EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201101387

Synthesis of Enantiopure 7-Substituted Azepane-2-carboxylic Acids as Templates for Conformationally Constrained Peptidomimetics

Elena Cini,[a] Giuseppe Bifulco,[b] Gloria Menchi,[c] Manuela Rodriquez,*[b] and Maurizio Taddei*[a]

Keywords: Amino acids / Peptidomimetics / Azepanes / Cyclization / Synthetic methods

The introduction of a cyclic amino acid in a peptide is one of the best methods to rigidify a strand. A general approach towards a new class of seven-membered ring amino acids is described starting from (*S*)-tribenzyl glutamic acid γ-aldehyde, which reacts with β-keto phosphonates to generate the Horner–Wadsworth–Emmons product. In the presence of H_2 and a Pd catalyst, a four-step process occurs involving double-bond hydrogenation, hydrogenolysis of three benzyl protecting groups, imine formation, and reductive amination to produce the 7-substituted azepane carboxylic acid in good overall yield and with good to excellent diastereomeric ratios. An amino function can be introduced in the 7-position as an additional orthogonal chemical handle for readily generating diversity on the cyclic amino acid scaffold by using a β-keto phosphonate derived from amino acids. A cyclic RGD (Arg-Gly-Asp) pentapeptide analogue containing this new class of noncoded amino acids was also prepared by microwave-assisted cyclization, showing a promising activity as $\alpha_v\beta_3$ integrin inhibitor.

Introduction

The development of new small molecules with high affinity and selectivity towards therapeutically relevant targets is one of the main efforts of contemporary bioorganic and medicinal chemists.[1] Peptides serve as leads in this discovery process; although flexible, they work in the so-called biologically active conformation adopted to bind proteins. A conformationally constrained peptide analogue is expected to have higher affinity for the target because the preorganized molecule should pay a lower entropic cost for complexation, assuming that the flexible and the constrained molecules interact in the same way with the solvent and the receptor.[2] Moreover, conformationally constrained molecules often show better selectivity profiles. Evidence of candidates with reduced flexibility showing improved high oral bioavailability have been also demonstrated.[3]

One of the possible options to rigidify a structure inserted into a flexible peptide is to prepare a cyclic analogue of the natural linear peptide. Such a cyclization is primarily intended to introduce a constraint into the backbone that forces the system into a predefined secondary structure, corresponding roughly to α-, β-, or γ-turns or β-strands, which are structural motifs frequently found in the biologically active conformations of peptides. Although the introduction of cyclic constraints into peptides has proven to be advantageous to organize the backbone, such cyclizations are generally not able to preferentially orient the amino acid side chains. Because these side chains contribute significantly to specificity and affinity, controlling their spatial orientation is an important aspect in the design of peptidomimetics.[4] One of the best methods to constrain a peptide, with simultaneous projection of the side chain orientations, is to introduce a cyclic amino acid into the strand. Besides proline, other kinds of mono-, bi-, and even tricyclic amino acids have been designed and synthesized to serve as useful templates for turn mimetics.[5]

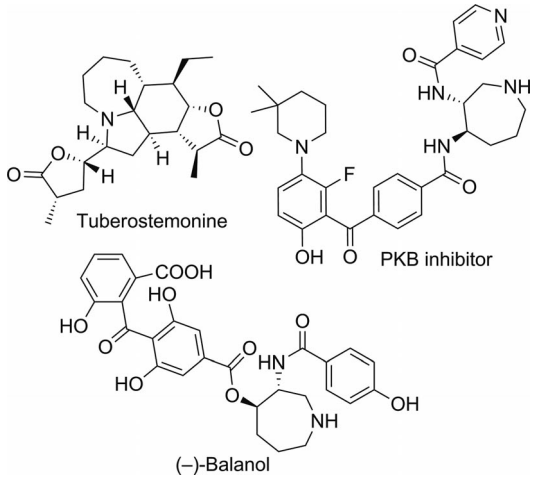
Results and Discussion

Azepanes are heterocyclic rings that are well represented in nature and in pharmaceutically relevant structures such as Balanol and Tuberostemonine (Scheme 1),[6] but no enantiopure substituted azepane amino acid has been synthesized until now, with the exception of simple azepane 2-carboxylic acid.[7]

Considering our experience in the application of glutamic acid as a synthon to access bioactive molecules,[8] we figured out a possible rapid approach to a collection of enantiomerically pure azepane carboxylic acids based on the

[a] Dipartimento Farmaco Chimico Tecnologico,
Università degli Studi di Siena,
Via A. Moro 2, 53100 Siena, Italy
Fax: +39-0577-234280
E-mail: taddei.m@unisi.it
[b] Dipartimento di Scienze Farmaceutiche e Biomediche,
Università degli Studi di Salerno,
Via Ponte don Melillo, 84084 Fisciano, Salerno, Italy
[c] Dipartimento di Chimica "Ugo Schiff",
Università degli Studi di Firenze,
Polo Scientifico di Sesto Fiorentino,
50019 Sesto Fiorentino, Firenze, Italy

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201101387.

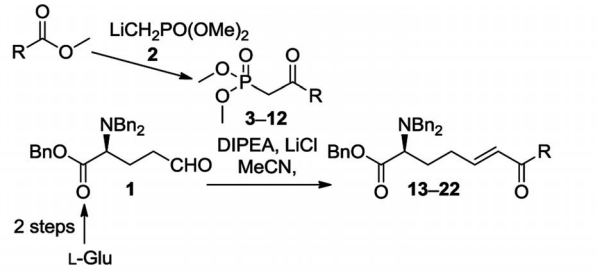


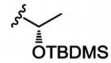
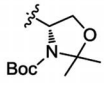
Scheme 1. Azepane-containing bioactive molecules.

reductive cyclization of 2-amino-7-ketocarboxylates. For their preparation, a HWE (Horner–Wadsworth–Emmons) reaction between the γ-aldehyde derived L-Glu and a phosphonate (derivable form the corresponding ester) was proposed (see the scheme in Table 1).

Aldehyde **1**, obtained in two steps from L-glutamic acid,[9] was treated with phosphonates **3**–**12**. With the exception of commercially available phosphonates **3**–**5**, compounds **6**–**12** were prepared by reaction of lithium methyl phosphonate **2** (generated in situ from dimethyl methylphosphonate with BuLi in THF at –78 °C) with different esters.[8a] β-Ketophosphonates **6**–**12** were obtained in good to acceptable yields, although some of them were unstable at room temperature and were prepared just before their use. An additional HWE reaction was carried out by employing DIPEA in the presence of dry LiCl in MeCN to

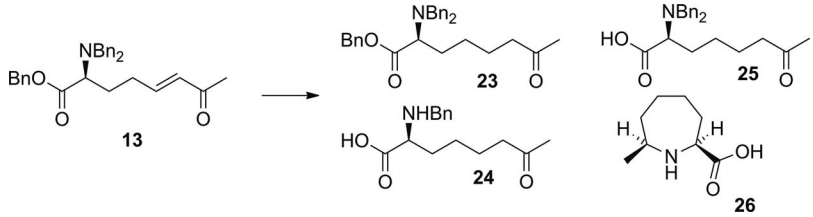
Table 1. Synthesis of benzyl 2-dibenzylamino-7-oxooct-5-enoates **13**–**22**.



Entry	R	Phosphonate	α,β-Unsaturated ketone	Yield (%)[a]
1	CH_3	**3**	**13**	78
2	CH_2CH_3	**4**	**14**	81
3	$(CH_2)_4CH_3$	**5**	**15**	75
4	$C(CH_3)_3$	**6**	**16**	71
5		**7**	**17**	80
6		**8**	**18**	77
7		**9**	**19**	71
8		**10**	**20**	75
9		**11**	**21**	73
10	CH_2CH_2Ph	**12**	**22**	80

[a] Yield of isolated and fully characterized product.

Table 2. Optimization of the cyclization step.



