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Phe-Ala-Based Diazaspirocyclic Lactam as Nucleator of Type II′ β-Turn

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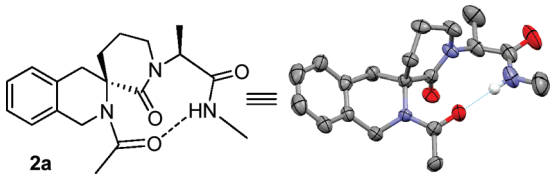

The synthesis of a novel Phe-Ala dipeptide mimic, built up on a diazaspirocyclic lactam core, is presented. This new scaffold was evaluated for conformational mimicry of reverse turn by combining molecular modeling, IR, NMR, and X-ray diffraction experiments. All these tools agree on the presence of a strong intramolecular hydrogen bond, thus demonstrating the ability of this spiro compound to act as a type II′ β-turn inducer.

Introduction

The importance of reverse turn for the biological properties of peptides has in recent years spurred the search for new scaffolds able to influence the secondary structure when placed into a peptide chain.[1] One of the most studied reverse motifs is the β-turn, a four amino acid fragment capable of inverting the chain direction, which is often stabilized by an intramolecular hydrogen bond between the carbonyl oxygen of the first residue (*i*) and the amide proton of the fourth one (*i* + 3).[2] Among the many proposed β-turn mimics,[3] spirocyclic scaffolds[4] have attracted attention as privileged structures, able to provide, upon the attachment of appropriate functional groups, useful high-affinity ligands, relevant to the field of drug discovery. The polysubstituted central atom common to the rings of spiro compounds confers on the overall molecular framework unique 3D properties that were early on recognized by medicinal chemists. For instance, in the simplest two-ring system, the almost mutually orthogonal position of the rings can be used to mimic structural motifs found in proteins, thus increasing the probability of interactions with biological systems. In particular, making reference to β-turns, proline-based spirocyclic lactams have proven to be very effective nucleators of type II−II′ β-turn conformations.[5]

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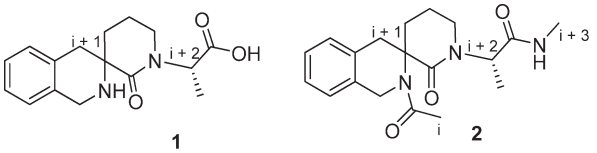


FIGURE 1. Proposed β-turn inducer **1** and the derived tetrapeptide mimic **2**.

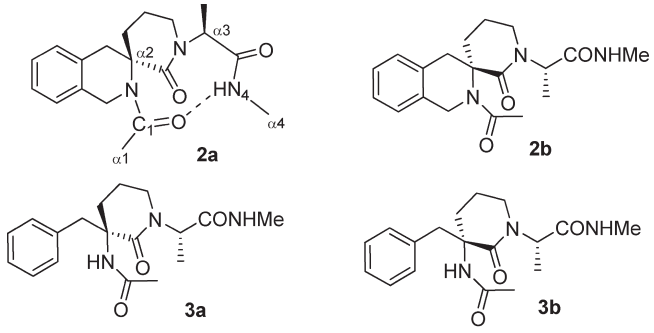
Recently, type II β-turn mimics based upon L-Pro-containing spirocyclic scaffolds have served for the synthesis of new Pro-Leu-Gly (PLG) peptidomimetics with efficient dopamine receptor modulating activities.[6] Among benzo-condensed compounds, benzazepinones-based spirocyclic derivatives, bearing mainly β-turn conformations, were recently reported as potent and selective μ-opioid agonists.[7]

Following our ongoing interest in the design and synthesis of new β-turn mimics,[8] we report here on the preparation of an unprecedented diazaspirocyclic peptidomimetic capable of efficiently stabilizing a type II′ β-turn. The stereogenic spiro carbon atom is common to a tetrahydroisoquinoline and to a piperidin-2-one ring and enables the construction of a benzo-condensed 1,8-diazaspiro[5.5]undecan-7-one ring system. The tetrahydroisoquinoline-3-carboxylic acid (Tic), a conformationally constrained mimic of Phe, is a key element in several peptide-based drugs, since its incorporation itself not only restricts the conformational freedom of the aromatic ring but also places constraints on the peptide backbone.[9] We reasoned that elaborating the Tic into a diazaspyrocyclic structure would lead to an efficient β-turn nucleator. For investigation purposes, we selected Ala as the $(i + 2)$ amino acid residue. The target β-turn inducer would consist of the Phe-Ala mimic **1** (Figure 1). The derived **2** could be considered as a simplified tetrapeptide model, in which the *N*-terminal amino acid (*i*) has been substituted by an acetyl group and the C-terminal residue $(i + 3)$ by an *N*-methyl moiety.

Results and Discussion

The tetrapeptide mimic **2** was first investigated by computational methods to determine the correct stereochemistry required to maximize the β-turn propensity.

