Spiro β-Lactams as β-Turn Mimetics. Design, Synthesis, and NMR Conformational Analysis

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Molecular modeling calculations using high-level ab initio methods (MP2/6-31+G*) of a new type of spiro β-lactams predict that these systems could adopt a β-turn secondary structure in solution. Strong intramolecular hydrogen bonds stabilize the β-turn conformation with a geometry that is very close to the ideal type II β-turns. The synthesis of the spiro β-lactams is achieved by Staudinger reaction of a cyclic ketene derived from *N*-bencyloxycarbonyl-L-proline acid chloride with an imine. This reaction allows the formation of the spiranic backbone in a single-step with high diastereoselectivity and good yields. The new spiro β-lactams obtained are the core for the preparation of different types of peptidomimetics using well-established peptide chemistry. The NMR conformational analysis shows that these compounds adopt β-turn conformation as predicted by the theoretical studies.

Introduction

In recent years many efforts have been directed to construct peptidomimetics that display more favorable pharmacological properties than the natural peptides.[1] In this sense, the design and synthesis of conformationally restricted peptidomimetics is an important approach toward improving the potency, selectivity, and metabolic stability of peptide-based drugs. On the other hand, structural constrained peptidomimetics have also found other applications, like the determination of the bioactive conformation of active peptides in biochemistry.[2] More recently, small peptides with a well-defined secondary structure have been employed as catalysts in asymmetric synthesis,[3] in a biomimetic approach to an enzymatic catalytic center.

Herein we report the theoretical design (using high level ab initio methods), synthesis and NMR conformational analysis of new spiro β-lactams, compounds that fold into β-turn like structure.

Among numerous strategies developed for the construction of β-turn mimics, the incorporation into a peptidic backbone either a "Freidinger" γ-lactam[4] structure **1** or a spiro system[5] **2** (Figure 1) has proven to be useful in the design of a variety of medicinally relevant targets. In addition, it has been recently reported that the use of α,α-disubstituted β-lactams **3** could also be a good approach to promote a β-turn folding in a peptidic chain.[6] At this point, we thought that the combination of the structural motifs of compounds **2** and **3** in a spiro β-lactam **4**, could be a very useful tool in the design of new β-turn mimetics, due to their special structural features: 1) the high rigidity of the spiro backbone which simultaneously restricts two (Φ_{i+1}, Ψ_{i+1}) of the four torsion angles, (2) the presence of proline in the ***i***+1 position of the β-turn mimic (proline is the preferred amino acid at the ***i***+1 position in most common protein

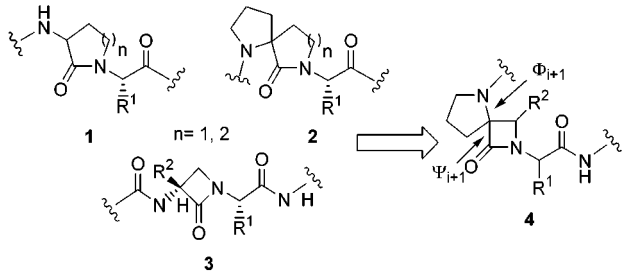

Figure 1. Previously described structural approaches to β-turns (**1**, **2**, **3**) and the combination of two of them in a spiro β-lactam (**4**).

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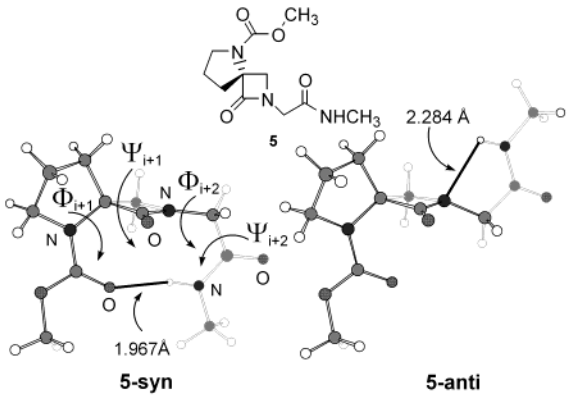


Φ_{i+1}= -44.8 (-60); Ψ_{i+1}= 126.5 (120); Φ_{i+2}= 104.1 (80); Ψ_{i+2}= -16.7 (0)

Figure 2. MP2/6-31+G* optimized syn and anti conformations of the model β-turn mimetic **5**. Dihedral angles obtained from the model geometry optimization (the ideal type II β-turn angles are in parentheses).

β-turns),[7] thus facilitating the recognition of these peptidomimetics by peptide receptors, and (3) they possess a quaternary center at the Cα position of the proline residue, which should increase the stability of these compounds toward the hydrolysis by proteases.