Entry	Catalyst	Conditions	Product (% Yield)
1	Pd/C	$HCOONH_4$, 2-propanol, MW, 150 °C, 2 min	**23** (87)[a]
2	Pd/C	H_2 (3 atm), MeOH, 150 °C, 60 min	**23** (48), **24** (32), **25** (8)[b]
3	Pd/C	H_2 (6 atm), MeOH, 150 °C, 4 h	**23** (6), **24** (27), **25** (41)[b]
4	$Pd(OH)_2/C$	H_2 (6 atm), MeOH, r.t., 4 h	**24** (81)[a]
5	$Pd(OH)_2/C$	H_2 (6 atm), MeOH, 150 °C, 4 h	**24** (85)[a]
6	$Pd(OH)_2/C$	H_2 (6 atm), MeOH, MW, 150 °C, 10 min	**24** (88)[a]
7	$Pd(OH)_2/C$	H_2 (6 atm), MeOH/AcOH (10:1), r.t., 12 h	**26** (79)[a] 95:5 *dr*[c]
8	$Pd(OH)_2/C$	H_2 (6 atm), MeOH/AcOH (10:1), MW, 100 °C, 20 min	**26** (91)[a] 70:30 *dr*[c]

[a] Yield of isolated and fully characterized product. [b] Yield determined by HPLC analysis by using *p*-aminobenzoic acid as an internal standard. [c] Ratio determined by HPLC analysis of the corresponding *N*-Cbz derivative prepared directly from the crude reaction mixture.

give α,β-unsaturated ketones **13**–**22** in good yields (Table 1). Careful inspection of the ^{1}H NMR (400 MHz) spectra showed that exclusively the *E* isomer was always formed.

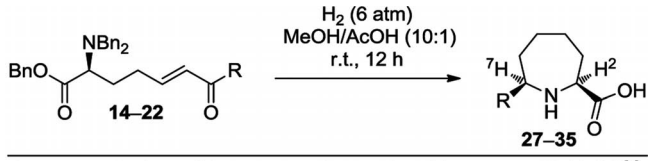
Compounds **13**–**22** were then submitted to double-bond hydrogenation/hydrogenolysis of the benzyl groups to obtain directly the cyclic azepane. The amine was first formed under reductive conditions and further cyclized to generate the imine, which was promptly reduced by hydrogen employed in the reaction. First attempts were carried out on compound **13**, used as a model, by transfer hydrogenolysis with $HCOONH_4$ and Pd/C as catalyst under microwave (MW) dielectric heating[10] (Table 2, Entry 1). Unfortunately, only reduction of the double bond occurred (compound **23**; Table 2, Entry 1). Also, the use of H_2 at a different pressure and temperature did not lead to removal of the benzyl groups (Table 2, Entries 2 and 3). Moving from Pd/C to $Pd(OH)_2$/C (Pearlman's catalyst), monobenzyl derivative **24** was obtained (Table 2, Entry 4). However, every attempt to remove the last benzyl group failed, even when working at a higher temperature or by using a MW apparatus equipped to operate under gas pressure[11] (Table 2, Entries 4–6). Compound **26** was finally obtained in 79% yield by using $Pd(OH)_2$/C in MeOH/AcOH (10:1) under 6 atm of hydrogen at room temperature for 12 h (Table 2, Entry 7).

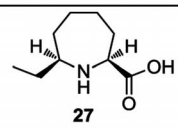
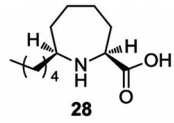
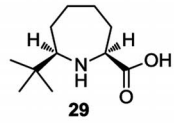
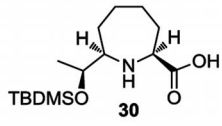
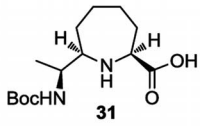
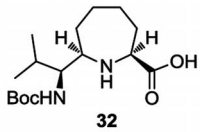
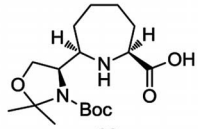
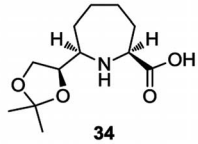
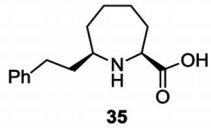
7-Methylazepane-2-carboxylic acid **26** was obtained as a mixture of diastereomers in 95:5 ratio (HPLC and NMR analysis). Under the optimized conditions, once the imine is formed, it is rapidly reduced avoiding racemization of the stereocenter at C2. The enantiomeric purity of compound **26** was proved by comparison of the NMR spectroscopic data and HPLC data of the (+)-Mosher acid amide with those obtained for the analogous racemic compound prepared in the same way starting from racemic glutamic acid (see the Supporting Information). To speed up the step, hydrogenation under MW dielectric heating was attempted under the same reaction conditions. Product **26** was obtained in very good yields but with lower diastereoselectivity (Table 2, Entry 8). The optimized reaction conditions were applied to ketones **14**–**22** and the corresponding azepane carboxylic acids **27**–**35** were obtained in very good yields after 12 h (see Table 3).

The reaction proceeded with a high level of stereoselectivity except in the case of compounds **32** and **34**. However, the isomer formed in higher quantity was always isolated by column chromatography on silica gel. The ^{1}H NMR spectra of the cycles show large and sharp signals relative to different parts of the molecule. Preliminary analysis of the ROESY spectra for assessing the relative configuration of the azepane sustituents in the 2- and 7-positions suggested their *cis* arrangement on the basis of a ROE peak between H-2 and H-7. This attribution was confirmed by a NOESY spectrum carry out on compound **26** showing a cross-peak between H-2 and H-7.

For securing this stereochemical assignment, we calculated, at the DFT MPW1PW91 level using the 6-31G(d,p) basis set, the ^{13}C NMR chemical shifts for the most significant conformers of the *trans* and *cis* stereoisomers, and their Boltzmann-weighted averaged shieldings were compared to the experimental values.[12] As shown in Table 4, a mean absolute error (MAE = Σ $[|(\delta_{exp.} - \delta_{calcd.})|]/n$) of 2.3 ppm for the *cis* stereoisomer vs. 4.6 ppm observed for the *trans* stereoisomer strongly suggested the *cis* configura-

Table 3. Synthesis of different 7-substituted-azepane 2-carboxylic acids.



Entry	Amino acid	Azepanic acid	Yield (%)[a] *dr*
1	**14**	 **27**	85 91:9[b]
2	**15**	 **28**	92 >99:1[b]
3	**16**	 **29**	93 90:10[b]
4	**17**	 **30**	92 83:17[b]
5	**18**	 **31**	90 89:11[c]
6	**19**	 **32**	89 65:35[c]
7	**20**	 **33**	94 92:8[c]
8	**21**	 **34**	92 68:32[b]
9	**22**	 **35**	81 90:10[c]

[a] Yield of isolated and fully characterized product. [b] Ratio determined by HPLC analysis of the corresponding *N*-Cbz derivative prepared directly from the crude reaction mixture. [c] Diastereomeric ratio determined by HPLC analysis.

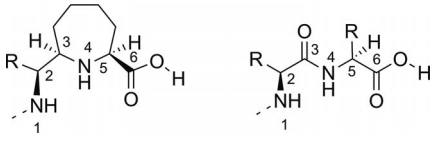
tion for the azepane ring. As the original stereocenter was derived from (*S*)-lactic acid, we could assign the absolute configuration (*S*) to the carbon atom in the 7-position of azepanes **26**–**28** and the (*R*) configuration to azepanes **29**–**35**, thus resulting in a *cis* relative configuration (for the most abundant isomer). This spatial arrangement is compatible with the mode of formation of the imine and attack of the H_2 adsorbed on the Pd surface form the less-hindered side (opposite to the substituent in the 2-position). The increase in the hindrance around the C=N gave an increase in the selectivity, whereas the presence of two substituents of comparable size at the adjacent stereocenter (as in **32** and **34**) reduced the stereoselection.

Table 4. ^{13}C NMR calculated [MPW1PW91/6-31G(d,p) level] chemical shifts for the *cis* and *trans* forms of **30** and the corresponding ^{13}C NMR chemical shift values (δ, ppm).

Atom	Calculated ^{13}C NMR chemical shifts		
	cis	*trans*	**30** (Exp.)
2	59.0	60.0	60.2
3	29.8	31.2	27.6
4	26.1	28.0	23.9
5	24.3	30.2	24.1
6	32.1	35.8	26.3
7	64.9	58.9	65.1
9	71.6	72.7	68.8
10	21.4	20.5	17.5
MAE[a]	2.3	4.6	

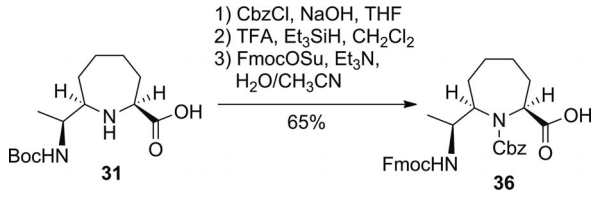
[a] Mean absolute error (MAE) found for ^{13}C NMR chemical shifts of *cis* and *trans* vs. **30** ^{13}C NMR experimental values: MAE = $\Sigma[|(\delta_{exp.} - \delta_{calcd.})|]/n$.