Having prearranged the *S* configuration on the $(i + 2)$ Ala component, we considered the two possible configurations at the spiro $C_{\alpha 2}$ atom (Figure 2) in model compounds **2a**,**b** (Figure 2). To assess the presence of a β-turn-like conformation, the pseudotorsion angle of the amide backbone β and



$d_\alpha = (C_{\alpha 1} - C_{\alpha 4})$ $\beta = (C_1\text{-}C_{\alpha 2}\text{-}C_{\alpha 3}\text{-}N_4)$

FIGURE 2. Structures submitted to computer-aided conformational analysis.

TABLE 1. Results from Computer-Aided Conformational Analysis[a]

compd	no. of conf < 6 kcal/mol	% d_α < 7 Å	% $\lvert\beta\rvert$ < 30°	% H bond C_1=O---HN_4
2a	6	66 (5.37 Å)	66 (−15.9°)	33 (present)
2b	16	44 (6.30 Å)	25 (−35.6°)	0 (absent)
3a	23	52 (7.58 Å)	69 (−34.7°)	8 (absent)
3b	43	16 (9.93 Å)	12 (−178.4°)	0 (absent)

[a]Results are reported as percentage of conformers which meet the indicated requirement. Values for the global minimum are reported in parentheses.

the interatomic distance d_α were evaluated. In addition, the presence of the intramolecular hydrogen bond C_1=O---HN_4, involved in a 10-membered ring, was assumed as a critical requirement.[10] To support the assumption that the spirocyclic moiety would be crucial for the β-turn stabilization, we also investigated compound **3a**,**b** in which the precursor Phe amino acid is not constrained into a Tic ring system. Structures **2a**,**b** and **3a**,**b** were submitted to a computational procedure[11] consisting of an unconstrained Monte-Carlo/Energy Minimization conformational search using the molecular mechanics MMFF94 force field[12] in vacuo. For each compound only conformations within 6 kcal/mol of the global minimum were kept. Results are reported in Table 1 as the percentage of conformers which meet the requirements for a generic β-turn.

The best candidate for our purpose was compound **2a**, embodying D-Phe and L-Ala and exhibiting both a low number of stable conformations and good percentages of conformers with the desired geometrical requirements. In particular, with respect to **2b**, the presence of D-Phe seems to promote an intramolecular 10-membered H-bond. Unconstrained mimics **3a**,**b** were shown to be less effective in stabilizing reverse turn conformations, and the minimum energy conformers for both compounds were not β-turns.

The stability of the H-bond in **2a** was also checked by means of molecular dynamics[11] simulations at 300 K (100 ps with 1.5 fs time step and without any imposed constraint) in

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(10) Hydrogen bonds are defined as nonbonded contacts between a nitrogen or oxygen and an hydrogen attached to nitrogen or oxygen, separated by a distance ranging from 1.6 to 2.1 Å and making an X−H--Y (X, Y = N, O) angle >120°.

(11) Conformational analysis and DFT geometry optimization were performed with the suite *Spartan'08* (Wavefunction, Inc.: Irvine, CA). Molecular dynamics were performed with *MacroModel*, version 9.7 (Schrödinger, LLC, New York, 2009).

