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Synthesis of Enantiopure Pyrrolidine-Derived Peptidomimetics and Oligo-β-peptides via Nucleophilic Ring-Opening of β-Lactams†

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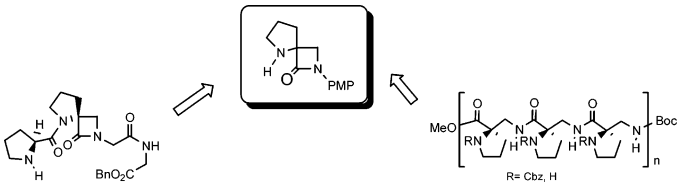

The synthesis of the two enantiomers of pyrrolidine-derived spiro β-lactams by resolution with D- and L-Boc phenylalanine is described. The potential of these optically active spiro β-lactams on the synthesis of peptidomimetics as analogues of melanostatin is evaluated. Theoretical studies of several models, at the Becke3LYP/6-31+G* level of theory, together with previous experimental evidences from our group, gathered by NMR, allow us to design structures that can efficiently mimic some biologically active peptide-type molecules. On the other hand, the spiro β-lactams have shown their utility in the preparation of β-peptides. As an example, a homo-tetra-β-peptide was synthesized. This research will continue in the future in order to obtain higher peptides with potential biological activity.

Introduction

The field of peptidomimetics is experiencing considerable development because of their potential as precursors for small, but efficient biologically important compounds that could lead to new therapeutically useful drugs. These compounds act by mimicking the activity of some biologically relevant peptides being, however, resistant to the hydrolytically activity of proteases. In addition, an important feature of peptidomimetics is the introduction of conformational restrictions that still allow them to be recognized by the potential acceptors interacting with them. There are a number of possible approaches for achieving this goal, but the introduction of cycles that bridge different parts of the molecule is one of the most popular alternatives. This strategy was pioneered by Freidinger[1] and followed by a number of practitioners in this field.[2] We have shown previously[3] that the introduction of a combination of a four-membered ring (such as a β-lactam) with a five-membered ring (such as pyrrolidine-derived ring) could produce a quite stable structure, able to form a type II β-turn (**1**, Figure 1).[2c,4]

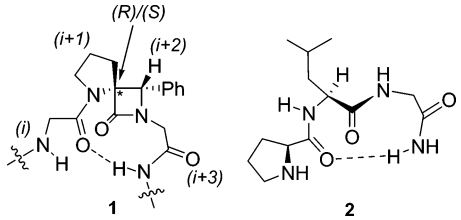

FIGURE 1. Spiro β-lactam forming a type II β-turn **1**, and melanostatin **2**.

The detailed analysis by NMR and the density-functional calculations indicate that **1** adopts in solution a type II β-turn conformation. In view of the potential interest of these types of compounds, we decided to further explore other possible spiro β-lactam-containing structures.

† Dedicated to Prof. Dr. Víctor M. Riera González on the occasion of his retirement from the Universidad de Oviedo.

‡ Universidad de Oviedo.

§ Universidad de Valencia.

(1) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656.

(2) For a review, see: (a) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699. (b) Giannis, A.; Kolter. T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244. (c) Robinson, J. A. *Synlett.* **1999**, *4*, 429.

(3) Alonso, A.; López-Ortiz, F.; Pozo, del C.; Peralta, E.; Macías, A. González, J. *J. Org. Chem.* **2001**, *66*, 6333.

(4) Muller, G.; Hessler, G.; Decornez, H. Y. *Chem. Int. Ed. Engl.* **2000**, *39*, 894.

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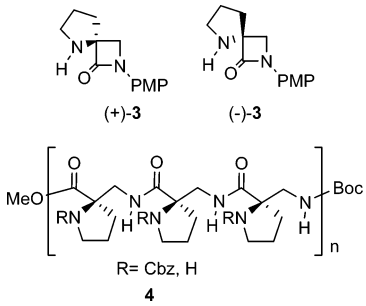


FIGURE 2. Optically active β-lactams **3** and β-peptides **4**.

More specifically, we decided to undertake the preparation of an optically active analogue of the neuropeptide melanostatin (PLG) **2** (see Figure 1), which has a related structure with the peptidomimetic **1** following the methodology we described previously, but making the necessary modifications that will allow us to obtain the C4-unsubstituted system.[5] The importance of the preparation of these analogues lies in the fact that they could serve as starting structures for the development of new types of compounds with the ability to modulate the activity of the dopamine receptor.[6] The essential step relies on the synthesis of optically active β-lactams (+)-**3** and (−)-**3** (Figure 2).

On the other hand, we wish to demonstrate the utility of the optically active β-lactams **3** on the preparation of β-peptides **4** (Figure 2) bearing a functionalized five-membered ring in its structure. Analogues of these compounds have shown to form secondary structures with a helix-type conformation and could have interesting biological properties.[7]

Results and Discussion

Molecular Modeling Studies. Before starting the synthesis of analogues of **2**, we carried out a theoretical study, at the Becke3LYP/6-31+G* level of theory, of the different structures in which we were interested (**5**−**8**, Figure 3), to test the possibility that these structures could be better candidates than the system containing the spiro **5**−**4** structure, previously studied in our group.[3]

The geometries of the structures **2**, **5**−**8** were fully optimized at the Becke3LYP/6-31+G* level of theory (see Supporting Information (SI) for the energies and Cartesian coordinates). The stationary points located were characterized as minima, by performing the corresponding frequency calculations. All the calculations were carried out with the Gaussian 98 suite of programs.[8] The optimized structures (showing some selected geometrical parameters) are shown in Figure 4.

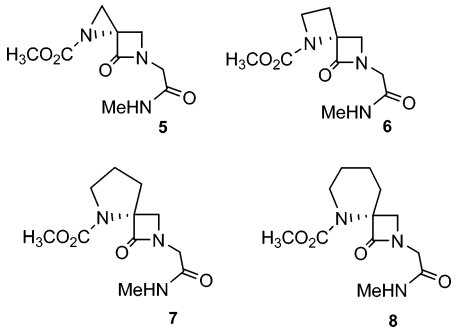
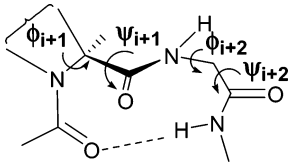


FIGURE 3. Spiro β-lactams **5**−**8** studied theoretically at the Becke3LYP/6-31+G* level of theory.

TABLE 1. Hydrogen Bond Lengths (Å), Energetic Preferences (kcal mol^{-1}), and Torsional Angles (deg) in Spiro β-Lactams and Related Compounds



	$d_{(C=O\cdots HN)}$	$\Delta E_{(anti-syn)}$	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}
ideal[a]	–	–	−60	120	80	0.0
2-syn	2.030	1.2	−69	113	109	−8.5
5-syn	2.346	2.3	−3.6	127.9	90.4	−22.7
6-syn	2.067	4.6	−38.3	123.0	101.5	−10.4
7-syn	2.014	4.7	−50.6	123.6	104.5	−13.6
8-syn	2.019	2.1	−57.8	122.9	108.6	−13.6

[a] See ref 9.

As can be seen, the syn conformations form a intramolecular hydrogen bond, which determine the formation of a β-turn.[9] Attending to the length of this hydrogen bond, the most favorable situation is in the case of melanostatin (**2-syn**), **7-syn,** and **8-syn** analogues. A summary of the results obtained are collected in Table 1.

As can be seen, the spiro system containing the pyrrolidine ring **7** is apparently the most favored, both in terms of the hydrogen bond length and the energetic preference for the syn conformation, having a β-turn. In addition, the analysis of the dihedral angles (see the figure in Table 1 for the definition of the dihedral angles) indicates that compounds **7-syn** and **8-syn** are close to the ideal case for a type II β-turn, while **5-syn** and **6-syn** are far from the ideal values, especially for the parameter ϕ_{i+1}. It seems that the introduction of three- and four-membered rings in the bicyclic system significantly alters the molecular geometry with respect to the ideal cases.

The geometry of the melanostatin **2**, in the syn conformation, forming a β-turn was optimized at the same level of theory, to

(5) The synthesis of the C4 unsubstituted β-lactams was accomplished using formaldehyde-derived imines, as described previously. See: (a) Kamiya, T.; Oku, T.; Nakaguchi, O.; Takeno, H.; Hashimoto, M. *Tetrahedron Lett.* **1978**, *51*, 5119. (b) Nakaguchi, O.; Oku, T.; Takeno, H.; Hashimoto, M. Kamiya, T. *Chem. Pharm. Bull.* **1987**, *35*, 3985. (c) Cainelli, G.; Galletti, P.; Giacomini, D. *Synlett.* **1998**, 611. (d) Cainelli, G.; Galletti, P.; Giacomini, D. *Tetrahedron Lett.* **1998**, *39*, 7779.

