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Synthesis of dibenzyl iminodiacetic derivatives as potential inhibitors of HIV-1 aspartyl protease

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1. Introduction

HIV-1 protease is an essential retroviral enzyme which is responsible for the processing of viral polyproteins to structural proteins and enzymes [1]. Extensive studies have been carried out in recent years giving rise to a number of potent HIV-1 protease inhibitors and many were designed using a classical approach by analogy with the transition state during the hydrolysis step [2]. The C_2 symmetry properties of the enzyme have also been used to prepare symmetrical compounds which complement the enzymatic site and are potent new inhibitors. They are characterized by a symmetrical spacer, a diamine [3–5] or a diacid [6–9], linking two natural amino-acids or analogues as in compounds **1**, **2**, and **3** (*figure 1*) respectively. However, these compounds possess one or two central secondary alcohols which can interact with the two catalytic Asp residues of the protease and their inhibitory properties may be due only to the mimicry of the transition state of the substrate during the hydrolysis step.

In our preliminary approach to prepare new inhibitors of HIV-1 protease, we were interested in examining whether the symmetrical properties related to those of the enzymatic site alone were sufficient to provide potent inhibitors. Recently, we reported [10] the weak inhibitory properties of a series of symmetrical diamido derivatives prepared from peptidic sequences derived from the P'_2, P'_1, P_1 or P_2 groups of HIV-1 protease substrates and simple diacid spacers. We present herein an extension of this work where an amino function and complementary groups of the S'1 and S1 subsites were introduced into the symmetrical iminodiacetic acid spacer **4** (X = H). We thought that the basic function could provide an additional and productive interaction with the carboxylic group of the aspartic acid of the enzymatic site, strengthening the inhibitory potency of the compounds. Morover, the corresponding hydroxylamine derivative **4** (*figure 2*, X = OH) could be regarded as an analogue of the monohydroxyl derivative **1** (A-74704) used by Abbott in the design of HIV-1 protease inhibitors. The hydroxyl function on the nitrogen atom could mimic the hydroxyl function of the transition state of the substrate in the hydrolysis step and the hydroxylamine derivatives could be considered as transition-state analogy-based inhibitors. To date, no hydroxylamine derivatives have been designed as protease inhibitors.

In a manner similar to the HIV-1 protease inhibitors already reported, the iminodiacetic acid linker was condensed with phenylalanine or valine derivatives which were the most suitable substituents to bind to the S2 and S'2 enzymatic subsites. However, comparison of the structures of compound **4** and the reference compound **1** (A-74704) (*figure 2*) indicated that the best superimposition would be obtained with iminodiacetic acid derivatives with the (R) configuration for the P'_2, P'_1, P_1 or P_2 groups as the peptidic backbone was in the opposite direction. Consequently, the iminodiacetic acid linker was synthesized with the (2R,2'R) configuration and condensed with the selected D-amino acids. However, for the purpose of comparison, compounds with the opposite configuration were also synthesized.

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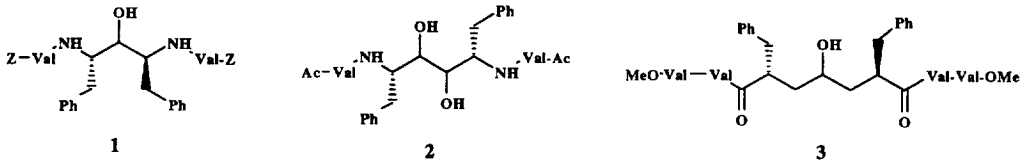

Figure 1. Structure of compounds **1–3**.

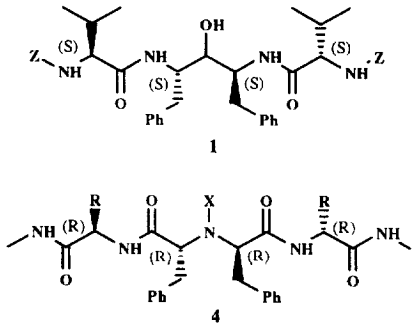

Figure 2. Comparison of the configuration of the P'_2, P'_1, P_1 or P_2 groups in the reference compound A-74704 **1** and the iminodiacetic acid derivatives **4**.