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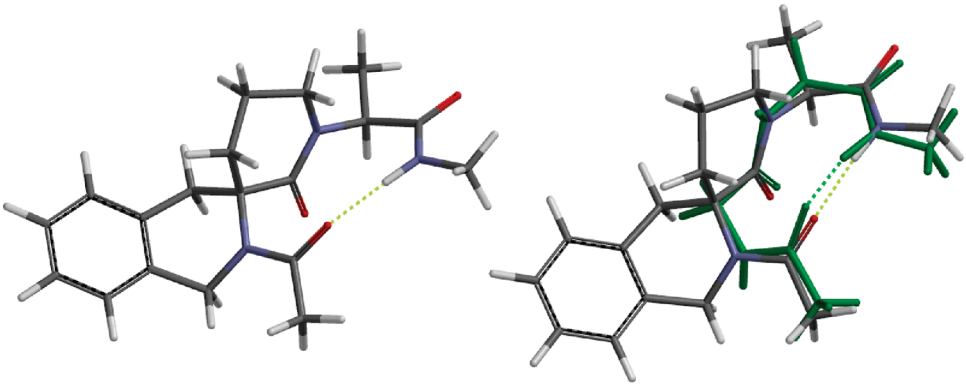
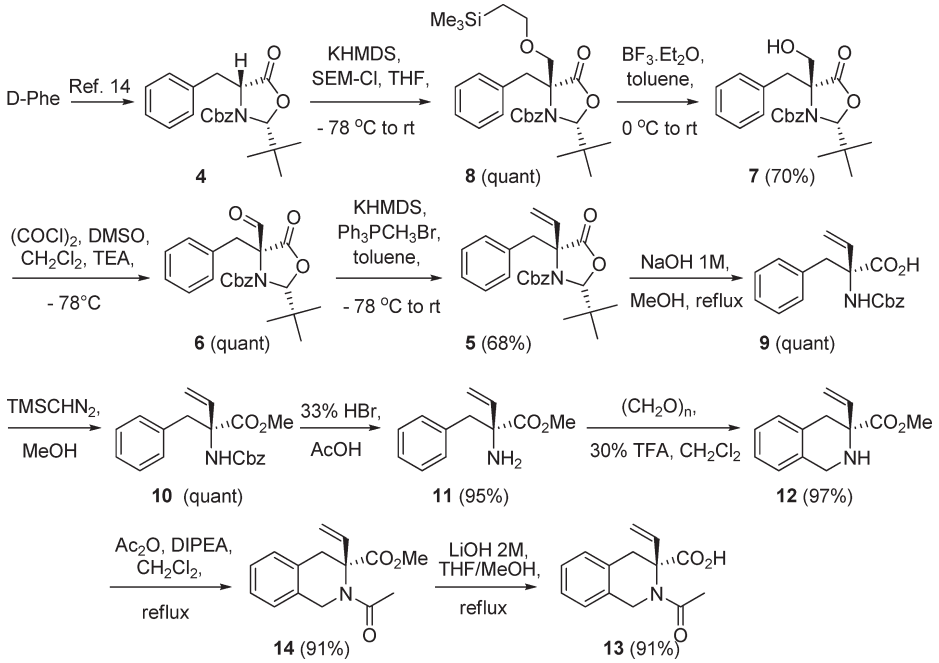


FIGURE 3. Plot of the structure of **2a** obtained by MC/MM analysis and then optimized at the B3LYP-6-31G* level and of its superimposition with a type II′ β-turn model (in green).

SCHEME 1. Synthetic Sequence



vacuo, in chloroform (noncoordinating solvent), and in water (coordinating solvent). The results confirmed the presence of this H-bond in 53%, 74%, and 21% of samples in vacuo, chloroform, and water, respectively. The minimum energy conformer was finally optimized at the B3LYP-6-31G* level, and the dihedral angles of the backbone amide were measured. The found values ($\Phi_{(i+1)} = 54.9°$; $\psi_{(i+1)} = -141.3°$; $\Phi_{(i+2)} = -96.9°$; $\psi_{(i+2)} = 43.9°$) pointed out a type II′ β-turn (Figure 3).

The computational evidence prompted us to undertake the synthesis of **2a**.

Following a reported procedure,[13,14] D-Phe was first converted into oxazolidinone **4** (Scheme 1). From this intermediate, we first meant to achieve vinyl derivative **5** through aldehyde **6**. Actually, all attempts to obtain **6** by direct formylation of **4** with different reagents (HCO_2Et, DMF, or formylpiperidine) and conditions were unsuccessful. So we planned to get **6** through alcohol **7** and subsequent Swern oxidation. The required hydroxymethylene moiety was inserted by alkylation of **4** with SEM-Cl to give **8**, and subsequent removal of the trimehylsilylethyl group with BF_3. Alcohol **7** was obtained in 70% overall yield as the single expected diastereoisomer. Swern oxidation of the primary alcohol to aldehyde **6**, followed by Wittig olefination with Ph_3PCH_3Br and KHMDS at low temperature smoothly afforded olefin **5**. Treatment of **5** with NaOH in MeOH gave acid **9**, which was esterified with diazomethane to *N*-Cbz,α-vinylphenylalanine methylester **10** in quantitative overall yield. To achieve the tetrahydroisoquinoline ring, the Cbz protecting group was first removed in acid conditions, and the obtained amino ester **11** was submitted to Pictet−Spengler cyclization with *p*-formaldehyde (30% TFA, CH_2Cl_2). The reaction proceeded smoothly to give the Tic derivative **12**, from which the α-vinyl acid **13** was derived in high overall yield by *N*-acetylation to **14** and subsequent methylester hydrolysis.

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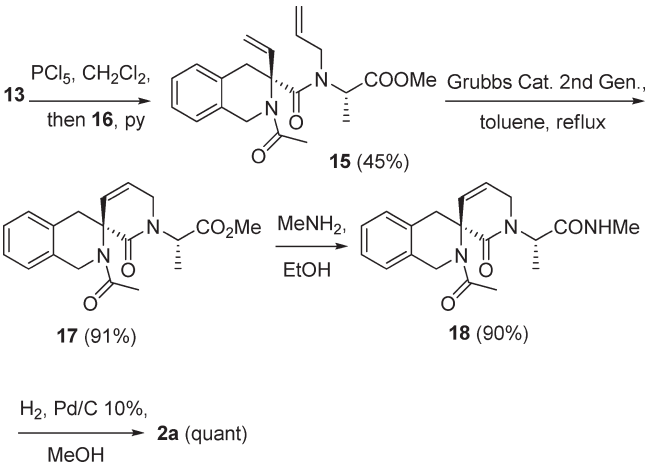
SCHEME 2. Synthetic Sequence