(6) (a) Khalil, E. M.; Ojala, W. H.; Pradhan, A.; Fair, V. D.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **1999**, *42*, 628. (b) Khalil, E. M.; Pradhan, A.; Ojala, W. H.; Gleason, W. B.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **1999**, *42*, 2977. (c) Dolbeare, K.; Pontoriero, G. F.; Gupta, S. K.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem.* **2003**, *46*, 727. (d) Palomo, C.; Aizpurua, J. M.; Benito, A.; Miranda, J. I.; Fratila, L. A.; Matute, C.; Domercq, M.; Gago, F.; Martin-Santamaría, S.; Linden, A. *J. Am. Chem. Soc.* **2003**, *125*, 16243. (e) Saitton, S.; Del Tredici, A. L.; Mohell, N.; Vollinga, R. C.; Bostrom, D.; Kihlberg, J.; Luthman, K. *J. Med. Chem.* **2004**, *47*, 6595. (f) Khasanov, A. B.; Ramirez-Weinhouse, M. M.; Webb, T. R.; Thiruvazhi, M. *J. Org. Chem.* **2004**, *69*, 5766. (g) Bitterman, H.; Gmeiner, P. *J. Org. Chem.* **2006**, *71*, 97. (h) Grison, C.; Coutrot, P.; Genève, S.; Didierjean, C.; Marraud, M. *J. Org. Chem.* **2005**, *70*, 10753.

(7) (a) Hintermann, T.; Seebach, D. *Chimia* **1997**, *51*, 244. (b) Gademann, K.; Ernst, M.; Hoyer, D.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1223. (c) Werder, M.; Hauser, H.; Abele, S.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1774. (d) Porter, E. A.; Wang, X.; Lee, H.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565.

(8) The calculations were carried out with the Gaussian 98 (Revision A.9) M. J. Frisch et al. Gaussian, Inc. Pittsburgh, PA, 1998.

(9) The different types of turns have analyzed by Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein. Chem.* **1985**, *37*, 1. See, also: Wilmot, C. M.; Thornton, J. M. *J. Mol. Biol.* **1988**, *203*, 221.

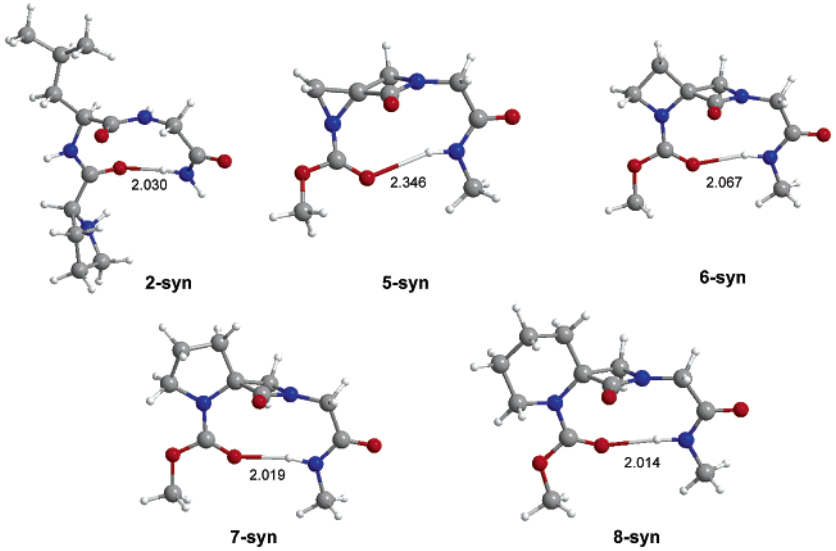


FIGURE 4. Becke3LYP/6-31+G* optimized structures, **2** and **5**−**8** (lengths are in Å).

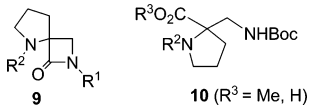
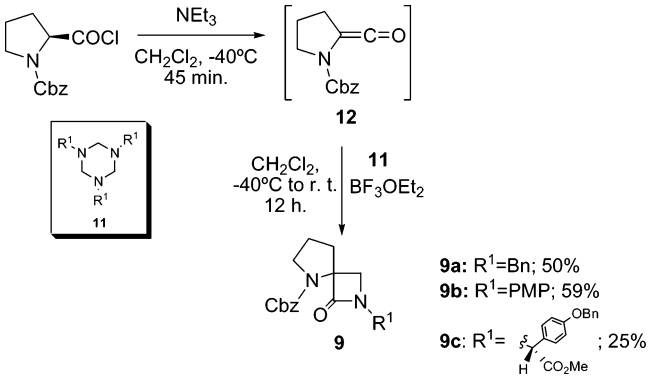


FIGURE 5. β-Lactam **9** and β-aminoesters and β-amino acids intermediates **10**.

SCHEME 1. Synthesis of Spiro β-Lactams 9 via Staudinger Reaction of Methyleneimines Derived from 11



be able to make comparisons with the other possible analogues; also, the anti conformer was fully optimized.

On the basis of these calculations and the experimental information available from our previous experiments,[3] we decided to accomplish the synthesis of the C4 unsubstituted and optically active β-lactam derivatives **9** (Figure 5), as a valuable intermediates in the preparation of different types of β-turn-inducing peptidomimetics, analogues to melanostatin **2**, and also as intermediates for the synthesis of the β-aminoesters and β-amino acids **10** (Figure 5), which could be used in the preparation of β-peptides **4**.

Synthesis of β-Lactams 3. The synthesis of C4 unsubstituted β-lactams involves the use of the extremely unstable imines of formaldehyde. These compounds, which are formed by condensation of a primary amine with an 30% aqueous solution of formaldehyde, rapidly trimerize to give the corresponding hexahydro-1,3,5-triazines, **11** (see Scheme 1).

The [2 + 2]-cycloaddition reaction between the ketene derived from Cbz-L-proline acid chloride and the methyleneimines was carried out in the following way: ketene **12** (see Scheme 1) was generated by dehydrochlorination of the acid chloride with triethylamine at −40 °C for 45 min, and then a dichloromethane solution of triazine **11** and boron trifluoride etherate (which depolymerize the triazine) was added. After 12 h, the corresponding spiro β-lactams **9** were obtained, with yields around 25−59%.

The β-lactams **9** can also be used in the preparation of analogues of *N*-sulfonyl-β-lactams, which in some cases have shown antibiotic activity (Scheme 2).[10] The oxidative removal of the PMP group from β-lactam **9b** gives the N-unsubstituted derivative **9e**, which upon treatment with the complex pyridine−sulfur trioxide gave the *N*-sulfonyl β-lactam **9f**.

Optical Resolution of the Racemic Mixture (±)-3. To obtain the enantiomerically pure spiro-β-lactam **3** (see Figure 2) as the pivotal intermediate on the synthesis of PLG analogues and β-peptides, we decided to resolve the corresponding racemic mixture. The β-lactam **9b** (R^1 = PMP; Scheme 2) was subjected to hydrogenolysis, to give the unprotected pyrrolidine system (±)-**3** (Scheme 3). The racemic mixture (±)-**3** was then treated with L-Boc-Phe-OH or D-Boc-Phe-OH, to give the corresponding salts **14** and **13**, respectively, which precipitates in the reaction medium. An additional crystallization gave the enantiopure salt, which upon treatment with $NaHCO_3$, gave the optically pure β-lactams (−)-**3** and (+)-**3**, respectively.

This synthetic procedure allows us to obtain each of the two enantiomers of the desired β-lactam **3**, in a procedure that can be carried out on a multigram scale.

Assignment of the Absolute Configuration to the β-Lactams 3. After we carried out the resolution of the racemic mixture of the β-lactams **3**, we had undertaken the task of determining their absolute configuration. Previously, the optical purity of the enantiomers obtained was assessed by HPLC analysis of the β-lactams (−)-**3** and (+)-**3** (see SI).