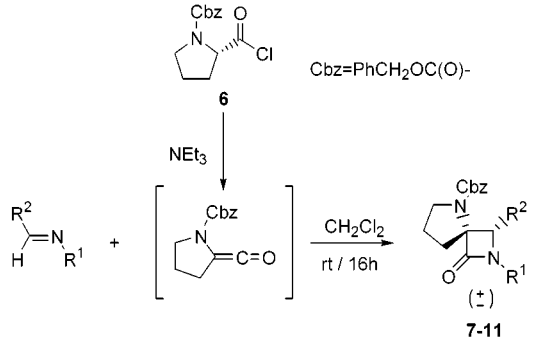
Results and Discussion

Molecular Modeling. Molecular mechanics and molecular dynamics are the conventional tools used for modeling the β-turn mimics.[5c,d] This is a very convenient approach from the computational point of view especially when the molecular size of the system under study is relatively large. However, the major drawback of these methodology is the heavy dependence of the reliability of the results obtained on the quality of the force field used. Therefore, we decided to use ab initio[8,9] methods in the design of the β-turn mimics, despite of their increased computational cost. As the hydrogen bond plays a very important role on these structures we carried out our calculations at the correlated level (MP2/6-31+G*). As a model compound for the calculations, we used the spiro β-lactam **5** (see Figure 2), with absolute configuration (*R*) on the quaternary center. Full geometry optimization of the model system **5** at the MP2/6-31+G* level of theory afforded two minima (see Figure 2): **5-syn**, showing a short (1.967 Å) intramolecular hydrogen-bond and **5-anti**, the non hydrogen-bonded structure (see Figure 2). The energy difference between **5-syn** and **5-anti** exceeds 5 kcal mol^{-1}, which implies that the model designed spiro β-lactams exhibits a strong preference for the intramolecular hydrogen-bonded conformation (**5-syn**).

Moreover, the geometry of **5-syn** shows dihedral angles, which are in good agreement with those expected for an ideal type II β-turn structure (Figure 2). The value of the angle formed for the atoms involved in the intramolecular hydrogen bond (the hydrogen of the amido group and the oxygen of the carbamate) is the one expected for an ideal type II β-turn (160°).[7] The analysis of the geometry of the two conformers of **5** also indicates that the nitrogen of the β-lactam in the less stable **5-anti** isomer is significantly pyramidalized. Thus, the dihedral angle formed by the side-chain carbon atom, the nitrogen, and the two carbon atoms of the ring is 142°, while in **5-syn**, the substituent attached to the β-lactam nitrogen lies on the plane defined by the heterocycle (dihedral angle = 176°). Furthermore, in **5-anti** the hydrogen atom bonded to the nitrogen of the side-chain is pointing to the pyramidal nitrogen of the β-lactam ring with a N···H distance of 2.284 Å, consistent with the existence of a weak hydrogen bond between both atoms. However, the pyramidalization of the β-lactam nitrogen decreases the contribution of the nitrogen lone pair to the amide resonance (the N−C bond length in **5-anti** is 0.012 Å longer compared to the N−C bond length in **5-syn**) making this conformation less stable.

In summary, the results of the modeling process carried out allow to conclude that spiro β-lactams **5** combine the structural features required for inducing a β-turn motif. The next step would be the validation of the initial hypothesis on the bench laboratory.

Scheme 1. Synthesis of Spiro β-lactams



Synthesis of Spiro β-Lactams and β-Turn Mimics. With these promising theoretical results in hand, a synthetic route to a series of spiro β-lactams was planned. In a previous work from our laboratory, we synthesized some spiro β-lactams through a Staudinger reaction of unsymmetrical cyclic ketenes.[10] For the application of this strategy to the present case we have selected the *N*-benzyloxycarbonyl-L-proline acid chloride **6** as precursor of the ketene involved in the Staudinger reaction with several imines (Scheme 1). The result would be the one-step synthesis of the desired spiro β-lactam backbone **4**. The synthesis was achieved by addition of the acid chloride **6** to a solution of the imine and triethylamine, in dichloromethane, at room temperature; the in situ generated cyclic ketene reacts with the imine to give, after aqueous workup and chromatographic purification, the spiro β-lactams **7**−**11** (Table 1). Good yields were

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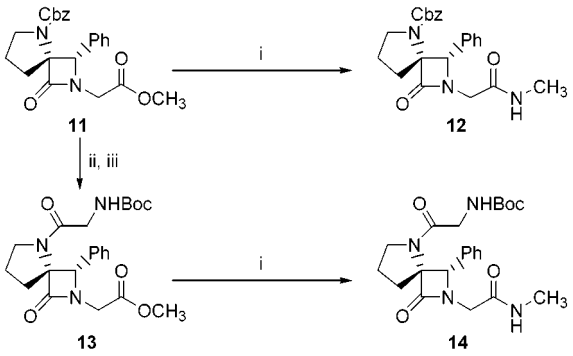
(10) Alonso, E.; Del Pozo, C.; Gonzalez, J. ***Abstracts of Papers, 219th National Meeting of the American Chemical Society, San Francisco, CA***; American Chemical Society: Washington, DC, 2000; ORGN 839.