Compounds **31**–**33** (derived from encoded amino acids and carrying a protected exocyclic NH_2) can be considered as potential cyclic analogues of dipeptides (Scheme 2). With the same 6-atom-distance between the amino and the carboxylic groups, 7-alkylaminoazepane 2-carboxylic acids retain the same topology of a dipeptide. Moreover, by introducing an alkylamino group derived from an amino acid, all the analogues of the coded amino acids can be potentially prepared.



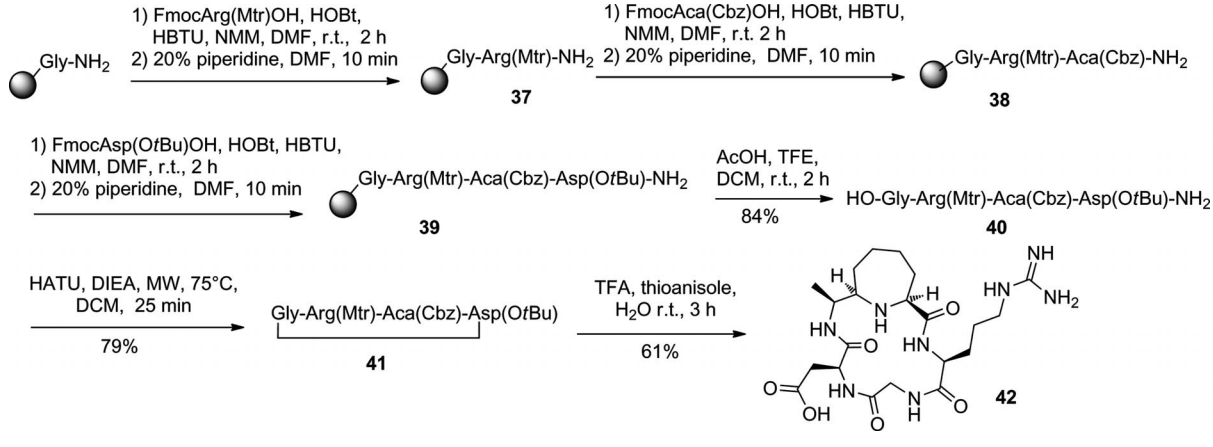
Scheme 2. Similarities between azepane carboxylic acids and dipeptides.

With the aim of proving that these azepane carboxylic acids could be successfully employed in the synthesis of conformationally constrained peptides, the preparation of a cyclic RGD (Arg-Gly-Asp) cyclic pseudopeptide, incorporating our seven-membered ring template, was attempted. To carry out a solid-phase synthesis of the cyclic peptide, the endocyclic nitrogen of **31** was protected as Cbz and the Boc protection of the exocyclic nitrogen was replaced with a Fmoc group through acid deprotection followed by reaction with Fmoc-OSu (Scheme 3).



Scheme 3. Preparation of Fmoc-Aca(Cbz)-OH.

Compound **36** [stated as Fmoc-Aca(Cbz)-OH] was isolated in 65% yield. The preparation of cyclic peptide **42** was carried out on solid phase starting with the acid-labile PS-DVB-2-ClTrt-Gly-NH_2 resin (Scheme 4). *N*-Fmoc-Arg-(Mtr)OH was loaded onto the resin by using HBTU/HOBT. Then the synthesis proceeded through a cycle of Fmoc deprotection (10% piperidine in DMF) and further coupling with Fmoc-Aca(Cbz)-OH (**36**) first and *N*-Fmoc-Asp(O*t*Bu)-OH after, until linear pseudopeptide **40** was obtained after removal from the resin by using AcOH/TFE/DCM (2:2:6) for 2 h. This product, obtained in good overall yields (84%), was cyclized in a 7×10^{-4} M solution in CH_2Cl_2 under microwave dielectric heating at 75 °C, 25 min, 25 W, using HATU/DIPEA as the coupling agent.[13] Product **41** (protected at the amino acid side chains) was obtained in exceptionally high yield (79%). Fi-



Scheme 4. Synthesis of a pentapeptide RGD analogue containing the Aca moiety.

nal removal of all the protections with TFA/thioanisole/H_2O (90:5:5) for 3 h at room temperature gave compound **42**, which was purified by reverse-phase chromatography on a C-18 cartridge followed by precipitation in diethyl ether. Product **42** was submitted to solid-phase binding competition studies[14] against ST1646,[15] a well-known integrin ligand, showing $IC_{50} = 1.8 \pm 0.4$ μM towards $\alpha_v\beta_3$ and $IC_{50} = 2.9 \pm 0.8$ μM towards $\alpha_v\beta_5$ receptors. Conformational analysis carried out on compound **42** suggested the possible existence of a turn consistent with the binding data (Figure 1). In particular, **42** showed a preferred cyclopeptide conformation very similar to the X-ray-binding conformation of EMD121974.[15]

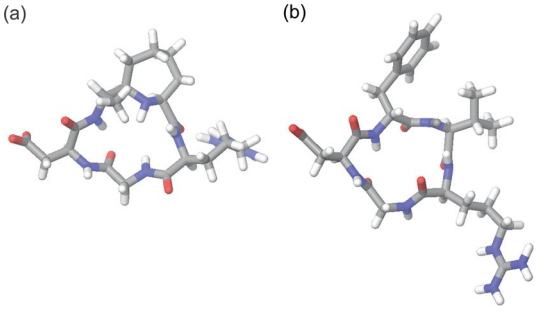


Figure 1. (a) Minimum energy conformation of **42** sampled during the 10-ns Monte Carlo/stochastic dynamics simulation after energy minimization. (b) X-ray conformation of $\alpha_v\beta_3$-bound EMD121974.[16]

Conclusions

In conclusion we have described the synthesis of a new class of enantiopure cyclic amino acid based on the azepane ring in four simple steps starting from readily available L-glutamic acid. These structures can be used as a template for the introduction of constraints into a peptide as a turn inducer. A cyclic RGD analogous cyclic pseudopeptide that showed micromolar binding affinity towards $\alpha_v\beta_3$ and $\alpha_v\beta_5$ receptors has also been prepared.

Experimental Section

General Methods: All reagents and solvents were used after purification: THF distilled from Na with benzophenone, CH_2Cl_2 distilled from dry $CaCl_2$, MeOH distilled from Na, DMF and MeCN dryed with molecular sieves (4 Å). The reactions were carried out in oven-dried or flamed vessels (vials) and performed under nitrogen. Flash column chromatography was performed with Merck silica gel 60 (0.040–0.063 mm, 230–400 mesh). Merck aluminum-backed plates pre-coated with silica gel 60 (UV254) were used for TLC and were visualized by staining with a solution of $KMnO_4$. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 27 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referred to the residual hydrogen in the solvent ($CHCl_3$, δ = 7.27 ppm; CD_2HOD, δ = 3.31 ppm; CHD_2SOCD_3, δ = 2.50 ppm). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sext = sextuplet, sept = septuplet m = multiplet and/or multiple resonances, br. s = broad singlet), coupling constant (*J*) in Hz and integration. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 27 °C. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$, δ = 77.0 ppm; CD_3OD, δ = 49.1 ppm, [D_6]DMSO, δ = 39.5 ppm). Mass spectrometry data of the products were collected with LC-MS ESI mass spectrometers. LC/MS conditions: ES ionisation after passage through a C-18, 35 × 5 mm, 3 μ column, elution: mixture A (99.9% water, 0.1% HCOOH); mixture B (99.9% acetonitrile, 0.1% HCOOH): 0–6.0 min, 95% mixture A; 6.0–9.0 min 95–0% mixture A; 9.0–15 min 0–95% mixture A, flow 1.0 mL/min, T = 40 °C. Reactions carried out under microwave dielectric heating were performed with a microwave oven (Discover from CEM) under monomode irradiation in a 10 mL sealed vial. The internal temperature was monitored through an internal IR sensor and the maximum internal pressure monitored and maintained under the value of 200 psi.