The method chosen for solving the problem of the absolute configuration, involved the analysis of the NOESY spectra of

(10) (a) Dückheimer, W.; Blumbach, J.; Lattrell, R.; Scheunemann, K. H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 180. (b) Stewart, W. J. *Nature* **1971**, *229*, 174. (c) Hashimoto, M.; Komori, T.; Kamiya, T. *J. Am. Chem. Soc.* **1976**, *98*, 3023. (d) Slucharsky, A.; Dejneka, T.; Gordon, E. M.; Weaver, E. R.; Koster, W. H. *Heterocycles* **1984**, *21*, 191. (e) Cimarusti, C. M.; Sykes, R. B. *Chem. Ber.* **1983**, *116*, 2.

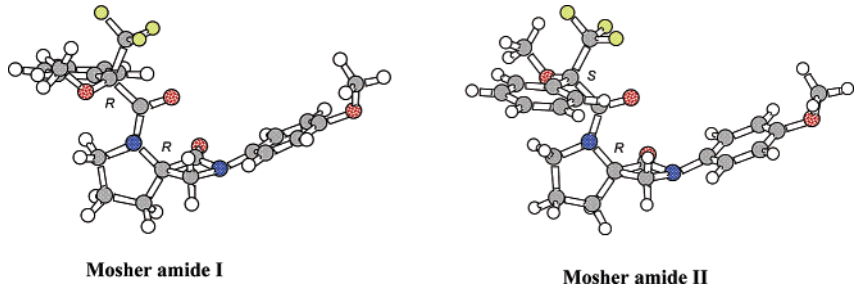
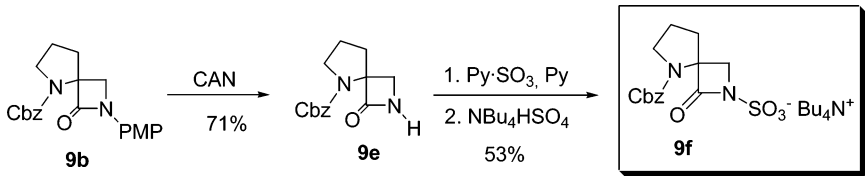
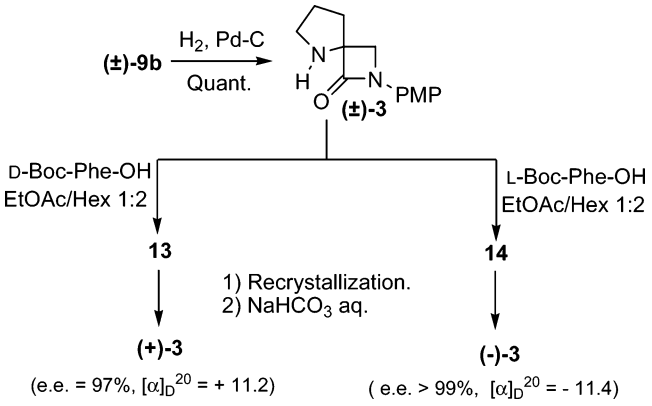


FIGURE 6. PM3-optimized structures of the Mosher amides **I** and **II**.

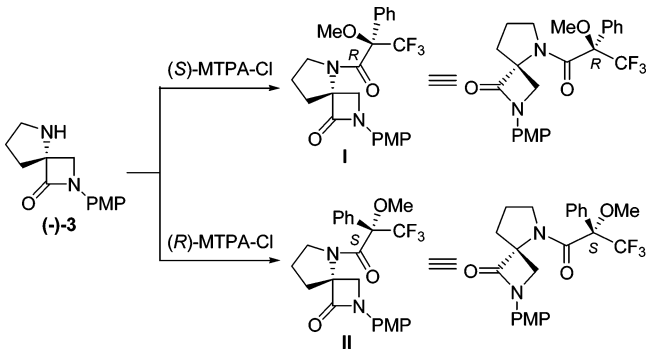
SCHEME 2. Synthesis of Spiro Analogs of *N*-sulfonyl-*β*-lactams, 9f



SCHEME 3. Optical Resolution of *β*-Lactam (±)-9b



SCHEME 4. Mosher's Amides, I and II of (−)-3



the corresponding Mosher's amides of (+)-**3** and (−)-**3**, as shown in Scheme 4.[11]

As the starting point, we optimized the geometry of the two diastereomeric amides **I** and **II**, using the PM3 method (see Figure 6),[12] assuming an (*R*) configuration for the stereocenter of the *β*-lactam (−)-**3**.

This model allowed us to tentatively establish what NOEs could be expected for each of the amides **I** and **II**, as a function of the configuration of the stereocenter of *β*-lactam (−)-**3**. The assignment of the ^{1}H signals in the spectra of the amides **I** and **II** was based on the basis of the analysis of the two-dimensional NMR experiments: COSY, HMQC, HMBC, and NOESY. The absolute stereochemistry of the quaternary center in *β*-lactam (−)-**3** was established from the data of the 2D NOESY spectra of amides **I** and **II**.

The 2D NOESY spectrum of amide **II** is shown in Figure 7. The most conclusive signal in the amide **II** (see Figure 7) is the correlation observed between the ortho hydrogens of the phenyl group coming from the Mosher moiety [H_{12}, $\delta = 7.56$ ppm, (d)] with one of the methylenic hydrogens (CH_2) of the *β*-lactam fragment [$H_{5\beta}$, $\delta = 4.48$ ppm, (d)], which implies that the phenyl ring and the *β*-lactam-CH_2, are in the same side. Here, we are using the α/*β* notation in order to differentiate the hydrogens placed bottom side (α-hydrogens) from the ones placed upper side (*β*-hydrogens).

In addition, $H_{5\beta}$ shows two cross-peaks with the hydrogens $H_{1\beta}$[$\delta = 2.77$ ppm, (ddd)] and $H_{2\beta}$ [$\delta = 1.63$ ppm, (ddd)]. From this result, and taking into account that the absolute stereochemistry of the Mosher fragment is known, we could assign the (*R*) absolute configuration to the stereogenic center of the *β*-lactam (−)-**3**.

The analysis of the 2D NOESY spectrum of the amide **I**, allowed us to confirm the assignment previously made. In this case, as it could be expected, it is not observed NOE between the phenyl ring and the CH_2 of the *β*-lactam moiety, because two fragments are oriented in opposite directions (see Figure 8).

It is interesting to note that the inversion on the absolute configuration of the stereogenic center of the Mosher reagent produces an inversion on the chemical shifts of the methylenic hydrogens of the carbon atom in the α position with respect to the nitrogen of the pyrrolidine ring (H_1), owing to the shielding effect exerted for the phenyl ring. Thus, the signals that in **II** appear at $\delta = 2.7$ ($H_{1\beta}$) and $\delta = 3.84$ ppm ($H_{1\alpha}$), now, in **I**, appear at $\delta = 3.47$ ($H_{1\beta}$) and $\delta = 2.80$ ppm ($H_{1\alpha}$). As can be seen in Figure 9, the phenyl ring of the Mosher reagent shields the hydrogen α in the pyrrolidine ring in **I** and the hydrogen *β* in the pyrrolidine ring in **II**.

Synthesis of the Peptidomimetic (−)-20. After obtaining the *β*-lactam (−)-**3** we proceeded to carry out the synthesis of

(11) a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1972**, *95*, 512. (b) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Am. Chem. Soc.* **1969**, *34*, 2543. (c) Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17.

(12) The PM3 method was used as implemented in Gaussian 98.

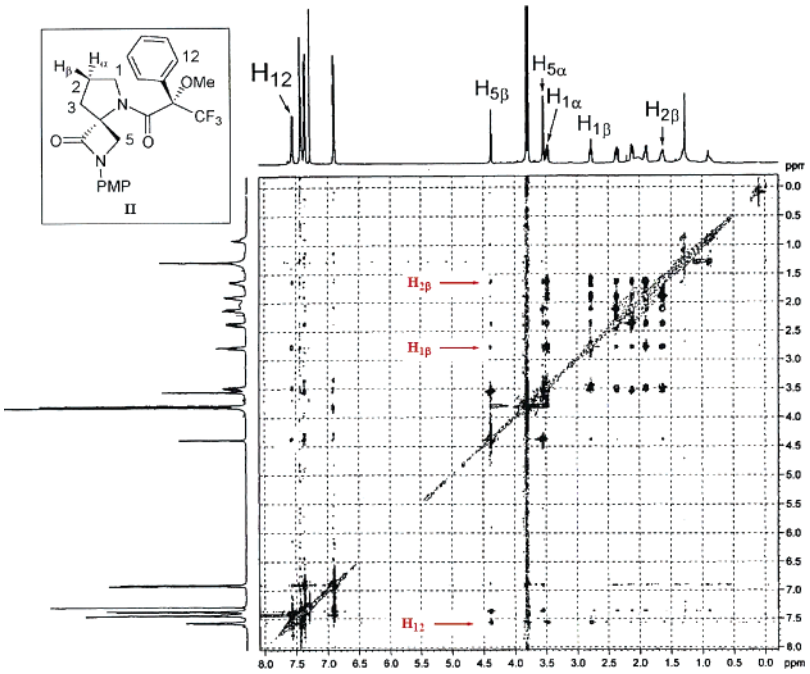


FIGURE 7. NOESY spectrum ($CDCl_3$) of the Mosher amide **II**.