Table 1. Preparation of Spiro β-lactams 7−11

	R^1	R^2	yield[a]
7	CH_3-	C_6H_5-	78%
8	p-$CH_3OC_6H_4-$	C_6H_5-	83%
9	p-$CH_3OC_6H_4-$	2-Furyl	66%
10	p-$CH_3OC_6H_4-$	$C_6H_5CH{=}CH-$	63%
11	$CH_3OC(O)CH_2-$	C_6H_5-	77%

[a] Yields after chromatographic purification.

Scheme 2[a]



[a] (i) H_3NCH_3Cl, NEt_3, MeOH, 7 h, >88% (ii) H_2, 1 atm, Pd−C, AcOEt/MeOH, 12 h; (iii) $BocNHCH_2(O)COCO_2Et$ (mixed anhydride), $CHCl_3$/Toluene 2 h, 50 °C, 77%.

obtained in all cases and only one diastereoisomer[11] was detected in the proton spectra of the crude reaction mixtures.[12]

To determine the ability of these compounds to adopt a folded β-turn pattern, we have synthesized the amides **12** and **14** by aminolysis of the methyl esters **11** and **13**, respectively, with an excess of methylamine in methanol (Scheme 2).

NMR Conformational Analysis. The NMR spectra of **14** showed the presence of only one species in $CDCl_3$ solution.[13] To our delight, the NOESY spectrum allowed to establish the configuration of the chiral centers and the conformation of the molecule (Figure 3). Thus, the correlation of the ortho protons of the phenyl ring with protons of both glycinamide moieties of **14** indicated that the three substituents of the spiranic system are arranged on the same side of the molecule. Furthermore, the glycinamide sidearms are spatially close as deduced from the interresidue correlation between the carbamate NH with the methyl protons.[14]

Further insight into the conformational preferences of **14** was obtained from the solvent[15] and temperature[16] chemical shift dependence of the NH protons. When using DMSO-d_6 as solvent instead of $CDCl_3$, a downfield shift of both the amide and carbamate NH protons ($\Delta\delta$= 0.59 and 1.28 ppm, respectively) is observed. In DMSO-d_6 the temperature coefficient ($\Delta\delta/\Delta T$) measured were −4 ppb/K for the amide proton and −9 ppb/K for the carbamate NH (Figure 4), whereas in $CDCl_3$ the respective values obtained were −5.3 ppb/K and −2.5 ppb/K.[17] According to the classification of hydrogen-bonding types described by Scolastico et al.[18] the figures obtained for the amide proton in $CDCl_3$ are characteristic of a peptide backbone hydrogen bonded, which is in equilibrium with a non-hydrogen-bonded state, while the data for the carbamate NH are in the expected range for a non-hydrogen-bonded proton. In DMSO-d_6 the results obtained from temperature coefficient ($\Delta\delta$ /ΔT) for both NH, their chemical shift values, and in addition the presence of rotamers in a 1:1 ratio, clearly indicate that in this solvent the amide hydrogen bond is broken and both NH amide and carbamate are solvent exposed.

The spiro β-lactam **12** exhibited an equilibrium mixture of rotamers about the carbamate bond either in deuteriochloroform or DMSO.[19] The NOESY spectrum measured in $CDCl_3$, showed the expected exchange cross-peaks between both rotamers. The correlation of the methine proton with the *pro-S* proton of the methylene group linked to the spiranic carbon indicated that the stereogenic centers of **12** have the same relative configuration than β-lactam **14**. Moreover, intramolecular hydrogen bonding in the major isomer is supported by the correlation of the *N*-methyl group with the methylene protons of the benzylic moiety.[20] These observations suggest the existence of an intramolecular hydrogen bond in the spiro β-lactams **12** and **14**, which is in agreement with the minimum energy calculated structure for the model compound **5-syn**.

Conclusions

In summary, we have described the rational design of novel type of β-turn mimetics employing high-level ab initio calculations. The spiro β-lactam backbone can be easily obtained from readily available starting materials with excellent stereoselectivity and good yields. The simple synthesis of these compounds makes them good candidates for the development of new peptidomimetics. NMR conformational studies indicate that these compounds adopt β-turn conformation. This methodology allows the construction of a wide variety of this type of peptidomimetics, just by combining different imines and cyclic ketenes. The effect of these modifications on the structural behavior and the possibility of obtain these compounds in their enantiopure form is currently underway.

Experimental Section

General. All melting points are uncorrected. Anhydrous methylene chloride was refluxed over phosphorus pentoxide and distilled immediately prior to use. Anhydrous ether was

(11) The relative configuration of the stereogenic centers of the compounds **7**−**11** was deduced from the corresponding NOESY spectra. In all cases the methine proton of the β-lactam showed a correlation with one proton of the methylene group linked to the spiranic carbon.