To achieve the RCM precursor **15**, the carboxylic acid **13** was reacted with *N*-allyl-alanine methylester **16**, readily prepared as described in the literature[15] (Scheme 2). Due to the steric demand of this condensation, the use of classical coupling agents (BOP-Cl, HATU, PyBrOP, BOP/HOBT) did not work, and the desired amide **15** could only be obtained in 45% yield by using PCl_5 as the condensing agent. With the aim of making the condensation step more efficient, we also tried to employ the primary amine alanine methylester, in place of the secondary amine *N*-allyl-alanine methylester. Condensation proceeded better (74% yield), but subsequent allylation of the amide nitrogen of the obtained dipeptide to give **15** could not be achieved, probably due again to steric hindrance.

Dipeptide **15** was then easily cyclized by a RCM reaction by using Grubbs' second generation catalyst, affording the desired diazaspiro ring system of compound **17**. By conversion of the methyl ester into methylamide **18** upon treatment with methylamine, and double bond reduction under hydrogen atmosphere in the presence of palladium, the target compound **2a** could be achieved.

With tetrapeptide mimic **2a** in hand, we performed a thorough conformational study to confirm the presence of the intramolecular hydrogen bond attesting a β-turn conformation. After full characterization by one- and two-dimensional NMR analyses, variable temperature (VT) ^{1}H NMR studies were performed on a 2.0 mM $CDCl_3$ solution of **2a**. The chemical shift of the NH amide proton ranged from 7.79 (T = 218 K) to 7.44 ppm (T = 328 K). These values, together with the low VT coefficient (−3.2 ppb/K), support the involvement of this proton in a hydrogen bond. Further, titration of the $CDCl_3$ 2.0 mM solution with up to 30% of DMSO-d_6, a strongly coordinating and hydrogen bond-acceptor solvent, produced a low variation of the NH chemical shift (from 7.53 to 7.72 ppm),[16] thus highlighting the stability of the intramolecular hydrogen bond.

Further evidence for the β-turn-like conformation came from the NOESY spectrum. Selective NOE contacts were

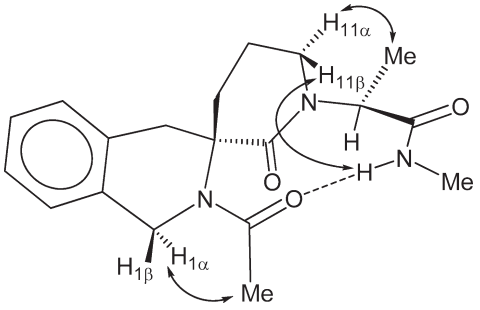


FIGURE 4. Relevant NOE contacts from NOESY (400 MHz, 301 K, 2.0 mM solution $CDCl_3$) of **2a**.

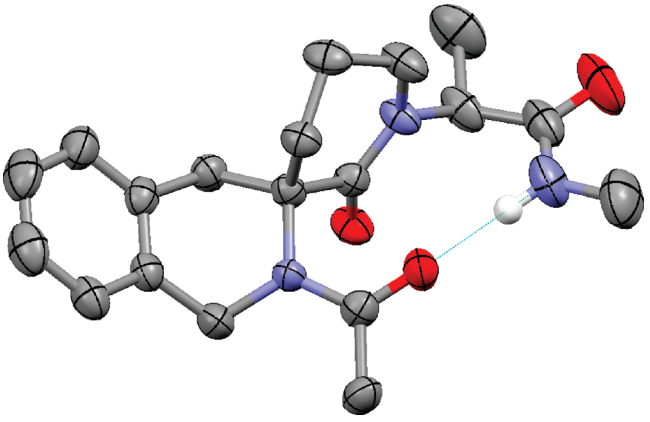


FIGURE 5. Plot of the molecular structure of compound **2a**, as determined by X-ray crystallography. Atomic displacement parameters at the 50% probability level (hydrogen atoms omitted: see the Supporting Information for details). β-Turned conformation and intramolecular 10-membered H bond are shown.

detected between both hydrogens H_1 and the methyl of the acetamide group, while two distinct NOE interactions were observed for $H_{11\beta}$ with the hydrogen bonded NHMe proton, and for $H_{11\alpha}$ with the methyl of the Ala residue (Figure 4). All these observations are in agreement with the presence of a stable hydrogen bonded conformation.

Also the FT-IR spectrum (2.0 mM solution $CHCl_3$), showing a unique extensive absorption band at 3345 cm^{-1} ascribable to an hydrogen-bonded NH stretching, further attests the presence of a stable secondary structure for **2a**.