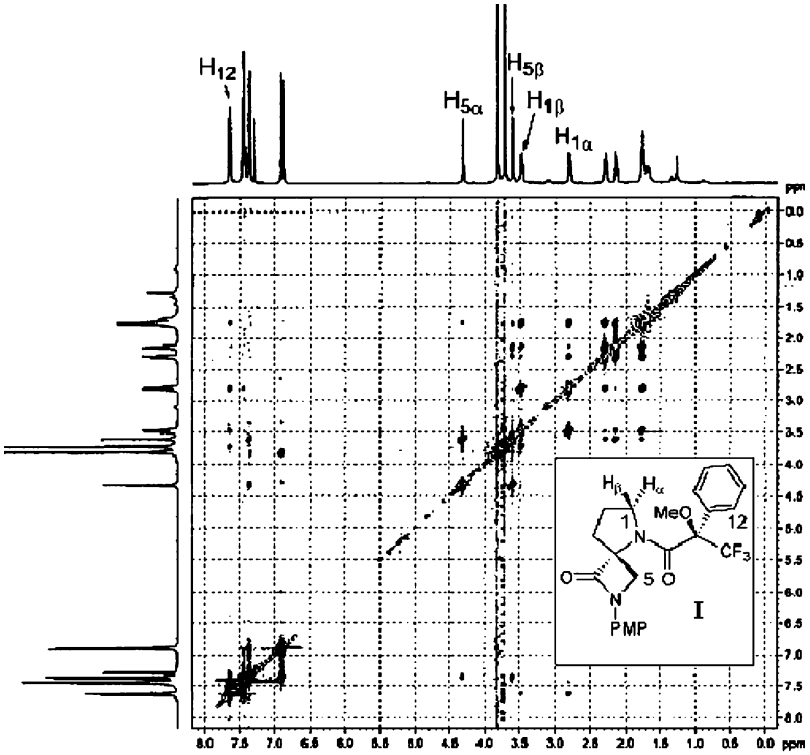


FIGURE 8. NOESY spectrum ($CDCl_3$) of the Mosher amide **I**.

(−)-**20**, the optically active analogue of melanostatin (see Figure 1), as described in Scheme 5.

After the coupling of the L-Boc-proline moiety, (−)-**15** was subjected to the oxidative removal of the PMP group. The reaction with benzyl bromoacetate and further hydrogenolysis gave the acid **18**, which was coupled with *O*-benzylglycine to introduce the side chain, affording (−)-**19**. The NMR study of (−)-**19** conducted in $CDCl_3$ showed that this compound is able, in solution, to adopt a type II β-turn conformation (Figure 10).

The structure of (−)-**19** was assigned through the analysis of the ^1H, ^{13}C, DEPT, COSY, HSQC, and HMBC spectra.^1H NMR spectrum of (−)-**19** showed an equilibrium mixture of rotamers about the carbamate bond. The ^1H NMR chemical shift of the NH_a amide proton ($\delta = 8.73$ and $\delta = 8.80$ ppm) is in the expected range for a hydrogen-bonded proton which is in agreement with the previously described theoretical studies and the correlations observed in the NOESY spectra (Figure 11).[4] Thus, the amide proton NH_a shows a correlation with proton

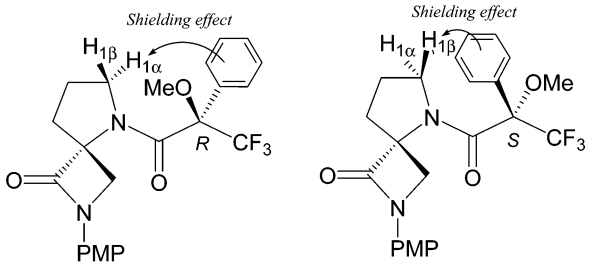


FIGURE 9. Opposite shielding effects of the phenyl ring on the H_1 hydrogens in **I** and **II**.

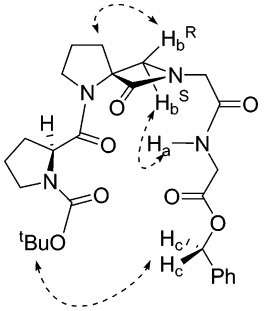
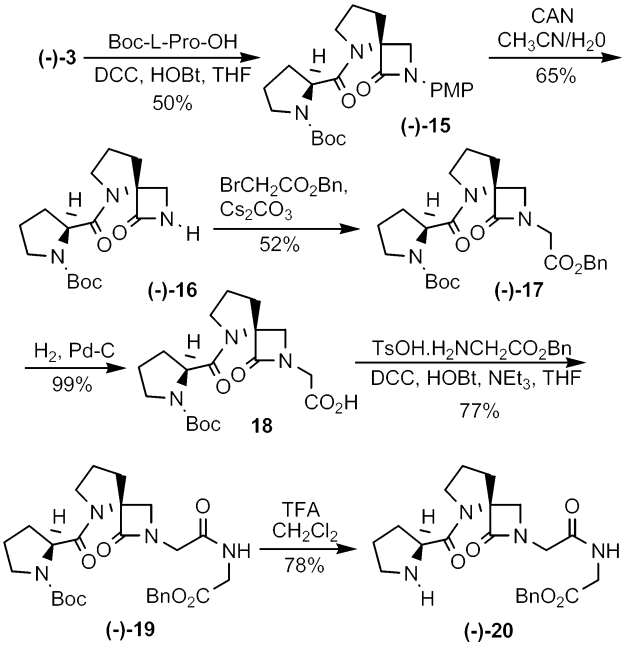


FIGURE 10. β-Turn conformation of (−)-**19** and NMR correlations.

SCHEME 5. Synthesis of the Melanostatin Analog(−)-20



H^S of β-lactam moiety at $\delta = 3.95$ and $\delta = 4.03$ ppm, suggesting the presence of a β-turned conformation. Moreover, the *tert*-butoxycarbonyl group ($\delta = 1.37$ and $\delta = 1.40$ ppm) correlates with methylene protons of benzyl group (H_c) at $\delta =$ 5.13 ppm, which is also consistent with a β-turn conformation (Figure 10).

Synthesis of the Oligo-β-peptide (−)-4c. As stated before, we want to demonstrate the utility of the optically active β-lactams **3** on the preparation of β-peptides **4** (Figure 2), because of the potential biological activity of these compounds.[7] The synthesis of β-peptides **4** (see Figure 2 and Scheme 6) was carried out with the standard techniques for peptide coupling.

It is interesting to note that the ring opening of the spiro β-lactam has to be carried out under mild conditions, as described previously,[13] using KCN in catalytic amounts to promote the β-lactam opening. The availability of the Boc-protected β-aminoester (+)-**10a** and the β-amino acid (+)-**10b**, allows us to proceed with the reiterative coupling, to obtain the desired tetra-β-peptide, (−)-**4c**.

In addition, we investigated the possibility of developing new peptidomimetics containing phosphorus or sulfur; thus, the geometry of compounds **22** and **23** was fully optimized (see Figure 12), and the results obtained indicate that these structures could be good starting points for developing new peptidomimetics, along the lines presented in the current work.

Conclusions

In summary, this research has established the utility of the optically pure spiro β-lactams for both the synthesis of optically active analogues of melanostatin, as (−)-**20**, and the new type of functionalized β-peptides **4**. The synthetic procedure developed allows us to obtain any of the two enantiomers of the critical intermediate **3** in a procedure that can be carried out in a multigram scale.