Synthesis of (*S*,*E*)-Benzyl 2-(Dibenzylamino)-7-oxooct-5-enoate (13) as a Representative Procedure: To a solution of phosphonate **3** (1.00 g, 1.3 mmol) in dry MeCN (40 mL) was added dry LiCl (254 mg, 6.0 mmol) followed by freshly distilled DIPEA (626 mg, 830 μL, 4.9 mmol) under an atmosphere of N_2. After stirring for 2 h at room temperature, aldehyde **1** (1.86 g, 4.6 mmol) in MeCN (25 mL) was added, and the mixture was stirred at room temperature for 12 h. A saturated solution of NaCl was used for quenching, and the organic layer was separated. The aqueous phase was extracted with EtOAc, and all the organic fractions were collected and dried with Na_2SO_4. The solvent was removed in vacuo. The crude mixture was purified by column chromatography (petroleum ether/EtOAc, 8:2) to give compound **13** as a colorless oil (1.56 g, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.22 (m, 15 H, ArH), 6.58 (dt, J = 15.6, 7.8 Hz, 1 H, CH), 5.90 (d, J = 15.6 Hz, 1 H, CH), 5.22 (AB system, 2 H, CH_2), 3.61 (AB system, 4 H, 2 CH_2), 3.37 (t, J = 7.0 Hz, 1 H, CH), 2.58–2.24 (m, 2 H, CH_2), 2.23 (s, 3 H, CH_3) 2.09–1.91 (m, 2 H, CH_2) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 197.7, 171.9, 146.6, 139.0, 135.8, 131.5, 128.7 (4 C), 128.4 (3 C), 128.3 (2 C), 128.1 (4 C), 128.0, 126.9 (2 C), 65.9, 59.9, 54.3 (2 C), 28.5, 27.7, 26.4 ppm. MS (ESI+, $C_{29}H_{31}NO_3$): m/z = 464 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{29}H_{31}NNaO_3$ 464.2202; found 464.2205.

(*S*,*E*)-Benzyl 2-(Dibenzylamino)-7-oxonon-5-enoate (14): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.20 (m, 15 H, ArH), 6.58 (dt, J = 15.6, 7.8 Hz, 1 H, CH), 5.90 (d, J = 15.8 Hz, 1 H, CH), 5.22 (AB system, 2 H, CH_2), 3.49 (AB system, 4 H, 2 CH_2), 3.37 (t, J = 7.0 Hz, 1 H, CH), 2.41 (q, J = 7.6 Hz, 2 H, CH_2), 2.27–2.11 (m, 2 H, CH_2), 1.96–1.84 (m, 2 H, CH_2), 1.04 (t, J = 7.6 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 200.9, 171.9, 145.2, 138.9 (2 C), 135.8, 130.4, 128.7 (4 C), 128.4 (2 C), 128.3, 128.2 (2 C), 128.1 (4 C), 126.8 (2 C), 65.9, 60.0, 54.3 (2 C), 32.7, 28.5, 21.4, 7.6 ppm. MS (ESI+): m/z = 456 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{30}H_{34}NO_3$ 456.2539; found 456.2537.

(*S*,*E*)-Benzyl 2-(Dibenzylamino)-7-oxododec-5-enoate (15): ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.44–7.20 (m, 15 H, ArH), 6.60 (dt, J = 15.0, 7.6 Hz, 1 H, CH), 5.93 (d, J = 15.0 Hz, 1 H, CH), 5.22 (AB system, 2 H, CH_2), 3.74 (AB system, 4 H, 2 CH_2), 3.37 (t, J = 7.0 Hz, 1 H, CH), 2.55 (t, J = 7.4 Hz, 2 H, CH_2), 2.46–2.15 (m, 2 H, CH_2), 2.11–1.91 (m, 2 H, CH_2), 1.60–1-50 (m, 2 H, CH_2), 1.54–1.28 (m, 4 H, 2 CH_2), 0.98 (t, J = 7.2 Hz, 3 H, CH_2) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 200.4, 172.2, 145.5, 139.2 (2 C), 135.9, 130.7, 128.8 (4 C), 128.5 (2 C), 128.4, 128.3 (2 C), 128.2 (4 C), 127.0, 66.1, 60.2, 59.9, 54.5 (2 C), 39.8, 31.4, 28.7, 27.9, 23.8,

22.4, 13.8 ppm. MS (ESI+): *m*/*z* = [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{33}H_{39}NNaO_3$ 520.2828; found 520.2830.

(*S*,*E*)-Benzyl 2-(Dibenzylamino)-8,8-dimethyl-7-oxonon-5-enoate (16): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.20 (m, 15 H, ArH), 6.80 (dt, *J* = 15.2, 7.8 Hz, 1 H, CH), 6.34 (d, *J* = 15.2 Hz, 1 H, CH), 5.22 (AB system, 2 H, CH_2), 3.71 (AB system, 4 H, 2 CH_2), 3.37 (t, *J* = 7.0 Hz, 1 H, CH), 2.44–2.01 (m, 2 H, CH_2), 1.94–1.83 (m, 2 H, CH_2), 1.09 (s, 9 H, 3 CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 203.7, 172.3, 146.1, 139.4 (2 C), 136.1, 128.8 (4 C), 128.6, 128.5 (2 C), 128.4, 128.3 (2 C), 127.1 (4 C), 124.5 (2 C), 66.1, 60.5, 54.7 (2 C), 42.7, 29.1, 28.1, 26.2 (3 C) ppm. MS (ESI+): *m*/*z* = 506 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{32}H_{37}NNaO_3$ 506.2671; found 506.2671.

(2*S*,8*S*,*E*)-Benzyl 8-(*tert*-Butyldimethylsilyloxy)-2-(dibenzylamino)-7-oxonon-5-enoate (17): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43–7.17 (m, 15 H, ArH), 6.87 (dt, *J* = 15.6, 6.8 Hz, 1 H, CH), 6.48 (d, *J* = 15.8 Hz, 1 H, CH), 5.21 (AB system, 2 H, CH_2), 4.21 (q, *J* = 6.8 Hz, 1 H, CH), 3.73 (AB system, 4 H, 2 CH_2), 3.38 (t, *J* = 7.0 Hz, 1 H, CH), 2.45–1.95 (m, 2 H, CH_2), 1.91–1.83 (m, 2 H, CH_2), 1.25 (d, *J* = 6.8 Hz, 3 H, CH_3), 0.92 (s, 9 H, 3 CH_3), 0.11 (s, 6 H, 2 CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 201.3, 172.1, 147.5, 139.1 (2 C), 135.8, 128.7 (4 C), 128.5, 128.2 (2 C), 128.1, 126.9 (2 C), 126.8 (4 C), 124.3 (2 C), 74.1, 66.0, 60.3, 54.4 (2 C), 29.2, 27.8, 25.6 (3 C), 20.9, 18.0, –4.9, –5.1 ppm. MS (ESI+): *m*/*z* = 608 [M + Na]$^+$. HRMS (ESI+) calcd. for $C_{36}H_{47}NNaO_4Si$ 608.3172; 608.3173.

(2*S*,8*S*,*E*)-Benzyl 8-(*tert*-Butoxycarbonylamino)-2-(dibenzylamino)-7-oxonon-5-enoate (18): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43–7.17 (m, 15 H, ArH), 6.87 (dt, *J* = 15.8, 6.8 Hz, 1 H, CH), 6.07 (d, *J* = 15.8 Hz, 1 H, CH), 5.48–5.40 (m, 1 H, NH) 5.25 (AB system, 2 H, CH_2), 4.61–4.48 (m, 1 H, CH), 3.72 (AB system, 4 H, 2 CH_2), 3.38 (t, *J* = 7.4 Hz, 1 H, CH), 2.55–2.05 (m, 2 H, CH_2), 2.01–1.83 (m, 2 H, CH_2), 1.60 (s, 9 H, 3 CH_3), 1.25 (d, *J* = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 197.8, 172.1, 155.0, 139.3, 139.1 (2 C), 136.0, 128.7 (4 C), 128.5, 128.4 (2 C), 128.3, 128.2 (2 C), 128.1 (2 C), 127.1 (2 C), 126.9 (2 C), 78.2, 65.9, 60.0, 59.8, 54.5 (2 C), 28.9, 28.2 (3 C), 27.7, 18.5 ppm. MS (ESI+): *m*/*z* = 593 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{35}H_{42}N_2NaO_5$ 593.2991; found 593.2990.

(2*S*,8*S*,*E*)-Benzyl 8-(*tert*-Butoxycarbonylamino)-2-(dibenzylamino)-9-methyl-7-oxodec-5-enoate (19): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.20 (m, 15 H, ArH), 6.82 (dt, *J* = 15.8, 7.6 Hz, 1 H, CH), 6.04 (d, *J* = 15.8 Hz, 1 H, CH), 5.22 (AB system, 2 H, CH_2), 4.47–4.39 (m, 1 H, CH), 3.73 (AB system, 4 H, 2 CH_2), 3.37 (t, *J* = 7.0 Hz, 1 H, CH), 2.44–2.01 (m, 3 H, CH, CH_3), 1.94–1.83 (m, 2 H, CH_2), 1.41 (s, 9 H, 3 CH_3), 0.94 (d, *J* = 7.4 Hz, 3 H, CH_3), 0.75 (d, *J* = 7.4 Hz, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 197.8, 172.0, 155.7, 147.7, 139.1 (2 C), 135.8, 128.7 (4 C), 128.5 (2 C), 128.4 (2 C), 128.3 (2 C), 128.2 (4 C), 127.0 (2 C), 79.2, 66.0, 60.1, 54.5, 30.5, 29.0, 28.2 (3 C), 27.7, 19.7 (2v) ppm. MS (ESI+): *m*/*z* = 621 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{37}H_{46}N_2NaO_5$ 631.3304; found 631.3302.