(12) This high steroselectivity was already observed with other amino ketenes in the Staudinger reaction. See: Georg, G. I.; Ravikumar, V. T. In *The Organic Chemistry of β-Lactams*; Georg, G. I., Ed.; VCH: New York, 1992; p 295.

(13) The structure of **12** and **14** was assigned through the analysis of the ^1H, ^{13}C, DEPT, HMQC, and HMBC spectra.

(14) The width of the amide proton signal was too large (short T_2) to observe any NOE correlation.

(15) (a) Pitner, T. P.; Urry, D. W. *J. Am. Chem. Soc.* **1972**, *94*, 1399. (b) Liang, B.; Rito, C. J.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 4440.

(16) (a) Kopple, K. D.; Ohnishi, M.; Go, A. *J. Am. Chem. Soc.* **1969**, *91*, 4264. (b) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512.

(17) The temperature coefficient for the amide proton in $CDCl_3$ has been calculated from the chemical shift difference of the signal measured at the lowest and highest temperatures. At intermediate temperatures the signal is overlapped with aromatic proton signals.

(18) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **1999**, 389.

(19) Ratio of rotamers measured at room temperature from the ^1H NMR spectrum: 1:1 in DMSO-d_6; 82:18 in $CDCl_3$.

(20) Increasing the temperature produced a progressive broadening of the signals due to the increased rate of exchange. At 110 °C in DMSO-d_6 the exchange is rapid on the NMR time scale and averaged signals for only one species were observed.

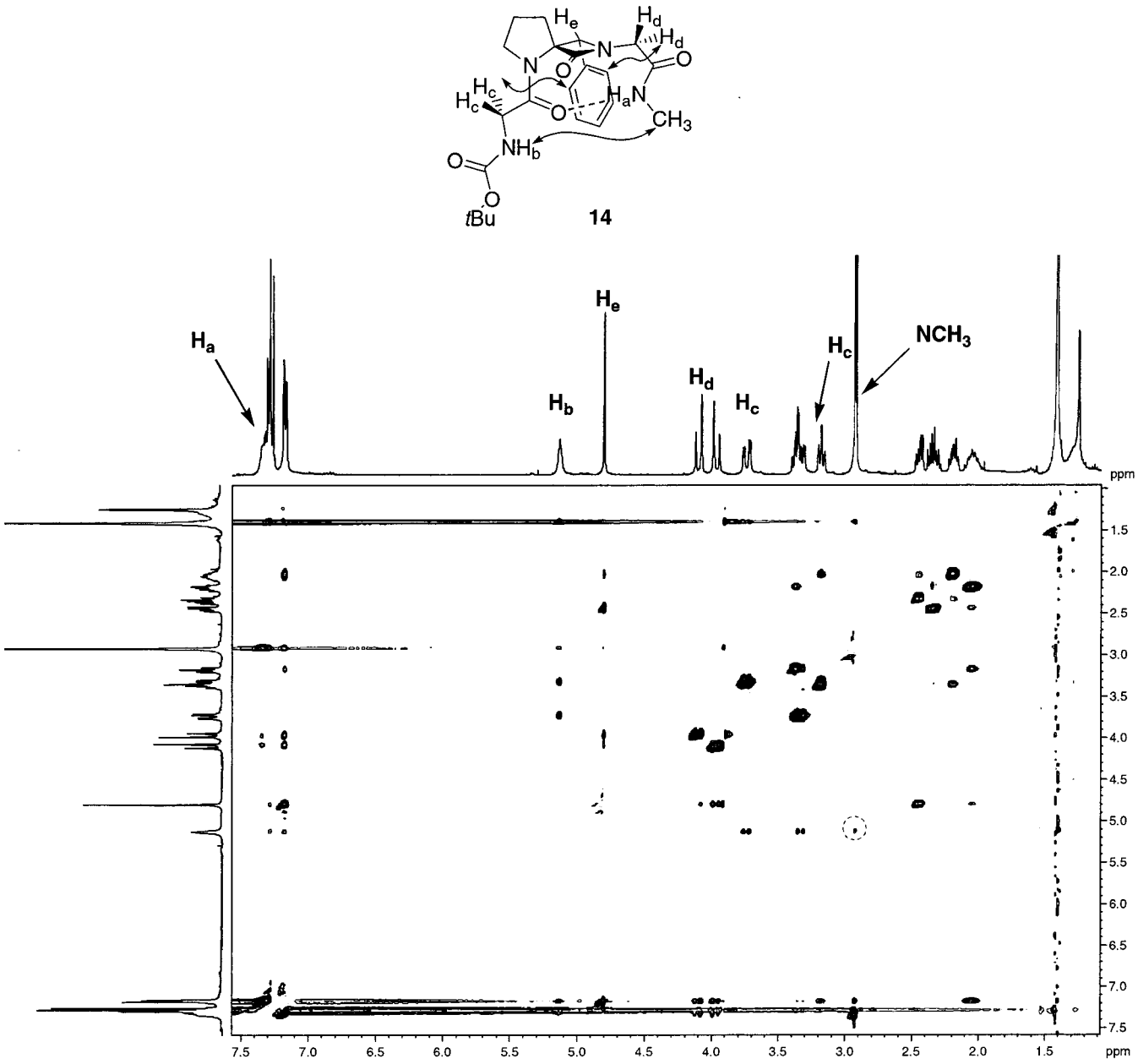


Figure 3. Selected NOEs of **14** used to assign the configuration of the chiral centers and the conformation of the molecule.