2. Chemistry

The influence of the P'_1 and P_1 substituents on the inhibitory activity was evaluated with the unsubstituted iminodiacetic derivatives **5a–b**. They were synthesized from *N*-benzyloxycarbonyl-iminodiacetic acid **6** and the corresponding amino acid esters using *i*Bu-chloroformate as the coupling agent to provide compounds **7a** and **7b** (*figure 3*). The benzyloxycarbonyl protection was removed by hydrogenolysis to give compounds **5a** and **5b**.

(2R,2'R)- and (2S,2'S)-(2,2')-dibenzyl-iminodiacetic acid **8b** was synthesized according to the synthetic pathway already reported [11] (*figure 4*). R- or S-phenyllactic acid was transformed into the methyl esters **9** (R = OMe) by refluxing MeOH in the presence of a catalytic amount of H_2SO_4. The corresponding triflates were prepared by the reaction of triflic anhydride in pyridine and reacted immediately with L- or D-phenylalanine methyl ester to give acid (2S,2'S) or (2R,2'R)-(2,2')-dibenzyl-iminodiacetic acid dimethyl esters **8a** which were hydrolyzed with 6 N aqueous HCl solution into the corresponding acids **8b**. The total inversion of the configuration occurred in the substitution reaction and the acids were obtained with more than 95% optical purity.

The condensation with D- or L-Val-OMe, D- or L-Phe-OMe and valinol was achieved using BOP as the coupling agent at 50 °C in acetonitrile to give compounds **10a–b** and **11a–c** which were characterized by their ^1H and ^{13}C NMR spectra.

The corresponding hydroxylamine derivatives were obtained either from (2,2')-dibenzyl-*N*-hydroxy-iminodiacetic acid or by direct oxidation of the corresponding amine derivatives **10** and **11**. Dimethyl (2R,2'R)- and (2S,2'S)-(2,2')-dibenzyl-*N*-hydroxy-iminodiacetates were synthesized with a poor yield (28%) by a similar pathway [12] to that described in *figure 4* from methyl (S)- or (R)-*O*-triflate-phenyl-lactate and methyl D- or L-*N*-hydroxy-phenylalanine. Unfortunately, hydrolysis of the diesters failed to give the corresponding diacids. This result agreed with those reported by Thenn et al. [13].

Consequently [14, 15], *meta*-chloro-perbenzoic acid was reacted directly with amino derivatives **10b** and **11b** in methylene chloride at 0 °C. Only the corresponding nitrones **12** and **13** could be isolated [11] from the reaction mixture. They were identified by their DCI-(NH_3) mass spectra and by the presence

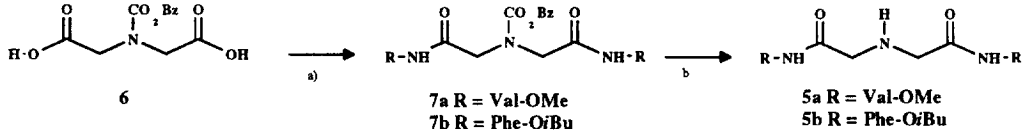

Figure 3. (a) 4-Methylmorpholine, *i*BuOCOCl, Val-OMe or Phe-O*i*Bu, Et_3N, THF. (b) H_2, 10% Pd/C.