Experimental Section

Preparation of Hexahydro-1,3-5-triazines 11a−c. These compounds were prepared according to the procedure described by Hegedus and co-workers.[14] To a solution of the corresponding primary amine (60 mmol) in a mixture (150 mL) of ethyl acetate/water (1:1), cooled at 0 °C, an aqueous solution (5 mL) of formaldehyde (37%) is added. The reaction mixture is stirred for 2 h at 0 °C, and then the organic layer is separated, washed with water (50 mL), and dried with anhd Na_2SO_4. The solvent is removed under vacuum, and a white solid is obtained. This solid is washed once with diethyl ether. Following this procedure, the hexahydro-1,3-5-triazines **11a−c** (Scheme 1) were prepared.

General Procedure for the Preparation of Spiro β-Lactams 9a,c.[3,13] To a stirred solution of the *N*-benzyloxycarbonyl L-proline acid chloride (2 mmol) in dry dichloromethane (20 mL) cooled to −40 °C, was added dropwise dry triethylamine (0.41 mL, 3 mmol). The solution became yellow to confirm that the ketene was formed. After 45 min at low temperature, a purple solution of the triazine **11a,c** (0.67 mmol) and $BF_3{\cdot}OEt_2$ (0.25 mL, 2 mmol), previously mixed in CH_2Cl_2 (10 mL), was added dropwise. The mixture was allowed to warm slowly to room temperature overnight and then quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted twice with CH_2Cl_2 (15 mL); the combined organic layers were washed with brine (20 mL) and dried over anhydrous Na_2SO_4. The solution was then concentrated and purified by column chromatography over SiO_2 with the appropriated mixture of ethyl acetate/hexane.

(±)-2-Benzyl-5-benzyloxycarbonyl-2,5-diazaspiro[3,4]octan-1-one (9a). $R_f = 0.25$ (hexane/EtOAc 1:1), 350 mg of **9a**, 50% yield, white foam. ^1H and ^{13}C NMR show the presence of rotamers about the carbamate bond in 1:1 ratio. ^1H NMR (300 MHz, $CDCl_3$): δ 1.70−2.15 (m, 3H), 2.34 (m, 1H), 3.01, 3.06 (d, $J =$ 4.8; $J = 4.6$ Hz, 1H), 3.35−3.78 (m, 3H), 4.27−4.70 (m, 2H), 4.98−5.21 (m, 2H), 7.00−7.40 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.7, 23.3, 33.5, 34.8, 45.5, 45.8, 47.6, 48.3, 52.9, 54.1, 67.0, 67.7, 72.2, 73.0, 127.5, 127.6, 127.9, 128.0, 128.2, 128.4,

(13) Macías, A. Alonso, E.; Pozo, del C. Venturini, A.; González, J. *J. Org. Chem.* **2004**, *69*, 7004.
(14) Hegedus, L. S.; D'Andrea, S. *J. Org. Chem.* **1988**, *53*, 3113.
(15) Floy, D. M.; Fritz, W.; Pluscec, J.; Weaver, E. R.; Cimarusti, C. M. *J. Org. Chem.* **1982**, *47*, 5160.

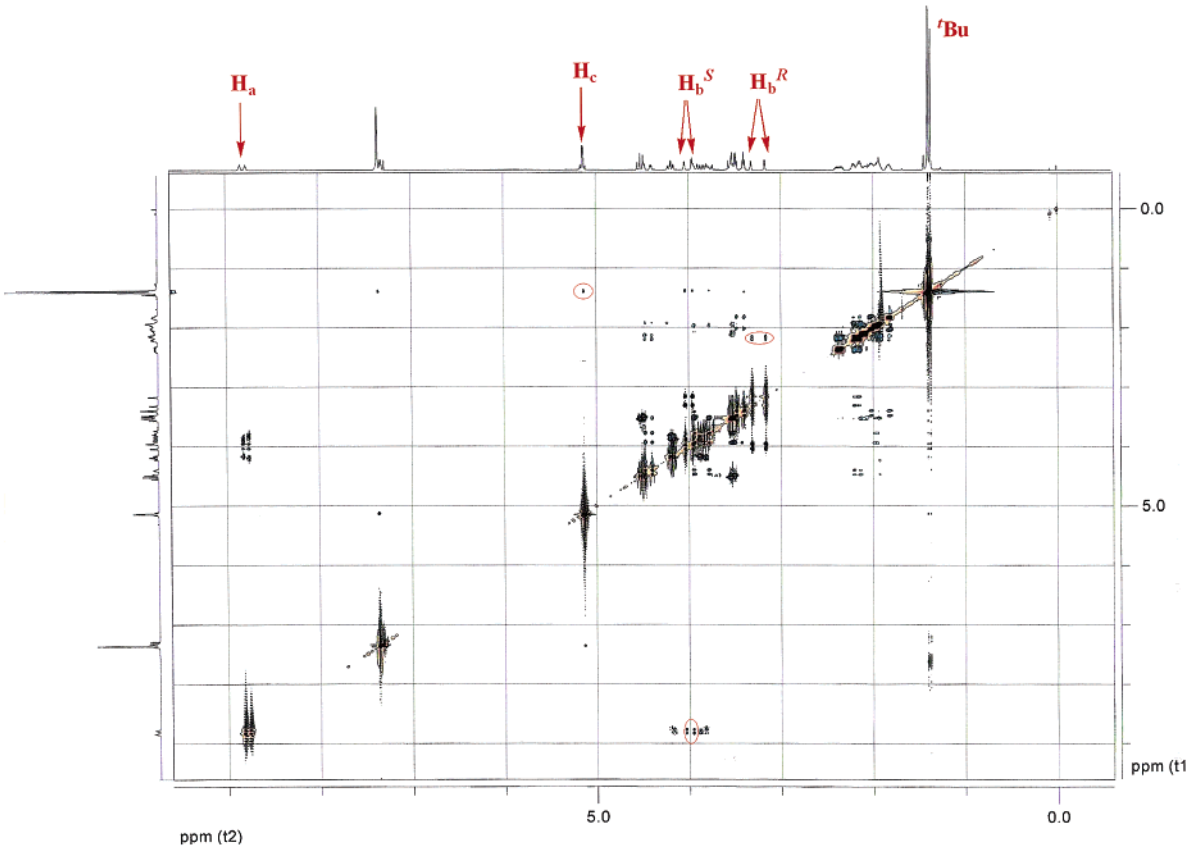
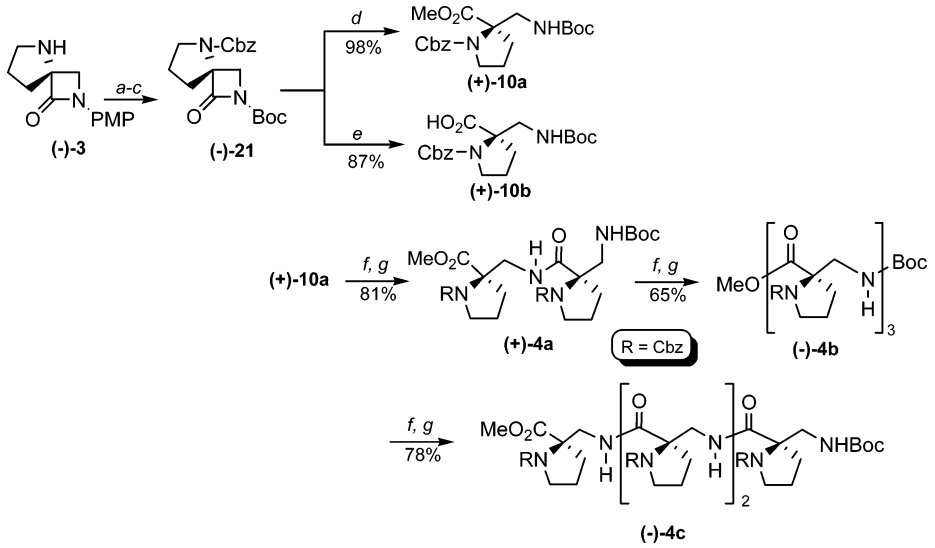


FIGURE 11. 2D NMR correlations observed in (−)-**19**.

SCHEME 6. Synthesis of β-Peptides 4[a]



[a] (a) $ClCO_2Bn$; (b) CAN; (c) $(Boc)_2O$; (d) KCN catalyst, MeOH; (e) NaOH 2 N, THF; (f) CF_3CO_2H, CH_2Cl_2, 0 °C; (g) (+)-**10b**, DCC, HOBt, THF, 0 °C to room temperature.