We could also isolate a single crystal of **2a** for crystallographic analysis from a 2-propanol solution (Figure 5). We were pleased to see that even in a potentially H-bond disrupting solvent such as 2-propanol, compound **2a** crystallizes in a β-turn conformation, characterized by a 2.084 Å intramolecular hydrogen bond between the CO_i oxygen atom acceptor and the NH_{i+3} hydrogen amide donor. The hydrogen bond directionality defined by the $_{(i)}O\cdots H{-}N_{(i+3)}$ angle of 160.65° is almost ideal. Also the $C\alpha_{(i)}\cdots C\alpha_{(i+3)}$ distance of 5.584 Å nicely complies with a β-turn arrangement. The dihedral angles ($\Phi_{(i+1)}$ = 49.42°; $\psi_{(i+1)}$ = −135.48°; $\Phi_{(i+2)}$ = −110.07°; $\psi_{(i+2)}$ = 40.74°), measured in the solid state, are very similar to the computed values, thus confirming a type II′ β-turn-like[17] structure for **2a**.

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(16) For DMSO-d_6/$CDCl_3$ solutions, chemical shifts were standardized by reference to residual proton resonance for CHD_2CD_3SO (2.49 ppm).

(17) Ideal values for type II′ β-turn: $\Phi_{(i+1)}$ = 60°; $\psi_{(i+1)}$ = −120°; $\Phi_{(i+2)}$ = −80°; $\psi_{(i+2)}$ = 0°.

Conclusions

In this work we described the design and synthesis of the new Phe-Ala based diazaspirocyclic lactam **2a**. Computational studies and spectroscopic conformational analyses clearly indicated that this scaffold adopts a type II′ β-turn conformation. This peptidomimetic, having a Tic core in the $i + 2$ position, represents a good candidate to replace the β-turn motif in various Phe-containing G-protein-coupled receptors ligands. Work to illustrate the application of this novel diazaspirocyclic lactam to the synthesis of analgesic tetrapeptide mimics is in progress in our laboratory.

Experimental Section

Compound 8. A solution of **4**[18] (1.69 g, 4.6 mmol) in anhydrous THF (30 mL) was treated dropwise with a 0.5 M KHMDS solution in toluene (12 mL, 6.0 mmol, 1.3 equiv) at −78 °C and under N_2 atmosphere. After 30 min, SEM-Cl (1.63 mL, 9.22 mmol, *d* 0.94 g mL^{-1}, 2 equiv) was added dropwise to the reaction mixture and stirring was continued at −78 °C for an additional 45 min and then for 2 h at room temperature. The reaction was quenched with 10% aqueous $NaHSO_3$ (30 mL), and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with 10% aqueous $NaHSO_3$, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo to afford compound **8** (2.30 g, quantitative yield) as a colorless oil, which was used without further purification. R_f (hexane/EtOAc 9:1) 0.41. $[\alpha]^{25}_D$ −23.6 (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (br s, 5H), 7.24 (br s, 5H), 5.50 (s, 1H), 5.25 (br s, 2H), 3.80−3.72 (m, 1H), 3.49 (d, J = 9.0 Hz, 1H), 3.41−3.15 (m, 4H), 0.90−0.46 (m, 2H), 0.53 (s, 9H), −0.02 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 155.4, 135.2, 134.6, 130.7 (2C), 128.4−126.9 (8C), 95.6, 71.9, 68.7, 68.3, 67.3, 38.5, 37.0, 24.7 (3C), 17.7, −1.8 (3C). HRMS (ESI) calcd for $C_{28}H_{39}NO_5Si$ 497.2597, found 497.2581.

Compound 7. To a solution of **8** (2.30 g, 4.6 mmol) in toluene (35 mL), at 0 °C and under N_2 atmosphere, was added $BF_3 \cdot Et_2O$ (645 μL, 5.1 mmol, *d* 1.1 g mL^{-1}, 1.1 equiv). The reaction mixture was stirred for 20 h, and then it was quenched by the addition of a saturated solution of $NaHCO_3$ (30 mL). The mixture was extracted with Et_2O (3 × 15 mL), dried over Na_2SO_4, and concentrated. Purification by Biotage Flash Cromatography (hexane/EtOAc 9:1) furnished compound **7** (1.28 g, 70% yield) as a colorless oil. R_f (hexane/EtOAc 4:1) 0.12. $[\alpha]^{25}_D$ −43.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (br s, 5H), 7.23 (br s, 5H), 5.59 (s, 1H), 5.25 (d, J = 12.2 Hz, 1H), 5.22 (d, J = 12.2 Hz, 1H), 4.18 (br d, J = 10.2 Hz, 1H), 3.80 (d, J = 10.7 Hz, 1H), 3.20 (d, J = 14.0 Hz, 1H), 3.16 (d, J = 13.8 Hz, 1H), 1.81 (br s, 1H), 0.64 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 155.6, 135.3, 134.7, 130.9−127.3 (10C), 96.1, 70.1, 67.9, 65.6, 38.3, 37.4, 24.9 (3C). HRMS (ESI) calcd for $C_{23}H_{27}NO_5Na$ (M + Na) 420.1781, found 420.1787.