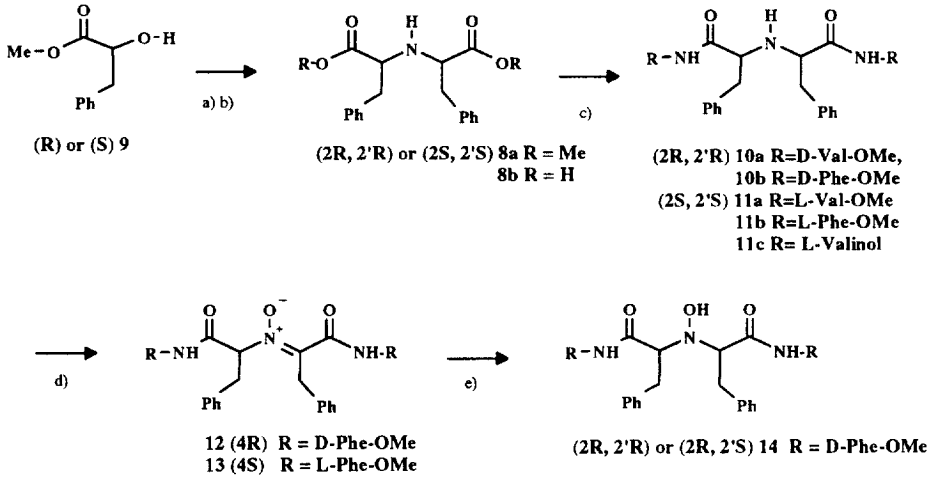

Figure 4. (a) $(CF_3SO_2)_2O$, pyridine, L- or D-Phe-OMe; (b) 6 N, HCl; (c) BOP, acetonitrile, L- or D-Val-OMe, L- or D-Phe-OMe, L-valinol; (d) m-$ClC_6H_4CO_3H$, CH_2Cl_2; (e) $NaBH_3CN$.

in their ^{1}H-NMR spectra of an AB system for the benzylic methylene group in the α position of the nitrone function. The time-dependent formation of another compound was observed and it was characterized by ^{1}H-NMR spectra as methyl 2-oximino-3-phenylpropanate resulting probably from oxidative cleavage. However, **12** and **13** were isolated with a 30% yield and, in particular **12** could be reduced with $NaBH_3CN$ to the hydroxylamino diastereoisomers (2R,2R')- and (2R,2'S)-**14** corresponding to the reduction of the double bond. The compounds were characterized by mass spectrometry and could be separated by preparative HPLC and identified by their ^{1}H-NMR spectra. The configurations of the molecules were confirmed by the symmetrical ^{1}H-NMR spectra which were due to the presence of a pseudo C_2 axis in (2R,2'R)-**14**.

3. Biological results and discussion

Enzymatic activity was determined using an HIV-1 aspartic protease expressed at high levels in *Escherichia coli* as previously reported [16].

The compounds **5a–b**, **10a–b**, **11a–c**, **12**, **13** and **14** were tested. They were found inactive or to possess a weak inhibitory activity. IC_{50} values could only be calculated for the enantiomers **10b** and **11b** (45 and 60 μM respectively) which were equipotent regardless of their configuration. No increase in the inhibitory activity was observed following the introduction of the hydroxyimino function while it was supposed to mimick the transition state analogue. These results show that in the design of HIV-1 protease inbitors, superimposition of the P_n groups in a suitable configuration with those of the active reference compounds is not a sufficient structural property for determining binding to the enzymatic site and that the nature of the chain should also be considered. Thus, the distance between the carbonyl functions of the central part of a number of potent inhibitors represents six or seven bonds and these groups have been implicated in the binding of these molecules with the enzymatic site by hydrogen bonds with a water molecule [17]. This distance produced a particular conformation of these inhibitors which was a very good fit for the binding site of the enzyme. However, this conformation could not exist in the imino acetic derivatives **4** where the distance between two carbonyl groups was too short. This structural difference could explain the weak inhibitory activity or the inactivity observed in the compounds with dicarboxylic spacers with four bonds which cannot adopt such a conformation.

4. Experimental protocols

4.1. Chemistry

Melting points were determined on a Mettler FP61 apparatus. NMR spectra were recorded using Bruker AC200 and

ARX400 spectrometers. Mass spectra were obtained using a Ribermag R10-10 mass spectrometer. IR spectra were performed on a Perkin Elmer 1420 spectrometer. Microanalyses were performed at the CNRS (Vernaison, France), and at the service de microanalyse of the Faculté de Pharmacie in Châtenay-Malabry; all the microanalyses were obtained within ± 0.4% of the theoretical values. All the amino acids were purchased from Novabiochem (Meudon, France). (R)- and (S)-methyl-phenyllactate **9** were prepared by reaction of the corresponding acid in the presence of H_2SO_4.

4.1.1. Benzyloxycarbonyl-iminodiacetic acid ***6***

To a solution of iminodiacetic acid (13.31 g, 0.1 mol) in 2 N NaOH (100 mL) was added dropwise benzyl chloroformate (5.8 mL, 0.11 mol) and a solution of 2 N NaOH (55 mL) at 5 °C. The mixture was stirred at room temperature for 2 h, washed with ether (3 x 50 mL), acidified to pH 2, and extracted with ether (3 x 70 mL). The organic layers were dried over $MgSO_4$ and concentrated in vacuo (0.1 mm Hg). The crude oil was crystallized in CCl_4 to provide 25 g of the diacid **6** (94%). ^{1}H NMR (CD_3OD) δ: 7.15 (m, 5H); 4.95 (s, 2H); 3.95 (2s, 4H). ^{13}C NMR (CD_3OD) δ: 173.2 (2C); 158.1 (1C); 137.7 (1C); 129.6, 129.2 and 128.8 (5C); 69.0 (1C); 50.4 (2C).