obtained by distillation from sodium and benzophenone. Chromatographic purifications were performed on silica gel (230−400 mesh) by flash technique. Analytical TLC plates (covered with silica gel 60 F_{254}) were viewed by UV light or developed by heating after treatment with an acidic solution of Ce(IV) and Mo(VI) or a basic solution of $KMnO_4$. Chemical shifts are expressed in parts per million (δ), refer to TMS in ^{1}H experiments and to deuterated solvent in ^{13}C experiments. Standard benchtop techniques were employed for handling air-sensitive reagents. The imines employed were prepared according to the general procedure published by Westheimer,[21] and were used without further purification.

Synthesis of *N*-Benzyloxycarbonyl L-Proline Acid Chloride (6). To a stirred solution of *N*-benzyloxycarbonyl L-proline (498 mg, 2 mmol) in dry ether (20 mL) was added PCl_5 (441 mg, 2.12 mmol) and the mixture was stirred for 1.5 h. at room temperature. Then the solvent was evaporated in vacuo to give a colorless oil. This oil was stirred in vacuo (0.1 mmHg) in a water bath at 50 °C for 1h to remove the $P(O)Cl_3$ formed during the reaction. NMR samples of the crude indicated purity higher than 95% and this compound was employed without further purification.

General Procedure for the Preparation of Spiro β-Lactams (7−11). To a stirred solution of the imine (2 mmol) and dry triethylamine (0.41 mL, 3 mmol) in dry dichloromethane (10 mL), was added dropwise the acid chloride **6** (535 mg, 2 mmol) dissolved in dry dichloromethane (5 mL). The mixture was stirred for 16 h at room temperature, and was 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/hexanes, affording the spiro β-lactams **7**−**11**.

(±)-(3*SR*,4*RS*)-5-Benzyloxycarbonyl-2-methyl-3-phenyl-2,5-diazaspiro[3,4]octan-1-one (7). Purified through flash chromatography (33% EtOAc/hexanes), 546 mg of **7,** 78% yield. White solid, mp = 137−139 °C. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in 1:1 ratio. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.86−2.12 (m, 4H), 2.15−2.47 (m, 4H), 2.84 (s, 3H), 3.01 (s, 3H), 3.21−3.51 (m, 4H), 4.47 (s,

(21) Taguchi, K.; Westheimer, F. H.; *J. Org. Chem.*, **1971**, *36*, 1570.

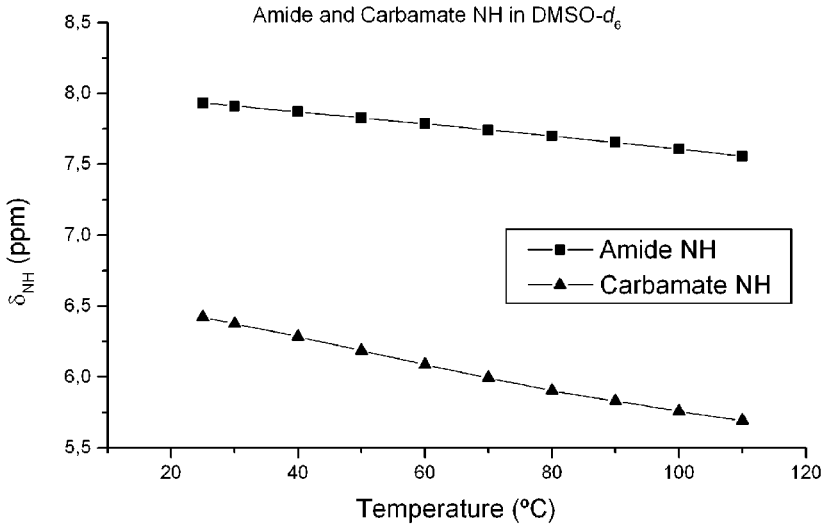


Figure 4. Variation of NH proton chemical shifts (ppm) of **14** as a function of temperature (°C) from a sample measured in DMSO-d_6.