(*S*)-*tert*-Butyl 4-[(*S*,*E*)-7-(Benzyloxy)-6-(dibenzylamino)-7-oxohept-2-enoyl]-2,2-dimethyloxazolidine-3-carboxylate (20): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.20 (m, 15 H, ArH), 6.75 (dt, *J* = 15.2, = 7.8 Hz, 1 H, CH), 6.20 (d, *J* = 15.2 Hz, 1 H, CH), 5.23 (AB system, 2 H, CH_2), 4.60–4.47 (m, 1 H, CH), 4.12–4.01 (m, 2 H, CH_2), 3.69 (AB system, 4 H, 2 CH_2), 3.37 (t, *J* = 6.8 Hz, 1 H, CH), 2.47–2.06 (m, 2 H, CH_2), 1.98–1.84 (m, 2 H, CH_2), 1.62–0.89 (m, 15 H, 5 CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 198.1, 172.0, 148.0, 139.2 (2 C), 135.9, 128.8 (4 C), 128.7 (2 C), 128.5 (2 C), 128.4 (2 C), 128.3 (4 C), 127.2 (2 C), 81.4, 80.7, 67.1, 66.2 (2 C), 65.8, 63.9, 60.2, 54.6 (2 C), 29.2, 28.3 (3 C), 27.9, 25.2 ppm. MS (ESI+): *m*/*z* = 649 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{38}H_{46}N_2NaO_6$ 649.3254; found 649.3257.

(*S*,*E*)-Benzyl 2-(Dibenzylamino)-7-[(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-7-oxohept-5-enoate (21): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.20 (m, 15 H, ArH), 6.83 (dt, *J* = 15.6, 7.6 Hz, 1 H, CH), 6.34 (d, *J* = 15.6 Hz, 1 H, CH), 5.22 (AB system, 2 H, CH_2), 4.49 (dd, *J* = 7.2, 6.8 Hz, 1 H, CH), 4.03–3.80 (m, 2 H, CH_2), 3.73 (AB system, 4 H, 2 CH_2), 3.37 (t, *J* = 6.8 Hz, 1 H, CH), 2.47–2.06 (m, 2 H, CH_2), 1.96–1.84 (m, 2 H, CH_2), 1.47 (s, 3 H, CH_3), 1.41 (s, 3 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 197.3, 172.1, 148.6, 139.2 (2 C), 135.9, 128.8 (4 C), 128.6 (2 C), 128.5 (2 C), 128.3, 128.2 (4 C), 127.1 (2 C), 125.4, 110.8, 79.3, 66.4, 66.1, 60.2, 54.6 (2 C), 29.2, 27.8, 25.9, 25.3 ppm. MS (ESI+): *m*/*z* = 528 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{33}H_{37}NNaO_5$ 550.2569; found 550.2569.

(*S*,*E*)-Benzyl 2-(Dibenzylamino)-7-oxo-9-phenylnon-5-enoate (22): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44–7.20 (m, 20 H, ArH), 6.09 (dt, *J* = 15.8, 7.6 Hz, 1 H, CH), 5.94 (d, *J* = 15.8 Hz, 1 H, CH), 5.22 (AB system, 2 H, CH_2), 3.71 (AB system, 4 H, 2 CH_2), 3.37 (t, *J* = 7.0 Hz, 1 H, CH), 2.94–2.65 (m, 4 H, 2 CH_2), 2.44–1.61 (m, 4 H, 2 CH_2) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 199.2, 172.3, 146.1, 141.3, 139.3 (2 C), 135.9, 130.1 (4 C), 128.9 (2 C), 128.7 (2 C), 128.6 (4 C), 128.5 (2 C), 128.4 (4 C), 127.2 (2 C), 126.1 (1 C), 66.2, 60.0, 54.6 (2 C), 41.5, 30.0, 28.7, 28.0 ppm. MS (ESI+): *m*/*z* = 554 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{36}H_{37}NNaO_3$ 554.2671; found 554.2674.

Synthesis of (2*S*,7*S*)-7-Methylazepane-2-carboxylic Acid (26) as a Representative Procedure: A solution of **13** (900 mg, 2.04 mmol) in MeOH (9 mL) was introduced into a bottle connected with a Parr apparatus. $Pd(OH)_2$/C (20%) (115 mg, 0.153 mmol) and acetic acid (61 mg, 58 μL, 1.02 mmol) were also added. The bottle was filled with H_2 (6 atm) and shaked at room temperature for 8 h. The bottle was degassed, the catalyst filtered (*ATTENTION: the Pd residue may be pyrophoric*) and washed several times with MeOH. The MeOH fractions were collected, and the solvent was removed under vacuum to give compound **26** as a yellow oil (310 mg, 79% yield, 95:5 *dr*). A portion of the crude (2 mg) was dissolved in THF (0.5 mL) and CbzCl (2 mg) was added followed by 10% NaOH (0.2 mL). The mixture was stirred in a vial for 4 h, and the product was analyzed at HPLC on a column EC 125/4.6 NUCLEODUR 100–5 C18 ec using the following eluent: 32% B (A: H_2O + 0.1% HCOOH, B: CH_3CN) at flow rate of 0.8 mL/min. The retention time of the minor isomer was 41.78 min and for the major isomer it was 44.39 min. The crude was then submitted to column chromatography on silica gel (EtOAc/MeOH, 95:5) to isolate the major isomer. ^{1}H NMR (300 MHz, CD_3OD): δ = 3.66 (dd, *J* = 3.2, 8.4 Hz, 1 H, CH), 3.40–3.37 (m, 1 H, CH), 2.24–2.19 (m, 1 H, *CH*H), 2.09–2.00 (m, 1 H, *CH*H), 1.91–1.88 (m, 1 H, *CH*H), 1.77–1.55 (m, 5 H, 2 CH2, *CH*H), 1.37 (d, *J* = 6.4 Hz, 3 H, CH3) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 173.6, 61.5, 54.8, 33.9, 28.9, 24.9, 24.7, 20.1 ppm. MS (ESI+): *m*/*z* = 158 [M + H]$^+$. HRMS (ESI+): calcd. for $C_8H_{16}NO_2$ 158.1181; found 158.1183. The diastereomeric ratio was determined by analytical LC–MS on the NCbz derivative.

(2*S*,7*S*)-7-Ethylazepane-2-carboxylic Acid (27): The diastereomeric ratio was determined by analytical LC–MS on the NCbz derivative (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following eluent: 40% B (A: H_2O + 0.1% HCOOH, B: CH_3CN) at flow rate of 0.8 mL/min. The retention time for the minor isomer was 43.78 min and for the major isomer it was 46.23 min. The crude was submitted to column chromatography on silica gel (EtOAc/MeOH, 95:5)

to isolate the major isomer. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 3.40 (dd, J = 4.0, 8.0 Hz, 1 H, CH), 2.98–2.96 (m, 1 H, CH), 2.03–1.38 (m, 10 *H*, 5 CH_2), 0.79 (t, J = 7.6 Hz, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 172.5, 60.4, 58.4, 30.3, 27.8, 26.8, 24.9, 24.4, 10.0 ppm. MS (ESI+): m/z = 194 [M + Na]$^+$. HRMS (ESI+) calcd. for $C_9H17NNaO_2$ 194.1157; found 194.1158.

(2*S*,7*S*)-7-Pentylazepane-2-carboxylic Acid (28): The diastereomeric ratio was determined by analytical LC–MS on the NCbz derivative (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following eluent: 45% B (A: H_2O + 0.1% HCOOH, B: CH_3CN) at flow rate of 0.8 mL/min. The retention time for the major isomer was 50.37 min; the minor was not detected. ^{1}H NMR (400 MHz, CD_3OD): δ = 3.67 (dd, J = 3.2, 8.4 Hz, 1 H, CH), 3.21–3.19 (m, 1 H, CH), 2.25–2.20 (m, 1 H, *CH*H), 2.06–1.94 (m, 3 H, *CH*H, CH_2), 1.77–1.26 (m, 12 H, 6 CH_2), 0.90 (t, J = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 173.6, 61.6, 58.9, 34.5, 31.7, 31.1, 28.6, 25.4, 25.1, 24.6, 22.5, 13.2 ppm. MS (ESI+): m/z = 236 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{12}H_{23}NNaO_2$ 236.1626; found 236.1626.