4.1.2. Benzyloxycarbonyl-iminodiacetyl-bis-(phenylalanine isobutyl ester) ***7b***

4-Methylmorpholine (1.1 mL, 1.01 g, 10 mmol), isobutyl chloroformate (1.3 mL, 1.37 g, 10 mmol), a solution of phenylalanine isobutyl ester tosylate (3.94 g, 10 mmol) and triethylamine (1.4 mL, 1.01 g, 10 mmol) in anhydrous THF (25 mL) were added at –15 °C to a suspension of the diacid **6** (1.34 g, 5 mmol) in anhydrous THF (100 mL). After 15 h at room temperature, the mixture was filtered and the filtrate concentrated. The residue was dissolved in CH_2Cl_2 (50 mL). This solution was washed (1 N HCl, H_2O, 1 N NaOH, H_2O), dried over $MgSO_4$ and concentrated. The crude oil was crystallized in a mixture of CH_2Cl_2 and hexane to provide a white powder (2.61 g, 78%). ^{1}H NMR ($CDCl_3$) δ: 8,25 (d, J = 7.5 Hz, 1H); 7.2–7.35 (m, 15H); 7.0 (d, J = 7.5 Hz, 1H); 5.1 (d, J = 12.5 Hz, 1H); 4.95 (d, J = 12.5 Hz, 1H); 4.8–4.9 (m, 2H); 3.8–4.1 (m, 8H); 3.0–3.2 (m, 4H); 1.85–1.95 (m, 2H); 0.9 (2d, J = 7.5 Hz, 12H). Anal. $C_{38}H_{47}N_3O_8$.

4.1.3. Benzyloxycarbonyl-iminodiacetyl-bis-(valine methyl ester) ***7a***

Compound **7a** was prepared according to the previous process described for compound **7b** from the diacid **6** and valine methyl ester hydrochloride followed by purification by column chromatography (SiO_2, CH_2Cl_2/*i*PrOH 93:7), 64% yield (2.54 g). ^{1}H NMR ($CDCl_3$) δ: 7.95 (d, J = 8 Hz, 1H); 7.15 (s, 5H); 7.0 (d, J = 8 Hz, 1H); 5.0 (s, 2H); 4.3 (dd, J = 6.5 Hz, J = 8 Hz, 1H); 4.45 (dd, J = 6.5 Hz, J = 8 Hz, 1H); 3.85 (s, 4H); 3.5 (s, 3H); 3.55 (s, 3H); 2.0 (m, 2H); 0.7–0.85 (m, 12H). Anal. $C_{24}H_{35}N_3O_8$.

4.1.4. Iminodiacetyl-bis-(valine methyl ester) hydrochloride ***5a***

Compound **7a** (0.500 g, 1.0 mmol) was stirred under an H_2 atmosphere at atmospheric pressure for 1 h in the presence of Pd/C (10%) (100 mg) in EtOH (10 mL). The catalyst was removed by filtration and the filtrate was concentrated. Compound **5a** was isolated by crystallization of its hydrochloride salt after acidification by an HCl solution in ether with an 85% yield (0.34 g). ^{1}H NMR of the free-base ($CDCl_3$) δ: 7.05 (d, J = 9 Hz, 2H); 4.6 (dd, J = 9 Hz, J = 5 Hz, 2H); 3.75 (s, 6H); 3.25 and 3.45 (AB, J = 16Hz, 4H); 2.1–2.3 (m, 2H); 0.9 (d, J = 7 Hz, 6H); 0.95 (d, J = 7 Hz, 6H). Anal. $C_{16}H_{29}N_3O_6$•HCl.