Compound 6. A solution of DMSO (1.0 mL, 14.1 mmol, *d* 1.1 g mL^{-1}, 3 equiv) in 2 mL of anhydrous CH_2Cl_2 was added dropwise, at −78 °C and under N_2 atmosphere, to a solution of $(COCl)_2$ (820 μL, 9.7 mmol, *d* 1.5 g mL^{-1}, 2 equiv) in 8 mL of anhydrous CH_2Cl_2. The mixture was stirred at −78 °C for 10 min. A solution of **7** (1.91 g, 4.8 mmol, 1 equiv) in 18 mL of anhydrous CH_2Cl_2 was added, and the reaction mixture was stirred at −78 °C and under N_2 atmosphere for 1.5 h. Et_3N (2.7 mL, 19.3 mmol, *d* 0.7 g mL^{-1}, 4 equiv) was added and the temperature was slowly warmed to rt. After 2 h of stirring at rt, the reaction mixture was diluted with 15 mL of CH_2Cl_2 and washed with 5% aqueous H_3PO_4 (20 mL), followed by extraction with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo, to afford **6** (1.99 g, quantitative yield) as a colorless oil. R_f (hexane/EtOAc 4:1) 0.36. $[\alpha]^{25}_D$ −38.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 1 H), 7.50−7.32 (m, 5H), 7.31−7.13 (m, 5H), 5.66 (s, 1H), 5.20 (d, J = 11.7 Hz, 1H), 5.08 (d, J = 11.7 Hz, 1H), 3.72 (d, J = 13.6 Hz, 1H), 3.56 (br s, 1H), 0.56 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.3, 166.4, 155.5, 134.7−127.8 (12C), 96.1, 76.5, 68.6, 37.4, 37.0, 24.5 (3C). HRMS (ESI) calcd for $C_{23}H_{25}NO_5$ 395.1733, found 395.1713.

Compound 5. The phosphonium salt Ph_3PCH_3Br (2 mmol) was quickly transferred to a flame-dried flask and purged with nitrogen; anhydrous distilled toluene (4 mL) was added. The resulting suspension was magnetically stirred and treated with a 0.5 M KHMDS solution in toluene (3.8 mL, 1.9 mmol), affording a brilliant yellow suspension. After 90 min, the reaction mixture was cooled to −78 °C and a solution of the crude aldehyde **6** (395 mg, 1.0 mmol, 1 equiv) in anhydrous toluene (3 mL) was added dropwise. After 50 min the temperature was slowly warmed to −40 °C and, after 30 min, to rt. After 1 h at rt, the reaction mixture was cooled to −20 °C and quenched with 3 N HCl (6 mL) and saturated NH_4Cl (6 mL) aqueous solutions. The reaction mixture was diluted with EtOAc, the organic layer separated, and the aqueous one extracted twice with the same solvent. The combined organic solvents were dried over Na_2SO_4 and evaporated to dryness under reduced pressure The crude product was purified by flash chromatography (hexane/EtOAc 9:1), affording the desired vinyl derivative **5** (267 mg, 68% yield) as an oil. R_f (hexane/EtOAc 9:1) 0.25. $[\alpha]^{25}_D$ −80.2 (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47−7.35 (4H), 7.30−7.16 (6H), 5.96 (dd, J = 17.3, 10.7 Hz, 1H), 5.54 (s, 1H), 5.31 (d, J = 11.8 Hz, 1H), 5.27 (d, J = 17.3 Hz, 1H), 5.25 (d, J = 10.7 Hz, 1H), 5.16 (d, J = 11.8 Hz, 1H), 3.50 (d, J = 13.7 Hz, 1H), 3.44 (d, J = 13.7 Hz, 1H), 0.50 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 155.7, 137.7, 134.9, 130.9, 128.6−127.0 (10C), 116.7, 94.64, 69.0, 67.8, 40.6, 37.3, 24.4 (3C). HRMS (EI) calcd for $C_{24}H_{27}NO_4$ 393.1940, found 393.1948.