135.1, 135.3, 135.9, 136.2, 153.7, 153.9, 169.5, 169.6. IR (KBr): 1711, 1757 cm^{-1}. MS (ESI^+): *m/z* 389 (M + K), 373 (M + Na), 351 (M + H). Anal. Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99. Found: C, 71.79; H, 6.31; N, 7.97.

(4*RS*)-5-Benzyloxycarbonyl-2-[(*R*)-(methoxycarbonyl)(4-benzyloxyphenyl)methyl]-2,5-diazaspiro[3,4]octan-1-one (9c). The analogue of nocardicine **9c** was prepared from triazine **11c** (0.67 mmol) according to the general procedure described above to afford (257 mg, 25%) of **9c** as a 1:1 mixture of diastereoisomers that could not be separated by column chromatography. R_f = 0.20 (hexane/EtOAc 1:1), white foam. ^1H NMR (300 MHz, $CDCl_3$): δ 1.70−2.49 (m, 4H), 3.25−3.80 (m, 7H), 4.79−5.22 (m, 4H), 5.42, 5.68 (s, 1H), 6.80−7.49 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.6, 23.2, 33.7, 34.9, 47.6, 48.3, 52.4, 52.5, 53.7, 56.5, 56.7, 66.8, 67.2, 69.8, 71.2, 72.0, 115.0, 115.1, 124.8, 125.0, 127.2, 127.3, 127.4, 127.7, 127.9, 128.2, 128.3, 128.4, 129.2, 129.4, 136.1, 136.3, 136.5, 153.5, 153.7, 158.8, 169.0, 169.2, 170.1, 170.4. IR (KBr): 1705, 1744, 1765 cm^{-1}. MS (ESI^+): *m/z* 553 (M + K), 537 (M + Na),

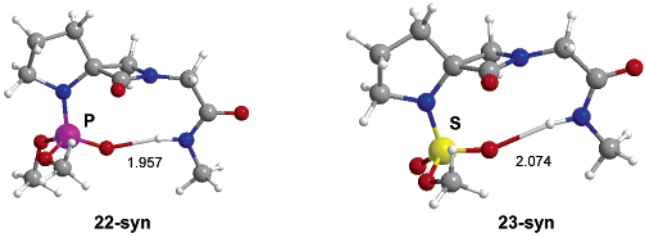


FIGURE 12. Becke3LYP/6-31+G* optimized structures, **22**- and **23-syn** (lengths are in Å).

515 (M + H). Anal. Calcd for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.20; H, 5.90; N, 5.42.

Modified Procedure for the Multigram Preparation of Spiro *β*-Lactam 9b. (±)-5-Benzyloxycarbonyl-2-(4-methoxyphenyl)-2,5-diazaspiro[3,4]octan-1-one (9b). To a stirred solution of the *N*-benzyloxycarbonyl L-proline acid chloride (20 mmol) in dry dichloromethane (70 mL) cooled to −40 °C, was added dropwise dry triethylamine (11.1 mL, 80 mmol). The solution became yellow to confirm that the ketene was formed. After 25 min at low temperature a purple solution of the triazine **11b** (6.6 mmol) and $BF_3{\cdot}OEt_2$ (2.51 mL, 20 mmol), previously mixed in CH_2Cl_2 (40 mL), was added dropwise. The mixture was allowed to warm slowly to room temperature overnight and then quenched with saturated aqueous HCl 1 N. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (1 × 20 mL); the combined organic layers were washed with brine (40 mL) and dried over anhydrous Na_2SO_4. The solution was then concentrated to give a red oil, which was dried under vaccum to eliminate the traces of dichloromethane. This red oil was then dissolved in methanol, and after several minutes, a white solid precipitated, and the mixture was stirred for 1 h; the solid was filtered and dried under vaccum. The product thus obtained (4.33 g, 59%) is pure enough to be used in the next step, or it can be purified by column chromatography over SiO_2 with the appropriated mixture of ethyl acetate/hexane. R_f = 0.35 (hexane/EtOAc 1:1), white solid, mp 95−97 °C. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in 1:1 ratio. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.80−2.10 (m, 2H), 2.23 (m, 1H), 2.45 (m, 1H), 3.42−4.18 (m, 7H), 4.91−5.18 (m, 2H), 6.70−7.40 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.8, 23.4, 33.7, 34.9, 47.6, 48.3, 52.5, 53.7, 55.4, 67.0, 67.7, 71.4, 72.1, 114.2, 117.6, 117.8, 127.7, 127.8, 127.9, 128.1, 131.6, 131.8, 135.1, 136.1, 153.7, 153.8, 155.9, 165.9, 166.0. IR (KBr): 1698, 1741 cm^{-1}. MS (ESI$^+$): *m*/*z* 405 (M + K), 389 (M + Na), 367 (M + H). Anal. Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 69.05; H, 6.03; N, 7.62.

General Procedure for Resolution of 9b with L- or D-*N*-Boc-Phenylalanine. General Procedure for the Cleavage of Cbz and Bn Groups. The corresponding *N*-Cbz or *O*-Bn substrate was dissolved in a 3:1 mixture of EtOAc/methanol and transferred via cannula to a flask under H_2 (1 atm) containing 20% weight of 10% Pd−C catalyst. The mixture was stirred overnight, the catalyst was filtered off on Celite, and the organic layer was concentrated in vacuo to afford the corresponding *N*H or *O*H compound.

(±)-2-(4-Methoxyphenyl)-2,5-diazaspiro[3,4]octan-1-one (±)-3. This compound was prepared according to the general procedure described above to remove the *N*-benzyloxycarbonyl group, using 10 mmol of the *N*-Cbz-*β*-lactam **9b**, to afford 2.28 g of (±)-**3**, in 98% yield as a white solid: mp 78−80 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.82−2.18 (m, 3H), 2.25 (m, 1H), 2.44 (broad s, 1H), 2.98 (m, 1H), 3.23 (m, 1H), 3.59 (d, *J* = 5.4 Hz, 1H), 3.67 (d, *J* = 5.4 Hz, 1H), 3.80 (s, 3H), 6.87 (m, 2H), 7.30 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.6, 32.5, 47.3, 55.4, 56.2, 74.1, 114.2, 117.7, 131.8, 156.0, 169.3. IR (KBr): 1731, 3263 cm^{-1}. MS (ESI$^+$): *m*/*z* 255 (M + Na), 233 (M + H). Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 67.30; H, 6.97; N, 12.03.

Resolution of (±)-3 with L- or D-*N*-Boc-Phenylalanine. To a stirred solution of *β*-lactam (±)-**3** (1.86 g, 8 mmol) in EtOAc/hexane 1:2 (30 mL) at 60 °C was added L- or D-*N*-Boc-phenylalanine (2.12 g, 8 mmol), and in a few minutes a white solid precipitated. To this solution, at 60 °C, was added a mixture hexane/EtOAc 1:2 (30 mL) and portions of EtOAc until the solid was dissolved completely. The solution was allowed to stand at room temperature for 24 h, and the resulting white crystals were filtered off and further recrystallized from hexane/EtOAc 1:2 and EtOAc, to afford the corresponding diastereomeric salt, **14** (yield 34%) or **13** (yield 32%), respectively.

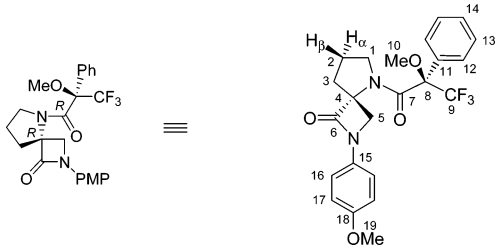
Synthesis of Optically Pure *β*-Lactams (−)-3 and (+)-3. Salt **14** or **13** (2 mmol) was dissolved in CH_2Cl_2 (25 mL), and saturated aqueous $NaHCO_3$ (25 mL) was added. The mixture was stirred for 10 min, and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to afford the optically active *β*-lactams (−)-**3** or (+)-**3**. The enantiomeric purity of this compounds was determined by HPLC (see SI).

(−)-(4*R*)-2-(4-Methoxyphenyl)-2,5-diazaspiro[3,4]octan-1-one (−)-3. Yield: 92%. $[\alpha]^{20}_D$ = −11.4 (c 1.0, $CHCl_3$); ee > 99%.

(+)−(4*S*)-2-(4-Methoxyphenyl)-2,5-diazaspiro[3,4]octan-1-one (+)-3. Yield: 90%. $[\alpha]^{20}_D$ = +11.2 (c 1.0, $CHCl_3$); ee = 97%.