4.1.5. Iminodiacetyl-bis-(phenylalanine isobutyl ester) hydrochloride ***5b***

Compound **7b** (1.26 g, 1.87 mmol) was stirred in EtOH (10 mL) under an H_2 atmosphere at atmospheric pressure for 4 h in the presence of $Pd(OH)_2$ (100 mg). The catalyst was removed by filtration and the filtrate was concentrated. Compound **5b** was isolated by crystallization of its hydrochloride after acidification by an HCl solution in ether with a 55% yield (0.60 g). ^{1}H NMR (CD_3OD) δ: 7.05–7.2 (m, 10H); 8.05 (d, J = 8 Hz, 2H); 4.75–4.9 (m, 2H); 3.75–3.9 (m, 4H); 2.9–3.2 (m, 8H); 1.75–1.9 (m, 2H); 0.85 (d, J = 6.5 Hz, 12H). Anal. $C_{30}H_{41}N_3O_6$•HCl.

4.1.6. (2S,2'S)-Dimethyl 2,2'-dibenzyl-iminodiacetate ***8a***

A solution of compound (R)-**9** (2.88 g, 16 mmol) and pyridine (1.29 mL, 1.26 g, 16 mmol) in CH_2Cl_2 (15 mL) was added dropwise over 30 min at 0 °C to a solution of trifluoromethane sulfonic anhydride (2.69 mL, 4.51 g, 16 mmol) in CH_2Cl_2 (15 mL). After returning to room temperature, the mixture was concentrated. Pentane (50 mL) was added and the solid formed was removed by filtration. The filtrate was concentrated. The oily residue was dissolved in CH_2Cl_2 (30 mL) and added dropwise at –70 °C over 1 h to a solution of L-phenylalanine methyl ester (5.71 g, 32 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred for 1 h at –70 °C and then for 16 h at room temperature. The solid formed was removed by filtration. The residue obtained by concentration of the filtrate was purified by column chromatography (SiO_2, CH_2Cl_2/*i*PrOH 99:1) to yield compound (2S,2'S)-**8a** (3.90 g, 71%) as an oil. ^{1}H NMR ($CDCl_3$) δ: 7.1–7.3 (m, 10H); 3.55 (X of ABX, J_{AB} = 13 Hz, J_{AX} = 6 Hz, J_{BX} = 7 Hz, 2H); 3.55 (s, 6H); 2.9 and 2.95 (AB of ABX, J_{AB} = 13 Hz, J_{AX} = 6 Hz, J_{BX} = 7 Hz, 4H). ^{13}C NMR ($CDCl_3$) δ: 173.9 (2C); 136.8 (2C); 129.1, 128.4 and 126.8 (10C); 61.0 (2C); 51.7 (2C); 39.6 (2C).

4.1.7. (2R,2'R)-Dimethyl 2,2'-dibenzyl-iminodiacetate ***8a***

Compound (2R,2'R)-**8a** was prepared as described previously from the alcohol (S)-**9** and D-phenylalanine methyl ester with a 73% yield. ^{1}H NMR ($CDCl_3$) δ: 7.0–7.2 (m, 10H); 3.45 (m, 2H); 3.45 (s, 6H); 2.75–2.95 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 173.8 (2C); 136.7 (2C); 129.0, 128.3 and 126.7 (10C); 60.85 (2C); 51.6 (2C); 39.5 (2C).

4.1.8. (2S,2'S)-2,2'-Dibenzyl-iminodiacetic acid ***8b***

The diester (2S,2'S)-**8a** (3.5 g, 10.25 mmol) was refluxed for 15 h in 6 N HCl (15 mL). The mixture was concentrated and the residue was dissolved in 5% NH_4OH (50 mL). The solution was washed (CH_2Cl_2) and acidified at 0 °C to pH 4 with 6 N HCl. The solid formed was isolated by filtration and dried in vacuo to yield the diacid (2S,2'S)-**8b** (2.09 g, 65%), m.p. > 250 °C. α_D^{25} = +32.5 (c = 0.5, 1 N NaOH). ^{1}H NMR (DMSO-d_6) δ: 7.1–7.25 (m, 10H); 3.4 (X of ABX, J_{AB} = 13.5 Hz, J_{AX} = 6.5 Hz, J_{BX} = 7 Hz, 2H); 2.75 and 2.8 (AB of ABX, J_{AB} = 13.5 Hz, J_{AX} = 6.5 Hz, J_{BX} = 7 Hz, 4H). ^{13}C NMR (DMSO-d_6) δ: 174.5 (2C); 137.8 (2C); 129.3, 128.3 and 126.5 (10C); 60.5 (2C); 38.9 (2C).