Compound 9. A solution of **5** (400 mg, 1.0 mmol) in NaOH 1 M (6.0 mL, 6.0 mmol, 6 equiv) and MeOH (6.0 mL) was stirred at reflux for 8 h, under N_2 atmosphere. After cooling to rt, the mixture was concentrated in vacuo and 5% aqueous H_3PO_4 (15 mL) was added until pH 1. The mixture was extracted three times with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo to afford **9** (331 mg, quantitative yield) as an oil. R_f (EtOAc/MeOH 9:1) 0.17. $[\alpha]^{25}_D$ −38.5 (*c* 1.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (br s, 1H), 7.48−7.27 (m, 5H), 7.26−7.16 (m, 3H), 7.13−7.02 (m, 2H), 6.12 (dd, J = 17.2, 10.6 Hz, 1H), 5.64 (br s, 1H), 5.38 (d, J = 10.0 Hz, 1H), 5.36 (d, J = 17.5 Hz, 1H), 5.23 (d, J = 12.2 Hz, 1H), 5.11 (d, J = 12.2 Hz, 1H), 3.63 (d, J = 13.5 Hz, 1H), 3.40 (d, J = 13.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6, 154.44 136.1, 134.8, 129.8−126.8 (10C), 116.3, 66.6, 64.6, 40.3, 27.6. HRMS (ESI) calcd for $C_{19}H_{19}NO_4$ 325.1314, found 325.1323.

Compound 10. At 0 °C, under N_2 atmosphere, a 2.0 M $TMSCHN_2$ solution in Et_2O (4.2 mL, 8.31 mmol, 4 equiv) was added dropwise to a stirred solution of **9** (675 mg, 2.08 mmol, 1 equiv) in MeOH (8 mL). After 1 h the reaction mixture was concentrated in vacuo, affording **10** (706 mg, quantitative yield) as an oil. R_f (EtOAc/MeOH 9:1) 0.69. $[\alpha]^{25}_D$ −35.6 (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47−7.32 (m, 5H), 7.27−7.15 (m, 3H), 7.06−6.97 (m, 2H), 6.10 (dd, J = 17.3, 10.6 Hz, 1H), 5.70 (br s, 1H), 5.33 (d, J = 10.4 Hz, 1H), 5.31 (d, J = 17.4 Hz, 1H), 5.23 (d, J = 12.3 Hz, 1H), 5.11 (d, J = 12.3 Hz, 1H), 3.79 (s, 3H), 3.66 (d, J = 13.5 Hz, 1H), 3.34 (d, J = 13.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 154.3, 136.6, 135.4, 129.9−126.9 (11C), 116.1, 66.5, 65.2, 52.8, 40.4. HRMS (ESI) calcd for $C_{20}H_{21}NO_4$ 339.1471, found 339.1466.

(18) Compound **4** was prepared according to ref 14. ^{1}H and ^{13}C NMR spectra are in accordance with literature. $[\alpha]^{25}_D$ + 10.4 (*c* 1.0, $CHCl_3$).

Compound 11. Under N_2 atmosphere, a 33% HBr solution in AcOH (3 mL) was added dropwise to a stirred solution of **10** (705 mg, 2.08 mmol) in AcOH (4.2 mL). After 36 h, the reaction mixture was diluted with H_2O (15 mL), aqueous $NaHCO_3$ solution was added until pH 9, and the mixture was extracted three times with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo to afford **11** (406 mg, 95% yield) as an oil. R_f (EtOAc/hexane 1:1) 0.23. $[\alpha]^{25}_D$ −21.5 (*c* 1.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.22 (m, 3H), 7.21−7.15 (m, 2H), 6.20 (dd, J = 17.3, 10.6 Hz, 1H), 5.37 (d, J = 17.2 Hz, 1H), 5.22 (d, J = 10.6 Hz, 1H), 3.75 (s, 3H), 3.30 (d, J = 13.2 Hz, 1H), 2.88 (d, J = 13.2 Hz, 1H), 1.87 (br s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 139.9, 135.4, 129.7−126.7 (5C), 114.1, 63.3, 51.9, 45.5. HRMS (ESI) calcd for $C_{12}H_{15}NO_2$ 205.1103, found 205.1102.

Compound 12. Under N_2 atmosphere, $(CH_2O)_n$ (80 mg, 2.53 mmol, 1.8 equiv) was added to a solution of **11** (283 mg, 1.38 mmol) in anhydrous CH_2Cl_2 (8 mL). The resulting suspension was treated with TFA (3.5 mL) and magnetically stirred for 24 h. A saturated aqueous solution of $NaHCO_3$ was added until pH 8. The reaction mixture was extracted three times with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/hexane 3:7), affording **12** (290 mg, 97% yield) as a foam. R_f (EtOAc/hexane 1:1) 0.23. $[\alpha]^{25}_D$ −23.9 (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22−7.09 (m, 3H), 7.07−6.99 (m, 1H), 5.98 (dd, J = 17.4, 10.7 Hz, 1H), 5.35 (d, J = 17.4 Hz, 1H), 5.28 (d, J = 10.7 Hz, 1H), 4.18 (d, J = 16.3 Hz, 1H), 4.10 (d, J = 16.0 Hz, 1H), 3.72 (s, 3H), 3.37 (d, J = 16.1 Hz, 1H), 3.03 (d, J = 16.1 Hz, 1H), 2.75 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 139.0, 133.9, 132.7, 129.1−126.3 (4C), 116.7, 62.9, 52.8, 44.8, 36.3. HRMS (ESI) calcd for $C_{13}H_{15}NO_2$ 217.1103, found 217.1114.