(2*S*,7*R*)-7-*tert*-Butylazepane-2-carboxylic Acid (29): The diastereomeric ratio was determined by analytical LC–MS on the NCbz derivative (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following gradient: from 50% to 80%B (A: H_2O + 0.1% HCOOH, B: CH_3CN) at flow rate of 0.8 mL/min. The retention time for the minor isomer was 7.18 min and for the major isomer it was 7.91 min. The crude was submitted to column chromatography on silica gel (EtOAc/MeOH, 95:5) to isolate the major isomer. ^{1}H NMR (300 MHz, CD_3OD): δ = 3.89–3.75 (m, 1 H, CH), 3.09–3.01 (m, 1 H, CH), 2.36–1.27 (m, 8 H, 4 CH_2), 1.09 (s, 9 H, 3 CH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 172.8, 65.3, 62.8, 34.2, 27.5, 27.2, 25.3 (3 C), 25.1, 24.8 ppm. MS (ESI+): m/z = 200 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{11}H_{22}NO_2$ 200.1651; found 200.1652.

(2*S*,7*R*)-7-[(*S*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]azepane-2-carboxylic Acid (30): The diastereomeric ratio was determined by analytical LC–MS (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following gradient: from 20% B to 75% B (A: H_2O + 0.1% HCOOH, B: CH_3CN) over 90 min, at flow rate of 0.8 mL/min. The retention time for the minor isomer was 29.79 min and for the major isomer it was 32.00 min. The crude was submitted to column chromatography on silica gel (EtOAc/MeOH, 90:10) to isolate the major isomer. ^{1}H NMR (400 MHz, CD_3OD): δ = 4.19–4.15 (m, 1 H, CH), 3.79–3.75 (m, 1 H, CH), 3.28–3.25 (m, 1 H, CH), 2.21–2.17 (m, 1 H, *CH*H), 2.04–1.99 (m, 1 H, *CH*H), 1.87–1.83 (m, 2 H, CH_2), 1.59–1.50 (m, 4 H, 2 CH_2), 1.24 (d, J = 6.0 Hz, 3 H, CH_3), 0.96 (s, 9 H, 3 CH_3), 0.17 (s, 6 H, 2 CH_3) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 175.9, 72.4, 67.6, 64.8, 30.6 (2 C), 28.4 (3 C), 28.2, 28.0, 27.6, 20.8, –2.1, –2.7 ppm. MS (ESI+): m/z = 302 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{15}H_{32}NO_3Si$ 302.2151; found 302.2149.

(2*S*,7*R*)-7-[(*S*)-1-(*tert*-Butoxycarbonylamino)ethyl]azepane-2-carboxylic Acid (31): The diastereomeric ratio was determined by analytical LC–MS (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following eluent: 82% B (A: H_2O + 0.1% HCOOH, B: CH_3CN) at flow rate of 0.8 mL/min. The retention time for the major isomer was 4.57 min and for the minor isomer it was 5.54 min. The crude was submitted to column chromatography on silica gel (EtOAc/MeOH, 90:10) to isolate the major isomer. ^{1}H NMR (300 MHz, CD_3OD): δ = 4.11–3.96 (m, 1 H, CH), 3.91–3.80 (m, 1 H, CH), 3.40–3.27 (m, 1 H, CH), 2.24–1.36 (m, 8 H, 4 CH_2), 1.33 (s, 9 H, 3 CH_3), 1.16 (d, J = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 172.9, 155.8, 80.1, 63.1, 61.5, 49.6, 27.8 (3 C), 26.3, 25.9, 25.0 (2 C), 16.3 ppm. MS (ESI+): m/z = 287 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{14}H_{27}N_2O_4$ 287.1971; found 287.1970.

(2*S*,7*R*)-7-[(*S*)-1-(*tert*-Butoxycarbonylamino)-2-methylpropyl]azepane-2-carboxylic Acid (32): The diastereomeric ratio was determined by analytical LC–MS (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following eluent: 75% B (A: H_2O + 0.1% HCOOH, B: CH_3CN) at flow rate of 0.8 mL/min. The retention time for the major isomer was 10.37 min and for the minor isomer it was 14.52 min. The crude was submitted to column chromatography on silica gel (EtOAc followed by EtOAc/MeOH, 95:5) to isolate the major isomer. ^{1}H NMR (400 MHz, CD_3OD): δ = 6.95 (d, J = 9.2 Hz, 1 H, NH), 3.83 (dd, J = 3.2, 6.8 Hz, 1 H, CH), 3.62–3.57 (m, 1 H, CH), 3.46 (d, J = 8.8 Hz, 1 H, CH), 2.25–2.01 (m, 2 H, CH_2), 1.90–1.33 (m, 16 H, CH, 3 CH_2, 3 CH_3), 0.98 (d, J = 6.4 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 172.9, 157.8, 79.1, 60.5, 59.5, 59.2, 29.4, 27.0 (3 C), 26.6, 25.1, 24.7, 24.4, 18.5, 18.1 ppm. MS (ESI+): m/z = 315 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{16}H_{31}N_2O_4$ 315.2284; found 315.2287.

(2*S*,7*R*)-7-[(*S*)-3-(*tert*-Butoxycarbonyl)-2,2-dimethyloxazolidin-4-yl]-azepane-2-carboxylic Acid (33): The diastereomeric ratio was determined by analytical LC–MS (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following eluent: 38% B (A: H_2O + 0.1% HCOOH, B: CH_3CN) at flow rate of 0.8 mL/min. The retention time for the major isomer was 21.26 min and for the minor isomer it was 23.57 min. The crude was submitted to column chromatography on silica gel (EtOAc/MeOH, 95:5) to isolate the major isomer. ^{1}H NMR (400 MHz, CD_3OD): δ = 4.24–4.20 (m, 2 H, CH_2), 3.97–3.92 (m, 1 H, CH), 3.77 (dd, J = 3.6, 8.0 Hz, 1 H, CH), 3.55–3.52 (m, 1 H, CH), 2.17–2.02 (m, 2 H, CH_2), 1.85–1.75 (m, 2 H), 1.65–1.43 (m, 19 H, 2 CH_2, 5 CH_3) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 173.2, 154.2, 94.9, 81.8, 65.1, 61.3, 59.7, 40.2, 27.7, 27.2 (3 C), 25.5, 25.1, 22.6 (2 C), 22.1 ppm. MS (ESI+): m/z = 365 [M + Na]$^+$. HRMS (ESI+): calcd. for $C_{17}H_{30}N_2NaO_5$ 365.2052; found 365.2053.

(2*S*,7*R*)-7-[(*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl]azepane-2-carboxylic Acid (34): The diastereomeric ratio was determined by analytical LC–MS on the NCbz derivative (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following eluent: 30% B (A: H_2O + 0.1% HCOOH, B: CH_3CN) at flow rate of 0.8 mL/min. The retention time for the major isomer was 42.32 min and for the minor isomer it was 45.63 min. The crude was submitted to column chromatography on silica gel (EtOAc followed by EtOAc/MeOH, 95:5) to isolate the major isomer. ^{1}H NMR (600 MHz, CD_3OD): δ = 4.21–4.08 (m, 2 H, CH_2), 3.91–3.85 (m, 1 H, CH), 3.71–3.66 (m, 1 H, CH), 3.56–3.49 (m, 1 H, CH), 2.24–1.97 (m, 4 H, 2CH_2), 1.81–1.72 (m, 2 H, CH_2), 1.67–1.54 (m, 8 H, CH_2, 2 CH_3) ppm. ^{13}C NMR (150 MHz, CD_3OD): δ = 178.6, 91.5, 81.9, 66.0, 59.7, 48.5, 27.2, 26.2, 24.5, 22.6, 20.1, 19.0 ppm. MS (ESI+): m/z = 244 [M + H]$^+$. HRMS (ESI+): calcd. for $C_{12}H_{22}NO_4$ 244.1549; found 244.1550.