4.1.9. (2R,2'R)-2,2'-Dibenzyl-iminodiacetic acid ***8b***

The diacid (2R,2R')-**8b** was prepared as described previously from the diester (2R,2R')-**8a** with a 42% yield, m.p. > 250 °C. α_D^{25} = –32.5 (c = 0.5, 1 N NaOH). ^{1}H NMR (DMSO-d_6) δ: 7.1–7.35 (m, 10H); 3.5 (m, 2H); 2.8–3.0 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 174.2 (2C); 137.55 (2C); 129.1, 128.65 and 126.25 (10C); 60.3 (2C); 38.7 (2C).

4.1.10. (2S,2'S)-2,2'-Dibenzyl-iminodiacetyl-bis-(L-valine methyl ester) ***11a***

The diacid (2S,2'S)-**8b** (0.741 g, 2.37 mmol), L-valine methyl ester (1.19 g, 7.10 mmol), 1-benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (2.292 g, 5.20 mmol) and 4-methylmorpholine (1.35 mL, 1.244 g, 12.3 mmol) in acetonitrile (55 mL) were stirred at 50 °C for 15 h. The mixture was concentrated and the residue dissolved in ether (50 mL). This solution was washed (1 N HCl, saturated $KHCO_3$, brine), dried over $MgSO_4$ and concentrated. The crude oil was crystallized in a mixture of MeOH/DIPO to yield compound **11a** (0.850 g, 67%), m.p.: 151 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.1–7.2 (m, 10H); 6.53 (d, J = 8.9 Hz, 2H); 4.37 (dd, J = 5.2 Hz, J = 8.8 Hz, 2H); 3.70 (s, 6H); 3.31 (m, 2H); 2.95 (dd, J = 13.5 Hz, J = 6.2 Hz, 2H); 2.87 (dd, J = 13.5 Hz, J = 7.4 Hz, 2H); 2.24 (bs, 1H); 2.00 (dsep, J = 5.2 Hz, J = 6.9 Hz, 2H); 0.85 (d, J = 6.9 Hz, 6H); 0.77 (d, J = 6.9 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 173.0 (2C); 172.0 (2C); 136.8 (2C); 129.2 (4C); 128.4 (4C); 126.7 (2C); 62.5 (2C); 56.1 (2C); 52.0 (2C); 40.1 (2C); 30.8 (2C); 19.0 (2C); 17.8 (2C). Anal. $C_{30}H_{41}N_3O_6$.

4.1.11. (2S,2'S)-2,2'-Dibenzyl-iminodiacetyl-bis-(L-phenylalanine methyl ester) ***11b****, (2S,2'S)-2,2'-Dibenzyl-iminodiacetyl-bis-(L-valinol)* ***11c****, (2R,2'R)-2,2'-Dibenzyl-iminodiacetyl-bis-(D-valine methyl ester)* ***10a****, (2R,2'R)-2,2'-Dibenzyl-iminodiacetyl-bis-(D-phenylalanine methyl ester)* ***10b*** [all compounds were prepared as previously described for compound **11a**]

11b; yield: 42%, m.p.: 131 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.0–7.2 (m, 20H); 6.34 (d, J = 8.2 Hz, 2H); 4.70 (ddd, J = 8.5 Hz, J = 7.2 Hz, J = 5.5 Hz, 2H); 3.66 (s, 6H); 3.13 (dd, J = 7.6 Hz, J = 5.9 Hz, 2H); 2.97 (dd, J = 13.8 Hz, J = 5.5 Hz, 2H); 2.83 (dd, J = 13.8 Hz, J = 7.2 Hz, 2H); 2.73 (dd, J = 13.7 Hz, J = 5.9 Hz, 2H); 2.54 (dd, J = 13.7 Hz, J = 7.7 Hz, 2H); 2.0 (bs, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 172.4 (2C); 171.4 (2C); 136.6 (2C); 135.7 (2C); 129.1 (8C); 128.3 (8C); 126.7 (4C); 61.9 (2C); 52.6 (2C); 52.1 (2C); 39.7 (2C); 37.6 (2C). Anal. $C_{38}H_{41}N_3O_6$.