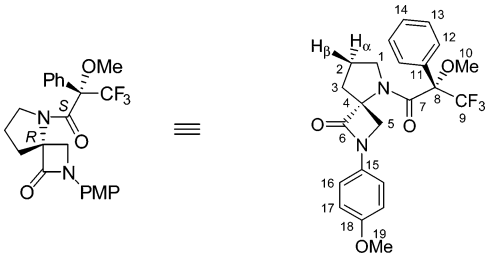
General Procedure for the Preparation of Mosher's Amides I and II. To a stirred solution of *β*-lactam (−)-**3** (0.05 mmol), in dry CH_2Cl_2 (2 mL), cooled to 0 °C, was added triethylamine (0.075 mmol) and the (*R*)- or (*S*)-MTPA-Cl (0.06 mmol). The mixture was allowed to warm to room temperature overnight and then quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted twice with CH_2Cl_2 (10 mL), and the combined organic layers were washed with brine (15 mL) and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to afford the diastereoisomeric Mosher's amides **(I** and **II)** which were studied by NMR to determine the absolute configuration of compound (−)-**3**.

Assignment of ^{1}H NMR Spectrum of Mosher's Amide (*R*,*R*)-I. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.76 (m, 2H, H2), 2.13 (dt, *J* = 13.1, *J* = 6.5 Hz, 1H, H3*β*), 2.30 (dt, *J* = 13.7, *J* = 7.1 Hz, 1H, H3α), 2.80 (dt, *J* = 11.2, *J* = 6.7 Hz, 1H, H1α), 3.47 (dt, *J* = 11.5, *J* = 6.4 Hz, 1H, H1*β*), 3.60 (d, *J* = 4.8 Hz, 1H, H5α), 3.74 (s, 3H, H10), 3.80 (s, 3H, H19), 4.32 (d, *J* = 4.8 Hz, 1H, H5*β*), 6.89 (d, *J* = 8.9 Hz, 2H, H17), 7.37 (d, *J* = 8.9 Hz, 2H, H16), 7.47 (m, 3H, H13,14), 7.63 (d, *J* = 6.9 Hz, 2H, H12).



Assignment of ^{1}H NMR Spectrum of Mosher's Amide (*R*,*S*)-II. ^{1}H NMR (500 MHz, $CDCl_3$): 1.63 (m, 1H, H2*β*), 1.89 (m, 1H, H2α), 2.11 (ddd, *J* = 10.2, *J* = 6.1, *J* = 4.4 Hz, 1H, H3*β*), 2.36 (ddd, *J* = 13.1, *J* = 10.5, *J* = 6.4 Hz, 1H, H3α), 2.77 (ddd, *J* = 11.1, *J* = 7.2, *J* = 3.8 Hz, 1H, H1*β*), 3.48 (ddd, *J* = 11.1, *J* = 9.1, *J* = 6.4 Hz, 1H, H1α), 3.54 (d, *J* = 4.7 Hz, 1H, H5α), 3.78 (s, 3H, H10), 3.82 (s, 3H, H19), 4.38 (d, *J* = 4.7 Hz, 1H, H5*β*), 6.91 (d, *J* = 9.0 Hz, 2H, H17), 7.36 (d, *J* = 9.0 Hz, 2H, H16), 7.43 (m, 3H, H13,14), 7.56 (m, 2H, H12).

Synthesis of *β*-Peptides 4. (−)-(4*R*)-5-Benzyloxycarbonyl-2-(4-methoxyphenyl)-2,5-diazaspiro[3,4]octan-1-one (−)-9b. To a stirred solution of the *β*-lactam (−)-**3** (4 mmol) in EtOAc/H_2O 1:1 (20 mL) at room temperature, $NaHCO_3$ (0.672 g, 8 mmol) and benzyl chloroformate (0.67 mL, 4.4 mmol) were added. The reaction was stirred vigorously for 2 h, and the aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (20 mL) and dried over anhydrous Na_2SO_4. The



solution was then concentrated and purified through column chromatography (hexane/EtOAc 2:1), to afford 1.42 g of (−)-**9b**, in 97% yield: white solid; $[\alpha]^{20}_D = -5.2$ (c 1.0, $CHCl_3$).

Synthesis of the *N*-Boc-*β*-Lactam (−)-21. (−)-(4*R*)-5-Benzyloxycarbonyl-2-(*tert*-butoxycarbonyl)-2,5-diazaspiro[3,4]octan-1-one (−)-21. Oxidative removal of *p*-methoxyphenyl group of *β*-lactam (−)-**9b** (1.39 g, 3.8 mmol) according to the general procedure described above afforded the N-unsubstituted derivative **9e** (see Scheme S1) which was employed in the next step without further purification. To a stirred solution of the N-unsubstituted-*β*-lactam **9e** (2.6 mmol), in acetonitrile (20 mL) cooled to 0 °C, di-*tert*butydicarbonate (5.2 mmol) and a catalytic amount of DMAP were added, and the mixture was stirred at room temperature overnight. Then, methylene chloride (40 mL) was added, and the mixture was washed with 1 M $KHSO_4$ (20 mL) and brine. The organic layer was dried over Na_2SO_4, and the solvent was removed in vacuo. The resulting crude was purified by column chromatography (hexane/EtOAc 2:1) to afford 0.652 g of (−)-**21** in 66% yield (two steps): colorless oil; $[\alpha]^{20}_D = -5.9$ (c 0.5, $CHCl_3$); $R_f = 0.20$ (hexane/EOAc 3:1). ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in ≈ 1.5:1 ratio. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39, 1.49 (s, 9H), 1.75−2.00 (m, 2H), 2.09−2.22 (m, 1H), 2.28−2.41 (m, 1H), 3.40−3.58 (m, 3H), 3.75, 4.03 (d, $J = 6.3$; $J = 6.5$ Hz, 1H), 4.99−5.19 (m, 2H), 7.21−7.35 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.7, 23.4, 27.7, 27.8, 33.9, 35.2, 47.4, 48.2, 51.7, 53.1, 67.1, 67.8, 71.3, 72.1, 82.9, 127.7, 127.8, 127.9, 128.0, 128.3, 135.2, 135.8, 147.7, 148.0, 153.3, 153.6, 166.9, 167.1. IR (KBr): 1694, 1731, 1822 cm^{-1}. MS (ESI$^+$): *m*/*z* 399 (M + K), 383 (M + Na). Anal. Calcd for $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.77. Found: C, 63.43; H, 6.69; N, 7.80.

Synthesis of α,α-disubstituted *β*-amino ester (+)-10a. Methyl (+)−-(2*R*)-2-[1-benzyloxycarbonylpyrrolidin-2-yl]-3-(*N*-*tert*-butoxycarbonylamino)propaneate (+)-10a. Prepared according to the published procedure,[5] using 1.2 mmol of the *N*-Boc-*β*-lactam (−)-**21**, to afford 461 mg of (+)-**10a** in 98% yield as a colorless oil: $[\alpha]^{20}_D = +45.8$ (c 0.5, $CHCl_3$); $R_f = 0.70$ (hexane/EtOAc 1:1). ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in ≈ 1.5:1 ratio. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H), 1.83−2.25 (m, 4H), 3.36−3.74 (m, 7H), 4.90−5.19 (m, 2H), 5.36 (m, 1H), 7.30 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.6, 23.1, 28.2, 34.1, 35.0, 43.2, 43.4, 48.0, 48.8, 52.1, 52.3, 66.8, 67.2, 67.9, 68.9, 79.1, 79.3, 127.6, 127.9, 128.1, 128.3, 128.4, 135.8, 136.4, 153.8, 154.7, 156.2, 156.4, 173.7, 174.0. IR (KBr): 1694, 1730, 3362 cm^{-1}. MS (ESI$^+$): *m*/*z* 393 (M + H). Anal. Calcd for $C_{20}H_{28}N_2O_6$: C, 61.21; H, 7.19; N, 7.14. Found: C, 61.30; H, 7.18; N, 7.16.

