*m*, 1.5 H); 3.20 – 3.03 (*m*, 1.5 H); 3.01 – 2.93 (*m*, 1.5 H); 2.92 – 2.82 (*m*, 1 H); 2.66 – 2.42 (*m*, 1.5 H); 2.39 (br. *d*, *J* = 11.2, 1 H); 2.10 – 1.92 (*m*, 2 H); 1.90 – 1.65 (*m*, 2 H); 1.62 – 1.44 (*m*, 3 H); 1.44 – 1.34 (*m*, 3 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 174.2 – 171.0 (2 C); 169.4; 155.9; 143.9 (2 C); 141.2 (2 C); 127.7 (2 C); 127.2 – 127.0 (2 C); 125.2 (2 C); 119.9 (2 C); 67.1; 62.6; 58.6 – 58.1 (2 C); 52.3; 50.2; 47.4 – 47.0 (2 C); 46.3; 46.0; 32.2; 29.0 – 28.5 (2 C); 18.8; 17.5. HR-ESI-MS: 563.2868 ($[M + H]^+$, $C_{31}H_{39}N_4O_6^+$; calc. 563.2864).

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