2H), 4.66 (d, J = 12.0 Hz, 1H), 4.75 (d, J = 12.5 Hz, 1H), 4.94 (d, J = 12.0 Hz, 1H), 5.04 (d, J = 12.5 Hz, 1H), 6.81 (m, 2H), 7.03 (m, 2H), 7.13−7.43 (m, 16H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.7, 22.4, 27.2, 33.9, 35.3, 46.8, 47.5, 66.0, 67.0, 72.0, 72.5, 78.0, 78.7, 126.2, 126.9, 127.0, 127.2, 127.3, 127.6, 127.8, 127.9, 134.2, 134.3, 135.4, 136.2, 153.2, 167.9, 168.3; IR (KBr) 1757, 1702 cm^{-1}; HRMS Calcd for $C_{21}H_{22}N_2O_3$ 350.1630. Found 350.1620. Anal. Calcd for $C_{21}H_{22}N_2O_3$ C, 71.98, H, 6.33, N, 7.99. Found C, 71.64, H, 6.08, N, 8.14.

(±)-(3*SR*,4*RS*)-5-Benzyloxycarbonyl-2-(4-methoxyphenyl)-3-phenyl-2,5-diazaspiro[3,4]octan-1-one (8). Purified through flash chromatography (33% EtOAc/hexanes), 734 mg of **8**, 83% yield. White solid, mp = 144−146 °C. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in a 1:1 ratio. mp 144−146 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.95−2.18 (m, 4H), 2.31−2.54 (m, 4H), 3.30−3.54 (m, 4H), 3.72 (s, 3H), 3.73 (s, 3H), 4.50 (d, J = 12.3 Hz, 1H), 4.73 (d, J = 12.6 Hz, 1H), 4.69−5.02 (m, 3H), 5.03 (d, J = 12.6 Hz, 1H), 6.77−6.87 (m, 6H), 7.00 (m, 2H), 7.14−7.41 (m, 20H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.8, 22.5, 34.3, 35.7, 47.0, 47.7, 55.0, 66.1, 67.1, 69.4, 70.0, 76.7, 77.4, 113.8, 118.1, 118.2, 126.5, 127.0, 127.3, 127.5, 127.8, 127.9, 130.7, 131.0, 133.1, 133.4, 135.1, 136.1, 153.1, 153.2, 155.6, 155.7, 164.6, 164.9; IR (KBr) 1751, 1695 cm^{-1}; HRMS Calcd for $C_{27}H_{26}N_2O_4$ 442.1892. Found 442.1892. Anal. Calcd for $C_{27}H_{26}N_2O_4$ C, 73.28, H, 5.92, N, 6.33. Found C, 73.44, H, 5.78, N, 6.39.

(±)-(3*SR*,4*SR*)-5-Benzyloxycarbonyl-2-(4-methoxyphenyl)-3-(2-furyl)-2,5-diazaspiro[3,4]octan-1-one (9). Purified through flash chromatography (33% EtOAc/hexanes), 570 mg of **9,** 66% yield. Pale yelow solid, mp =118−120 °C. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in a 1:1 ratio.; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.91−2.13 (m, 4H), 2.28−2.49 (m, 4H), 3.39−3.55 (m, 4H), 3.77 (s, 3H), 3.79 (s, 3H), 4.82 (d, J = 12.1 Hz, 1H), 4.91 (d, J = 12.5 Hz, 1H), 4.92 (s, 1H), 4.95 (s, 1H), 5.07 (d, J = 12.1 Hz, 1H), 5.17 (d, J = 12.5 Hz, 1H), 5.73 (d, J = 3.3 Hz, 1H), 6.20 (m, 2H), 6.29 (d, J = 3.3 Hz, 1H), 6.82 (m, 4 H), 7.18−7.34 (m, 16H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.8, 22.7, 34.0, 35.4, 47.3, 47.9, 55.3, 63.9, 64.2, 66.7, 67.6, 77.1, 77.8, 109.0, 110.1, 110.2, 110.6, 114.0, 118.5, 127.7, 128.1, 128.2, 130.5, 130.8, 135.4, 136.5, 142.2, 142.7, 148.0, 148.1, 153.7, 156.0, 156.1, 164.5, 164.6; IR (KBr) 1755, 1701 cm^{-1}; HRMS Calcd for $C_{25}H_{24}N_2O_5$ 432.1685. Found 432.1687. Anal. Calcd for $C_{25}H_{24}N_2O_5$ C, 69.43, H, 5.59, N, 6.48. Found C, 69.54, H, 5.78, N, 6.39.