(2*S*,7*R*)-7-Phenethylazepane-2-carboxylic Acid (35): The diastereomeric ratio was determined by analytical LC–MS on the NCbz derivative (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following eluent: 65% B (A: H_2O + 0.1% HCOOH, B: CH_3CN) at flow rate of 0.8 mL/min. The retention time for the major isomer was 11.04 min and for the minor isomer it was 12.32 min. The crude was submitted to column chromatography on silica gel (EtOAc/MeOH, 90:10) to isolate the major isomer. ^{1}H NMR (600 MHz, CD_3OD): δ = 7.28–7.23 (m, 5 H, ArH), 3.69–3.62 (m, 1 H, CH), 3.30–3.14 (m, 1 H, CH), 2.79–2.64 (m, 2 H, CH_2), 2.27–1.51 (m, 10 H, 5 CH_2) ppm. ^{13}C NMR (150 MHz, CD_3OD): δ = 173.3, 141.9, 126.7 (2 C), 126.4 (2 C), 125.1, 61.2, 57.8, 37.4, 35.8,

31.7, 26.3, 24.6, 23.9 ppm. MS (ESI+): m/z = 248 $[M + H]^+$. HRMS (ESI+): calcd. for $C_{15}H_{22}NO_2$ 248.1651; found 248.1654.

(2*S*,7*R*)-7-[(*S*)-1-{[(9*H*-Fluoren-9-yl)methoxy]carbonylamino}ethyl]-1-(benzyloxycarbonyl)azepane-2-carboxylic Acid (36): To a solution of **31** (286 mg, 1.0 mmol) in THF (4 mL) was added 10% NaOH (2.7 mL). The mixture was cooled to –5 °C, and then CbzCl (205 mg, 168 μL, 1.2 mmol) was added. The mixture was warmed up to room temperature and stirred for 1 h. A saturated aqueous solution of NH_4Cl was added until the solution reached pH 4, and the organic layer was separated. The aqueous phase was extracted with EtOAc, and all the organic fractions were collected and dried with Na_2SO_4. The solvent was removed in vacuo. The crude mixture was purified by column chromatography ($CHCl_3$/MeOH, 9:1) to give the Cbz derivative as a colorless oil (286 mg, 68% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.51–7.22 (m, 5 H, ArH), 5.25 (s, 2 H, CH_2), 4.71–3.93 (m, 3 H, 3 CH), 2.33–1.95 (m, 2 H, CH_2), 1.93–0.98 (m, 18 H, 3 CH_2, 4 CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.8, 157.1, 137.3, 129.0 (2 C), 128.4 (2 C), 127.8, 127.7, 80.1, 70.1, 68.0, 64.5, 50.7, 31.4, 29.9, 28.0 (3 C), 25.9, 24.7, 19.2 ppm. MS (ESI+): m/z = 442 $[M + Na]^+$. HRMS (ESI+): calcd. for $C_{22}H_{32}N_2NaO_6$ 443.2158; found 443.2158. To a solution of the NCbz derived previously prepared (130 mg, 0.31 mmol) in CH_2Cl_2 (1.2 mL) was added Et_3SiH (150 mg, 207 μL, 1.30 mmol) and TFA (1.705 g, 1.305 mL, 17.05 mmol), and the mixture was stirred at room temperature for 3 h. The solvent was removed in vacuo. To the residue was added H_2O (4 mL), Et_3N (84 mg, 116 μL, 0.83 mmol), and FmocOSu (136 mg, 0.40 mmol, in 4 mL of CH_3CN), and the mixture was stirred for 1 h. HCl (1 N) was added until pH 4, and the organic layer was separated. The aqueous phase was extracted with EtOAc, and all the organic fractions were collected and dried with Na_2SO_4. The solvent was removed in vacuo. The crude mixture was purified by column chromatography ($CHCl_3$/MeOH, 9:1) to give compound **36** as colorless oil (112 mg, 67% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 9.02, (br. s, 1 H, OH), 7.77–7.63 (m, 2 H, ArH), 7.61–7.49 (m, 2 H, ArH), 7.47–7.21 (m, 9 H, ArH), 5.20 (s, 2 H, CH_2), 4.52–3.89 (m, 6 H, CH_2, 4 CH), 2.31–1.06 (m, 11 H, 4 CH_2, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 174.1, 155.4, 154.8, 143.4, 143.1 (2 C), 135.1 (2 C), 128.9 (2 C), 128.6 (2 C), 128.0, 127.7 (4 C), 127.8 (2 C), 126.9 (2 C), 65.9, 64.7, 56.0, 50.8, 47.1, 37.6, 39.4, 28.9, 25.6, 24.1, 19.2 ppm. MS (ESI+): m/z = 565 $[M + Na]^+$. HRMS (ESI+): calcd. for $C_{32}H_{34}N_2NaO_6$ 565.2315; found 565.2315.

Solid-Phase Synthesis of the Linear Precursor and Cyclization of cyclo[-L-Gly-L-Asp-2-(*S*)-ACA-L-Arg-] (42): The 2-ClTrt-gly-NH_2 resin was placed in a 25 mL polypropylene ISOSOLUTE syringe on a VAC MASTER system, swollen in DMF (3 mL) for 1 h, and then washed with 2 × 3 mL of DCM.

(a) Peptide Coupling Conditions: A mixture of Fmoc-amino acid (3–4 equiv.), HOBt (3–4 equiv.), HBTU (3–4 equiv.), and NMM or DIEA (4–5 equiv.) was stirred under an atmosphere of N_2 in DMF (2.5 mL) for 2 h. After each coupling, washings were carried out with DMF (3 mL, 3 × 1.5 min), and DCM (3 mL, 3 × 1.5 min), and the Kaiser test was used to assess coupling efficiency.

(b) Fmoc Deprotection: Removal of the Fmoc protecting group was carried out using 20% piperidine in DMF (3 mL, 1 × 1.5 min), 20% piperidine in DMF (3 mL, 1 × 10 min or 1 × 5 min); washings in DMF 2 × 3 mL, DCM 2 × 3 mL, DMF 2 × 3 mL, (1.5 min each). First coupling: Fmoc-Arg(Mtr)-OH (850 mg, 1.4 mmol), HOBt (190 mg, 1.4 mmol), HBTU (530 mg, 1.4 mmol), and DIEA (226 mg, 300 μL, 1.75 mmol). After incorporation of Fmoc-Arg(Mtr)-OH, the Fmoc protecting group was removed according to general procedure b. Second coupling: Fmoc-Aca(Cbz)-OH (850 mg, 1.4 mmol), HOBt (190 mg, 1.4 mmol), HBTU (530 mg, 1.4 mmol), and DIEA (226 mg, 300 μL, 1.75 mmol). After incorporation of Fmoc-Aca-OH, the Fmoc protecting group was removed according to general procedure b. Third coupling: Fmoc-Asp(O*t*Bu)-OH (850 mg, 1.4 mmol), HOBt (190 mg, 1.4 mmol), HBTU (530 mg, 1.4 mmol), and DIEA (226 mg, 300 μL, 1.75 mmol). After incorporation of Fmoc-Asp(O*t*Bu)-OH, the Fmoc protecting group was removed according to general procedure b.

(c) Resin Cleavage (2-ClTrt-Gly-NH_2): The dried peptide resin was treated for 2 h, whilst stirring, with AcOH/TFE/DCM (2:2:6; 10 μL × 1 mg of resin) cleavage mixture. Then the resin was filtered off and washed with neat cleavage mixture (3 mL, 3 × 1.5 min). After addition of hexane (15 times volume) to remove acetic acid as an azeotrope, the filtrate was concentrated and lyophilized to give 270 mg of the crude linear peptide **40** (84% overall yield). MS (ESI+): m/z = 917 $[M + H]^+$.

(d) Cyclization: The cyclization step was performed on 65 mg of the crude linear peptide dissolved in dry CH_2Cl_2 (7×10^{-4} M) and to this solution, cooled to 0 °C, HATU (1.5 equiv.) and DIEA (2 equiv.) were added. The vial was inserted in the cavity of a Discover synthesizer (CEM Corporation) and heated at 75 °C (25 W power, max internal pressure 5 atm) for two cycles of 10 min each (with a no irradiation interval of 2 min). The mixture was concentrated in vacuo and washed with 1 N HCl and purified by crystallization from ether to give 49 mg of crude protected cyclopeptide **41** (79% yield). MS (ESI+): m/z = 899 $[M + H]^+$. HRMS: calcd. for $C_{43}H_{63}N_8O_{11}S^+$ 899.4332; found 899.4329.

(e) Protections Cleavage: Side chain deprotection was obtained by treatment with TFA/thioanisole/H_2O (90:5:5) for 3 h whilst stirring. The solvent was removed in vacuo to give the crude cyclopeptide that was purified by crystallization from ether (17 mg, 61%). The product was analyzed by analytical LC–MS (EC 125/4.6 NUCLEODUR 100–5 C18 ec) using the following gradient: from 0% B to 15% B over 15 min, at flow rate of 1 mL/min. The binary solvent system (A/B) was as follows: water (A), MeOH (B). The absorbance was detected at 214 nm. The HPLC analysis showed one main peak (t_R = 2.573 min) that was identified as pure **42** on the basis of MS (ESI). The product was found to be over 95% pure by HPLC analysis. MS (ESI+): m/z = 417 $[M + H]^+$.