Synthesis of α,α-Disubstituted *β*-Amino Acid (+)-10b. (+)-(2*R*)-2-[1-Benzyloxycarbonylpyrrolidin-2-yl]-3-(*N*-*tert*-butoxycarbonylamino)propanoic acid (+)-10b. To a stirred solution of *N*-Boc *β*-lactam (−)-**21** (1.2 mmol) in THF (3 mL), was added a 2 N aqueous solution of NaOH (15 mL), and the mixture was stirred overnight. The tetrahydrofuran was removed in vacuo and the aqueous phase was acidified (pH 2) with 1 N HCl (aq) and extracted twice with CH_2Cl_2 (20 mL). The combined organic layers were washed with brine (20 mL) and dried over anhydrous Na_2SO_4. The solution was then concentrated and purified through column chromatography to afford 395 mg of (+)-**10b** in 87% yield: white solid; mp 112−114 °C; $[\alpha]^{20}_D = +43.4$ (c 0.5, $CHCl_3$); $R_f = 0.20$ (EtOAc). ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in ≈ 3:1 ratio. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H), 1.90−2.27 (m, 4H), 3.46−3.85 (m, 4H), 5.09−5.20 (m, 2H), 5.38 (m, 1H), 7.35 (m, 5H), 9.00 (broad s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.5, 23.1, 28.2, 34.1, 35.2, 42.9, 43.2, 48.2, 48.9, 67.2, 67.4, 67.9, 68.7, 79.4, 80.8, 127.6, 127.8, 127.9, 128.3, 135.8, 136.2, 154.1, 155.1, 156.3, 156.5, 177.3, 178.1. IR (KBr): 1715, 3398 cm^{-1}. MS (ESI$^+$): *m*/*z* 417 (M + K), 401 (M + Na), 379 (M + H). Anal. Calcd for $C_{19}H_{26}N_2O_6$: C, 60.30; H, 6.93; N, 7.40. Found: C, 60.11; H, 6.92; N, 7.43.

Synthesis of (+)-(*R*,*R*)-*β*-Dipeptide (+)-4a. To a solution of the *N*-Boc *β*-aminoester (+)-**10a** (0.392 g, 1 mmol) in CH_2Cl_2 (5 mL) cooled to 0 °C was added TFA (1 mL), and the mixture was stirred until total disappearance of the starting material (2 h, monitored by TLC). The solution was evaporated and the residue was dissolved in CH_2Cl_2 (15 mL). Saturated aqueous $NaHCO_3$ (15 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were washed with brine (25 mL) and dried over anhydrous Na_2SO_4 to afford the NH_2 *β*-aminoester which was employed in the next step without further purification. To a stirred solution of this compound in dry THF (5 mL) cooled to 0 °C were added HOBt (153 mg, 1 mmol), *β*-amino acid (+)-**10b** (378 mg, 1 mmol), and dicyclohexylcarbodiimide (DCC) (206 mg, 1.05 mmol). The mixture was allowed to warm to room temperature overnight. After this time, the solution was filtered and the solid (*N*,*N*-dicyclohexylurea) discarded. The solvent was evaporated, and the residue was dissolved in EtOAc (20 mL). The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (15 mL), HCl (aq) 1 N, and brine (15 mL). The organic layer was dried over anhydrous Na_2SO_4. The solution was then concentrated and purified through column chromatography (hexane/EtOAc 1:1) to afford 0.529 g of *β*-dipeptide (+)-**4a** in 81% yield: white solid; mp 54−57 °C; $[\alpha]^{20}_D = +7.4$ (c 0.7, $CHCl_3$); $R_f = 0.20$ (hexane/EtOAc 1:1). ^{1}H and ^{13}C NMR show the presence of rotamers (see Figure S10). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 9H), 1.60−2.31 (m, 8H), 3.30−3.92 (m, 11H), 4.90−5.35 (m, 4H), 5.58 (m, 1H), 7.12, 7.49 (d, $J = 7.1$; $J = 6.3$ Hz, 1H), 7.22−7.40 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.5, 23.1, 24.8, 25.5, 28.2, 34.4, 34.9, 41.9, 42.4, 43.1, 43.4, 43.6, 48.1, 48.3, 48.8, 48.9, 51.9, 52.3, 66.8, 66.9, 68.3, 68.6, 69.6, 78.9, 79.0, 127.4, 127.6, 127.9, 128.1, 128.3, 136.0, 136.2, 136.4, 153.7, 154.3, 154.4, 154.9, 155.1, 156.4, 156.8, 173.7, 173.8. IR (KBr): 1705, 3330, 3437 cm^{-1}. MS (ESI$^+$): *m*/*z* 675 (M + Na), 653 (M + H). Anal. Calcd for $C_{34}H_{44}N_4O_9$: C, 62.56; H, 6.79; N, 8.58. Found: C, 62.69; H, 6.77; N, 7.45.

(−)-(*R*,*R*,*R*)-*β*-Tripeptide (−)-4b. The *tert*-butoxycarbonyl group of *β*-dipeptide (−)-**4a** (196 mg, 0.3 mmol) was removed according to the general procedure to afford the corresponding NH_2 *β*-dipeptide, which was employed in the next step without further purification. The coupling reaction of this compound with *β*-amino acid (+)-**10b** (113 mg, 0.3 mmol) according to the general procedure described above afforded, after column chromatography, 178 mg of *β*-tripeptide (−)-**4b** in 65% yield: white foam; $[\alpha]^{20}_D = -19.9$ (c 0.3, $CHCl_3$); $R_f = 0.20$ (hexane/EtOAc 1:2). ^{1}H and ^{13}C NMR show the presence of rotamers (see Figure S11). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05−2.30 (m, 21H), 3.28−3.90 (m, 15H), 4.91−5.25 (m, 6H), 5.30, 5.58 (m, 1H), 6.92, 7.65 (m, 1H), 7.05−7.50 (m, 16H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.7, 23.0, 23.3, 25.0, 25.6, 28.4, 29.4, 29.7, 34.0, 34.7, 35.2, 35.4, 36.5, 42.0, 42.2, 42.8, 43.6, 43.9, 48.2, 48.4, 48.8, 49.1, 52.1, 52.5, 67.1, 67.6, 67.9, 68.4, 68.5, 68.6, 68.9, 69.7, 79.0, 127.6, 127.8, 128.0, 128.5, 136.4, 136.6, 153.8, 154.3, 154.4, 155.1, 156.6, 156.9, 173.9, 174.6, 175.0. IR (KBr): 1701, 3417 cm^{-1}. MS (EI): *m*/*z* 913 (M). Anal. Calcd for $C_{48}H_{60}N_6O_{12}$: C, 63.14; H, 6.62; N, 9.20. Found: C, 63.00; H, 6.79; N, 7.43.

Synthesis of (−)-(*R,R,R,R*)-*β*-Tetrapeptide (−)-4c. The *tert*-butoxycarbonyl group of *β*-tripeptide (−)-**4b** (183 mg, 0.2 mmol) was removed according to the general procedure to afford the corresponding NH_2 *β*-tripeptide, which was employed in the next step without further purification. The coupling reaction of this compound with *β*-amino acid (+)-**10b** (76 mg, 0.2 mmol) according to the general procedure described above afforded, after column chromatography, 183 mg of *β*-tetrapeptide **(−)-4c** in 78% yield: white foam; $[\alpha]^{20}_D = -12.6$ (c 0.3, $CHCl_3$); $R_f = 0.20$ (hexane/EtOAc 1:2). ^1H and ^{13}C NMR show the presence of rotamers (see Figure S12). ^1H NMR (300 MHz, $CDCl_3$): δ 1.22, 1.40 (s, 9H), 1.50−2.33 (m, 16H), 3.25−3.87 (m, 19H), 4.80−5.29 (m, 8H), 5.31, 5.58 (m, 1H), 6.90−7.80 (m, 23H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.2, 22.3, 22.7, 23.0, 23.3, 28.4, 29.7, 34.7, 35.0, 35.2, 35.4, 36.5, 36.8, 42.1, 42.2, 42.8, 43.1, 43.6, 44.0, 48.2, 48.4, 49.0, 52.1, 52.3, 52.5, 67.1, 67.2, 67.5, 67.6, 67.9, 68.1, 68.3, 68.4, 68.9, 70.1, 78.9, 127.6, 127.7, 127.8, 128.0, 128.4, 128.5, 128.6, 135.9, 136.1, 136.2, 136.4, 136.5, 136.6, 153.8, 154.1, 154.2, 154.4, 155.2, 156.6, 173.3, 173.9, 174.7, 175.0. IR (KBr): 1705, 3416 cm^{-1}. MS (ESI^+): *m*/*z* 1196 (M + Na), 1174 (M + H). Anal. Calcd for $C_{62}H_{76}N_8O_{15}$: C, 63.47; H, 6.53; N, 9.55. Found: C, 63.59; H, 6.51; N, 9.52.

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Supporting Information Available: Experimental procedures and computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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