(±)-(3*SR*,4*RS*)-5-Benzyloxycarbonyl-2-(4-methoxyphenyl)-3-(2-phenylethylen)-2,5-diazaspiro[3,4]octan-1-one (10). Purified through flash chromatography (33% EtOAc/hexanes), 569 mg of **10,** 63% yield. White solid, mp = 142−144 °C. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in a 1:1 ratio. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.89 (m, 2H), 2.04 (m, 2H), 2.28 (m, 2H), 2.46 (m, 2H), 3.52 (m, 4H), 3.75 (s, 3H), 3.79 (s, 3H), 4.49 (d, J = 8.6 Hz, 1H), 4.55 (d, J = 8.5 Hz, 1H), 5.02 (m, 2H), 5.31 (m, 2H), 6.15 (dd, J = 16 and 8.5 Hz, 1H), 6.52−6.84 (m, 7H), 7.02 (m, 2H), 7.24−7.34 (m, 20H), 7.46 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.3, 23.0, 34.6, 35.8, 47.8, 48.3, 55.3, 55.4, 66.9, 67.7, 69.8, 70.3, 76.5, 76.8, 76.8, 77.1, 114.1, 118.3, 124.4, 125.5, 126.4, 126.5, 127.7, 127.8, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6, 131.3, 131.6, 135.4, 135.5, 135.8, 135.9, 136.4, 153.8, 154.3, 155.9, 156.0, 164.9, 165.0; IR (KBr) 1752, 1708 cm^{-1}; HRMS Calcd for $C_{29}H_{28}N_2O_4$ 468.2049. Found 468.2027. Anal. Calcd for $C_{29}H_{28}N_2O_4$ C, 74.34, H, 6.02, N, 5.98. Found C, 74.64, H, 6.28, N, 6.14.

(±)-(3*SR*,4*RS*)-5-Benzyloxycarbonyl-2-methoxycarbonylmethylen-3-phenyl-2,5-diazaspiro[3,4]octan-1-one (11). Purified through flash chromatography (33% EtOAc/hexanes), 628 mg of **11**, 77% yield. White solid, mp = 95−97 °C. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in a 1:1 ratio. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.9−2.1 (m, 4H), 2.37−2.53 (m, 4H), 3.23−3.47 (m, 4H), 3.48 (d, J = 18.2 Hz, 1H), 3.70 (d, J = 18.2 Hz, 1H), 3.70 (s, 6H), 4.43 (d, J = 18.2 Hz, 1H), 4.61−4.83 (m, 5H), 4.93 (d, J = 12.5 Hz, 1H), 5.03 (d, J = 12.5 Hz, 1H), 6.79 (m, 2H), 7.01 (m, 2H), 7.11−7.35 (m, 16H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.1, 22.7, 34.4, 35.8, 41.4, 41.5, 47.2, 47.9, 52.1, 52.2, 66.4, 67.4, 70.7, 71.2, 78.7, 79.4, 126.4, 127.1, 127.3, 127.5, 127.7, 128.00, 128.06, 128.12, 128.17, 134.1, 134.2, 135.6, 136.4, 153.4, 153.6, 168.3, 168.6, 169.1, 169.5; IR (KBr) 1781, 1750, 1702 cm^{-1}; HRMS calcd for $C_{23}H_{24}N_2O_5$ 408.1685, found 408.1694. Anal. Calcd for $C_{23}H_{24}N_2O_5$ C, 67.63; H, 5.92; N 6.86. Found: C, 67.54; H, 5.87; N, 6.99.

(±)-(3*SR*,4*RS*)-5-(*N*-*tert*-butoxycarbonylaminoacetyl)-2-Methoxycarbonylmethylen-3-phenyl-2,5-diazaspiro-[3,4]octan-1-one (13). 2 mmol (816 mg) of **11** were dissolved in a 3/1 mixture of EtOAc/methanol (16 mL) and transferred via cannula to a flask under H_2 (1 atm.) containing 50 mg of 10% Pd−C catalyst. The mixture was stirred overnight and then the catalyst was filtered off on Celite. The organic layer was concentrated in vacuo to afford 537 mg of a pale yellow oil, which was employed without further purification. *N*-*tert*-butoxycarbonylglycine (350 mg, 2 mmol), was dissolved in 4 mL of a 1:1 mixture of $CHCl_3$ and toluene, 0.28 mL of NEt_3 (2 mmol) was then added and the solution was cooled to −15 °C. To this mixture was added 217 mg (2 mmol) of ethyl chloroformate and stirred at this temperature for 30 min. Then the aforementioned crude oil was dissolved in 2 mL of $CHCl_3$ and added via cannula to the mixed anhydride previously formed. The mixture was heated to 50 °C and stirred for 2h. The solution was cooled to room temperature and washed with 5% aqueous $NaHCO_3$, 1M HCl and brine. The organic layer was