(2*S*,7*S*)-7-Methyl-1-[(*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl]azepane-2-carboxylic Acid: To a solution of **26** (10 mg, 0.064 mmol) in CH_2Cl_2 (1 mL) was added (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (19 mg, 0.077 mmol). The mixture was cooled to –5 °C, and then Et_3N (10 mg, 13 μL, 0.096 mmol) was added. The mixture was warmed up to room temperature and stirred for 1 h. HCl (1 N) was added, the organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2. All the organic fractions were collected and dried with Na_2SO_4. The solvent was removed in vacuo. The crude mixture was purified by column chromatography ($CHCl_3$/MeOH, 9:1) to give the chiral amide as a colorless oil (21 mg, 51% yield). ^{1}H NMR (400 MHz, [D_6]benzene): δ = 7.78 (d, J = 7.6 Hz, 1 H CH), 7.15–7.02 (m, 4 H, ArH), 4.05–4.00 (m, 1 H, CH), 3.87 (s, 3 H, CH_3), 3.21 (d, J = 10.4 Hz, 1 H, CH), 2.52–2.40 (m, 2 H, CH_2), 1.61–1.11 (m, 6 H, 3CH_2), 0.99 (d, J = 6.0 Hz, 3 H, CH_3) ppm. MS (ESI+): m/z = 396 $[M + Na]^+$.

Computational Details: To allow a full exploration of the conformational space, stochastic dynamics (10 ns) calculations at 300 K were performed with a Pentium-4 at 2.8 GHz processor using the AMBER force field included in the MacroModel package. All the structures so obtained (in number of 100) were minimized by using

the Polak-Ribier Conjugate Gradient algorithm (PRCG, 1000 steps, maximum derivative less than 0.05 kcal/mol). This led to the selection of the lowest-energy minimum conformer for **42** (Figure 1a). The initial geometries of the minimum energy conformers for **30** were optimized at the hybrid DFT MPW1PW91 level using the 6-31G(d) basis set (Gaussian 03 Software Package). GIAO ^{13}C calculations were performed using the MPW1PW91 functional and the 6-31G(d,p) basis set, using as input the geometry previously optimized at MPW1PW91/6-31G(d) level.

Supporting Information (see footnote on the first page of this article): Copies of the ^{1}H NMR and ^{13}C NMR spectra.

Acknowledgments

This work was financially supported by Ministero dell'Università e della Ricerca (MIUR) (Rome) within the PRIN 2009 Project number 2009RMW3Z5_006.

[1] a) A. Mann, *The Practice of Medicinal Chemistry*, 2nd ed. (Ed.: C. G. Wermuth), Academic Press, London, U. K., **2003**, pp. 233–250; b) R. E. Babine, S. L. Bender, *Chem. Rev.* **1997**, *97*, 1359–1472; See also: c) W. R. J. D. Galloway, M. Diáz-Gavilán, A. Isidro-Llobet, D. R. Spring, *Angew. Chem.* **2009**, *121*, 1216; *Angew. Chem. Int. Ed.* **2009**, *48*, 1194–1196.

[2] a) A. Giannis, T. Kolter, *Angew. Chem.* **1993**, *105*, 1303; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244–1267; b) J. Gante, *Angew. Chem.* **1994**, *106*, 1780; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699–1720; c) K. Suat, S. D. S. Jois, *Curr. Pharm. Des.* **2003**, *9*, 1209–1224; d) A. Perdih, D. Kikelj, *Curr. Med. Chem.* **2006**, *13*, 1525–1556.

[3] a) D. F. Veber, S. R. Johnson, H.-Y. Cheng, B. R. Smith, K. W. Ward, K. D. Kopple, *J. Med. Chem.* **2002**, *45*, 2615–26623; b) A. Reichelt, S. F. Martin, *Acc. Chem. Res.* **2006**, *39*, 433–442.

[4] a) S. Hanessian, G. McNaughton-Smith, H.-G. Lombart, W. D. Lubell, *Tetrahedron* **1997**, *53*, 12789–12854; b) S. Cowell, Y. S. Lee, J. P. Cain, V. J. Hruby, *Curr. Med. Chem.* **2004**, *11*, 2785–2798; c) A. Trabocchi, D. Scarpi, A. Guarna, *Amino Acids* **2008**, *34*, 1–24.

[5] S. Hanessian, L. Auzzas, *Acc. Chem. Res.* **2008**, *41*, 1241–1252.

[6] a) P. Wipf, S. R. Spencer, *J. Am. Chem. Soc.* **2005**, *127*, 225–235; b) P. Mungkornasawakul, S. Chalyong, T. Sastrauji, A. Jatisatienr, C. Jatisatienr, S. G. Pyne, A. T. Ung, J. Korth, W. Lie, *J. Nat. Prod.* **2009**, *72*, 848–851; c) D. L. Boger, P. Turnbull, *J. Org. Chem.* **1997**, *62*, 5849–5863; d) J. W. Lampe, P. F. Hughes, C. K. Biggers, S. H. Smith, H. Hu, *J. Org. Chem.* **1996**, *61*, 4572–4581; e) S. Torssell, E. Wanngren, P. Somfai, *J. Org. Chem.* **2007**, *72*, 4246–4249; f) C. B. Breiteniechner, T. Wegge, L. Berillon, K. Graul, K. Marzenell, W.-G. Friebe, U. Thomas, R. Schumacher, R. Huber, R. A. Engh, B. Masjost, *J. Med. Chem.* **2004**, *47*, 1375–1390.

[7] a) The single example of a 7-substituted azepane amino acid is described in: P. T. Cheng, Y. Jeon, W. Wang, WO 2002096357 A2, **2002**; for the synthesis of azepane 2-carboxylic acid see: b) H. T. Nagasawa, J. A. Elberling, P. S. Fraser, *J. Med. Chem.* **1973**, *14*, 501–508; c) M. Yasuda, M. Ueda, H. Muramatsu, H. Mihara, N. Esaki, *Tetrahedron: Asymmetry* **2006**, *17*, 1775–1779; d) G. T. Notte, T. Sammakia, *J. Am. Chem. Soc.* **2006**, *125*, 4230.

[8] a) M. Rodriquez, I. Bruno, E. Cini, M. Marchetti, M. Taddei, L. Gomez-Paloma, *J. Org. Chem.* **2006**, *71*, 103–107; b) E. Cini, L. R. Lampariello, M. Rodriquez, M. Taddei, *Tetrahedron* **2009**, *65*, 844–848; c) E. Cini, G. Giorgi, M. Rodriquez, M. Taddei, *Synlett* **2009**, 1562–1566; d) D. Conti, M. Rodriquez, A. Sega, M. Taddei, *Tetrahedron Lett.* **2003**, *44*, 5327–5330.

[9] M. Rodriquez, M. Taddei, *Synthesis* **2005**, 493–496.

[10] M. C. Daga, M. Taddei, G. Varchi, *Tetrahedron Lett.* **2001**, *42*, 5191–5194.

[11] a) E. Petricci, A. Mann, A. Rota, A. Schoenfelder, M. Taddei, *Org. Lett.* **2006**, *8*, 3725–3727; b) L. Piras, E. Genesio, C. Ghiron, M. Taddei, *Synlett* **2008**, 1125–1128.

[12] G. Bifulco, P. Dambuoso, L. Gomez-Paloma, R. Riccio, *Chem. Rev.* **2007**, *107*, 3744–3779.

[13] E. Cini, C. B. Botta, M. Rodriquez, M. Taddei, *Tetrahedron Lett.* **2009**, *50*, 7159–7161.

[14] A. Trabocchi, G. Menchi, E. Danieli, D. Potenza, N. Cini, A. Bottoncetti, S. Raspanti, A. Pupi, A. Guarna, *Amino Acids* **2010**, *38*, 329–337 and references cited therein.

[15] L. Belvisi, T. Riccioni, M. Marcellini, L. Vesci, I. Chiarucci, D. Efrati, D. Potenza, C. Scolastico, L. Manzoni, K. Lombardo, M. A. Stasi, A. Orlandi, A. Ciucci, B. Nico, D. Ribatti, G. Giannini, M. Presta, P. Carminati, C. Pisano, *Mol. Cancer Ther.* **2005**, *4*, 1670–1680.

[16] J.-P. Xiong, T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S. L. Goodman, M. A. Arnaout, *Science* **2002**, *296*, 151–155.

Received: September 22, 2011
Published Online: February 28, 2012