11c; 35% yield , m.p. < 60 °C. ^1H NMR ($CDCl_3$) δ: 7.2–7.35 (m, 10H); 6.6 (d, J = 8.5 Hz, 2H); 3.45–3.55 (m, 4H); 3.2–3.25 (m, 2H); 2.6–3.0 (m, 6H); 2.0 (bs, 2H); 1.6 (sep, J = 6.5 Hz, 2H); 0.85 (d, J = 6.5 Hz, 6H); 0.80 (d, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ: 172.5 (2C); 129.2 and 128.5 (8C); 127.0 (2C); 63.0 (2C); 62.7 (2C); 56.9 (2C); 37.0 (2C); 28.6 (2C); 19.2 (2C); 18.8 (2C). Anal. $C_{28}H_{41}N_3O_4$, 1.5 H_2O.

10a; 39% yield, mp: 154 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.1–7.2 (m, 10H); 6.53 (d, J = 8.7 Hz, 2H); 4.37 (dd, J = 5.2 Hz, J = 8.7 Hz, 2H); 3.70 (s, 6H); 3.30 (m, 2H); 2.95 (dd, J = 13.6 Hz, J = 6.3 Hz, 2H); 2.86 (dd, J = 13.6 Hz, J = 7.3 Hz, 2H); 2.24 (bs, 1H); 2.00 (dsep, J = 5.2 Hz, J = 6.9 Hz, 2H); 0.85 (d, J = 6.9 Hz, 6H); 0.77 (d, J = 6.9 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 173.0 (2C); 172.0 (2C); 136.8 (2C); 129.2 (4C); 128.4 (4C); 126.7 (2C); 62.5 (2C); 57.3 (2C); 52.0 (2C); 40.0 (2C); 30.8 (2C); 19.0 (2C); 17.8 (2C). Anal. $C_{30}H_{41}N_3O_6$.

10b; 61% yield, m.p.: 133 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.0–7.2 (m, 20H); 6.33 (d, J = 8.4 Hz, 2H); 4.70 (ddd, J = 8.4 Hz, J = 7.2 Hz, J = 5.5 Hz, 2H); 3.60 (s, 6H); 3.13 (dd, J = 7.6 Hz, J = 5.9 Hz, 2H); 2.97 (dd, J = 13.8 Hz, J = 5.5 Hz, 2H); 2.84 (dd, J = 13.8 Hz, J = 7.2 Hz, 2H); 2.73 (dd, J = 13.6 Hz, J = 6.0 Hz, 2H); 2.54 (dd, J = 13.6 Hz, J = 7.7 Hz, 2H); 2.0 (bs, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 172.4 (2C); 171.4 (2C); 136.9 (2C); 135.7 (2C); 129.6 (4C); 129.3 (4C); 127.5 (8C); 126.9 (4C); 61.9 (2C); 52.6 (4C); 39.8 (2C); 36.7 (2C). Anal. $C_{38}H_{41}N_3O_6$.

4.1.12. (4S)-3-(N-oxyde-aza)-2,4-dibenzylpent-2-enedioyl-bis-(phenylalanine methyl ester) ***13***

A solution of (2S,2'S)-**11b** (0.210 g, 0.329 mmol) and *meta*-chloro-perbenzoic acid (80%) (0.078 g, 0.362 mmol) in CH_2Cl_2 (5 mL) was stirred for 6 h at 0–5 °C, washed (1 N $NaHCO_3$, brine), dried over $MgSO_4$ and concentrated. The crude oil was purified by thin-layer chromatography (SiO_2, CH_2Cl_2/Et_2O 90:10) to yield an oil (0.066 g, 31%). DCI-MS(NH_3): 651 (M + H+). ^1H NMR (400 MHz, $CDCl_3$) δ: 8.3 (d, J = 7.4 Hz, 1H); 6.8–7.0 (m, 20H); 5.36 (dd, J = 11.4 Hz, J = 3.4 Hz, 1H); 5.1 (d, J = 7.8 Hz, 1H); 4.85 (ddd, J = 7.4 Hz, J = 7.2 Hz, J = 5.5 Hz, 1H); 4.6 (ddd, J = 8.0 Hz, J = 7.8 Hz, J = 5.0 Hz, 1H); 4.12 (d, J = 14.7 Hz, 1H); 3.73 (s, 3H); 3.72 (s, 3H); 3.5 (dd, J = 14.0 Hz, J = 11.4 Hz, 1H); 3.3 (d, J = 14.7 Hz, 1H); 3.25 (dd, J = 14.0 Hz, J = 5.5 Hz, 1H); 3.05 (dd, J = 14.0 Hz, J = 7.2 Hz, 1H); 2.99 (dd, J = 14.3 Hz, J = 5.0 Hz, 1H); 2.67 (dd, J = 14.3 Hz, J = 8.0 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 171.2 (1C); 170.7 (1C); 166.0 (1C); 162.0 (1C); 145.4 (1C); 135.6 (3C); 135.2 (1C); 128–129 (18C); 127 (2C); 75 (1C); 53.4 (2C); 52.4 (2C); 37.4 (1C); 36.0 (2C); 34.6 (1C).