dried over anhydrous Na_2SO_4, concentrated in vacuo and purified by column chromatography over SiO_2 (50% EtOAc/hexanes) to give 661 mg (77%, overall yield) of the pure β-lactam **13** as a white foam. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (s, 9H), 2.02−2.25 (m, 2H), 2.31 (m, 1H), 2.52 (m, 1H), 3.14−3.37 (m, 3H), 3.68 (m, 1H), 3.71 (s, 3H), 3.72 (d, J = 18.2 Hz, 1H), 4.68 (d, J = 18.2 Hz, 1H), 4.83 (s, 1H), 5.17 (broad t, 1H), 7.20−7.29 (m, 5H);^{13}C NMR (50 MHz, $CDCl_3$) δ 23.1, 28.1, 33.6, 41.6, 42.5, 46.3, 52.2, 71.0, 79.3, 79.5, 127.0, 128.1, 128.2, 134.0, 155.4, 167.1, 168.5, 168.6; IR (KBr) 3408, 1774, 1744, 1664, 1210, 1169 cm^{-1}; HRMS Calcd for $C_{18}H_{21}N_3O_6$ ($M^+ - C_4H_9 + H^+$) 375.1430. Found 375.1432. Anal. Calcd for $C_{22}H_{29}N_3O_6$ C, 61.24, H, 6.77, N, 9.74. Found C, 61.34, H, 6.98, N, 9.54.

Aminolysis of the compounds 11 and 13. To a stirred solution of **11** or **13** (1 mmol) in methanol (5 mL) was added methylamine hydrochloride (675 mg, 10 mmol) and triethylamine (2.10 mL, 15 mmol). This mixture was stirred for 7h at room temperature, the methanol was evaporated under reduced pressure and then saturated aqueous $NaHCO_3$ (10 mL) and ethyl acetate (10 mL) were added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 × 15 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The crude reaction was purified using a short silica gel column to afford the pure compounds **12** and **14**.

(±)-(3*SR*,4*RS*)-5-Benzyloxycarbonyl-2-methylaminocarbonylmethylen-3-phenyl-2,5-diazaspiro[3,4]octan-1-one (12). Purified through, flash chromatography (EtOAc), 378 mg of **12**, 93% yield. White solid, mp = 149−151 °C. ^{1}H and ^{13}C NMR show the presence of rotamers about the carbamate bond in a 4:1 ratio. mp 149−151 °C; Mayor: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89−2.16 (m, 2H), 2.34−2.45 (m, 2H), 2.88 (d, J = 4.8 Hz, 3H), 3.22 (m, 1H), 3.38 (m, 1H), 3.94 (d, J = 11.2 Hz, 1H), 4.10 (d, J = 11.2 Hz, 1H), 4.78 (d, J = 12.5 Hz, 1H), 4.80 (s, 1H), 5.07 (d, J = 12.5 Hz, 1H), 7.02 (m, 2H), 7.10−7.41 (m, 8H), 7.44(broad q, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8, 26.2, 33.6, 45.4, 47.4, 66.8, 72.8, 79.1, 126.9, 127.5, 127.8, 127.9, 128.3, 128.4, 134.1, 136.3, 154.1, 168.1, 168.8; IR (KBr) 3581, 3502, 3338, 1766, 1690 cm^{-1}; HRMS Calcd for $C_{23}H_{25}N_3O_4$ 407.1845, found 407.1847. Anal. Calcd for $C_{23}H_{25}N_3O_4$ C, 67.80, H, 6.18, N, 10.31. Found C, 67.74, H, 6.13, N, 10.33.

(±)-(3*SR*,4*RS*)-5-(*N*-*tert*-butoxycarbonylaminoacetyl)-2-Methylaminocarbonylmethylen-3-phenyl-2,5-diazaspiro-[3,4]octan-1-one (14). Purified through flash chromatography (10% MeOH/EtOAc), 378 mg of **14**, 88% yield. White solid, mp 106−108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.98−2.11 (m, 1H), 2.19 (m, 1H), 2.29−2.38 (m, 1H), 2.42 (m, 1H), 2.91 (d, J = 4.8 Hz, 3H), 3.17 (m, 1H), 3.30−3.40 (m, 2H), 3.74 (dd, J = 17.5, 4.4 Hz, 1H), 3.96 (d, J = 17.3 Hz, 1H), 4.10 (d, J = 17.3 Hz, 1H), 4.80 (s, 1H), 5.13 (broad t, NH), 7.15−7.19 (m, 2H), 7.24−7.33 (m, 3H), 7.35 (broad q, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.1, 26.4, 28.2, 33.0, 42.9, 45.4, 46.5, 72.5, 79.2, 79.7, 126.8, 128.3, 128.5, 133.8, 155.4, 167.6, 168.0, 168.2; IR (KBr) 3427, 1764, 1668, 1162 cm^{-1}; HRMS Calcd for $C_{18}H_{22}N_4O_5$ ($M^+ - C_4H_9 + H^+$) 374.1590. Anal. Calcd for $C_{22}H_{30}N_4O_5$ C, 61.38, H, 7.02, N, 13.01. Found C, 61.49, H, 6.88, N, 12.94.

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Supporting Information Available: Cartesian coordinates and computed total energies of **5-syn** and **5-anti**. This material is available free of charge via the Internet at http://pubs.acs.org.

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