4.1.13. (4R)-3-(N-oxyde-aza)-2,4-dibenzylpent-2-enedioyl-bis-((D)-phenylalanine methyl ester) ***12***

It was prepared according to the process described for compound **13** from the derivative **10b** with a 33% yield (0.107 g). DCI-MS(NH_3): 651 (M + H+). ^1H NMR ($CDCl_3$) δ: 8.35 (d, J = 7.5 Hz, 1H); 6.8–7.4 (m, 20H); 5.35 (dd, J = 3.5 Hz, J = 11.5 Hz, 1H); 5.1 (d, J = 7.7 Hz, 1H); 4.85 (m, 1H); 4.6 (m, 1H); 4.15 (d, J = 14.5 Hz, 1H); 3.73 (s, 3H); 3.72 (s, 3H); 2.6–3.6 (m, 7H).

4.1.14. 2,2'-Dibenzyl-N-hydroxy-iminodiacetyl-bis-(D-phenylalanine methyl ester) ***14***

Compound **12** (65 mg, 0.1 mmol) in THF (2 mL) was reduced by the addition of a solution of $NaBH_3CN$ (7 mg) in THF (1 mL) in the presence of AcOH (0.2 mL). After 2 h of stirring, the mixture was concentrated. The residue was dissolved in AcOEt (5 mL). The solution was washed (1 N $NaHCO_3$, brine), dried over $MgSO_4$ and concentrated. The crude oil was purified by thin-layer chromatography (SiO_2, CH_2Cl_2/EtO_2 90:10) to yield hydroxylamine as a mixture of diastereoisomers (37 mg, 55%). DCI-MS(NH_3): 653 (M + H+). Two fractions were separated by HPLC (SiO_2, CH_2Cl_2/MeOH 99.2:0.8). Compound (2R,2'R)-**14** (20%) eluted first: ^1H NMR (400 MHz, $CDCl_3$) δ: 7.0–7.3 (m, 21H); 6.13 (d, J = 8 Hz, 2H); 4.61 (dt, J = 5.6 Hz, J = 7.6Hz, 2H); 3.64 (m, 2H); 3.62 (s, 6H); 2.9 (m, 4H); 2.8 (dd, J = 7,7 Hz, J = 13.6 Hz, 2H); 2.5 (dd, J = 8.6 Hz, J = 13.6 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 173 (2C); 172 (2C); 135 (4C); 129 (8C); 128 (12C); 70 (2C); 54 (4C); 37 (2C); 36 (2C). Compound (2R,2'S)-**14a** (80%) eluted second: ^1H NMR (400 MHz, $CDCl_3$) δ: 6.7–7.3 (m, 21H); 6.45 (d, J = 8.6 Hz, 1H); 6.38 (bs, 1H); 4.8 (dt, J = 8.4 Hz, J = 6.8 Hz, 1H); 4.7 (dt, J = 4.9 Hz, J = 9.2 Hz, 1H); 3.64 (s, 3H); 3.68 (s, 3H); 3.72 (m, 1H); 3.70 (m, 1H); 3.10 (dd, J = 13. 9 Hz, J = 4.9 Hz, 1H); 3.05 (m, 2H); 2.95 (m, 4H); 2.76 (dd, J = 13.9 Hz, J = 9.4 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 173 (2C); 172 (2C); 135 (4C); 129 (8C); 128 (12C); 68 (2C); 54 (4C); 38 (2C); 35 (2C).

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