Compound 14. Under N_2 atmosphere, DIPEA (2.2 mL, 12.63 mmol, d 0.7 g mL^{-1}, 4 equiv) was added dropwise to a solution of **12** (686 mg, 3.15 mmol) in anhydrous CH_2Cl_2 (25 mL). The reaction mixture was cooled to 0 °C and Ac_2O (1.2 mL, 12.63 mmol, d 1.1 g mL^{-1}, 4 equiv) was added dropwise. The temperature was slowly warmed to rt and the mixture was magnetically stirred for 20 h, then at reflux for 10 h. The reaction mixture was diluted with H_2O (20 mL), 5% aqueous H_3PO_4 (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with sat. aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/hexane 2:3), affording **14** (747 mg, 91% yield) as a foam. R_f (EtOAc/hexane 8:2) 0.25. $[\alpha]^{25}_D$ +17.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.18 (m, 3H), 7.18−7.06 (m, 1H), 6.27 (dd, J = 17.3, 10.6 Hz, 1H), 4.90 (d, J = 10.6 Hz, 1H), 4.69 (d, J = 14.6 Hz, 1H), 4.64 (d, J = 14.3 Hz, 1H), 4.60 (d, J = 17.2 Hz, 1H), 3.77 (s, 3H), 3.28 (d, J = 14.8 Hz, 1H), 3.01 (d, J = 14.8 Hz, 1H), 2.27 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 169.1, 135.6, 133.5, 133.1, 127.6−125.2 (4C), 113.8, 64.7, 52.1, 47.8, 38.0, 22.2. HRMS (EI) calcd for $C_{15}H_{17}NO_3$ 259.1208, found 259.1197.

Compound 13. At 0 °C, under N_2 atmosphere, a 2.0 M LiOH solution in H_2O (7.44 mL, 14.88 mmol, 6 equiv) was added to a solution of **14** (644 mg, 2.48 mmol) in THF (30 mL) and MeOH (4 mL). The reaction mixture was stirred at reflux for 8 h, then concentrated in vacuo, diluted with H_2O (20 mL), and extracted with EtOAc (2 × 20 mL). A 5% aqueous H_3PO_4 solution (20 mL) was added to the aqueous layer and the mixture was extracted with EtOAc (2 × 20 mL), washed with brine, dried over Na_2SO_4, and concentrated in vacuo to afford **13** (554 mg, 91% yield) as a foam. R_f (EtOAc/MeOH 9:1) 0.01. $[\alpha]^{25}_D$ +3.2 (*c* 1.3, CH_3OH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (br s, 1H), 7.41−6.98 (m, 4H), 6.26 (dd, J = 17.3, 10.7 Hz, 1H), 4.93 (d, J = 10.7 Hz, 1H), 4.66 (br s, 2H), 4.63 (d, J = 17.5 Hz, 1H), 3.36 (d, J = 14.8 Hz, 1H), 3.05 (d, J = 14.9 Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 170.5, 135.6, 133.3, 133.2, 127.8−125.1 (4C), 114.1, 65.3, 48.0, 38.2, 22.3. HRMS (ESI) calcd for $C_{14}H_{15}NO_3$ 245.1052, found 245.1056.

Compound 15. At 0 °C, under N_2 atmosphere, a solution of **13** (308 mg, 1.26 mmol) in anhydrous CH_2Cl_2 (7 mL) was added to a solution of PCl_5 (262 mg, 1.26 mmol, 1 equiv) in anhydrous CH_2Cl_2 (6 mL). After stirring at 0 °C for 10 min and at rt for 3 h, the reaction mixture was cooled to −10 °C and added to a suspension of **16** (232 mg, 1.29 mmol, 1 equiv) in dry pyridine (1.65 mL, 20.43 mmol, d 1.0 g mL^{-1}, 16 equiv) and anhydrous CH_2Cl_2 (12 mL). After stirring at −10 °C for 10 min, and at rt for 20 h, water (25 mL) was added, the organic layer was washed with saturated aqueous $NaHCO_3$ solution, water, and 2.5% aqueous H_3PO_4 (15 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/hexane 9:1), affording **15** (209 mg, 45% yield) as a foam. R_f (AcOEt/MeOH 9:1) 0.48. $[\alpha]^{25}_D$ −14.0 (*c* 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3CN, 77 °C) δ 7.36−7.13 (m, 4H), 6.37 (dd, J = 17.3, 10.8 Hz, 1H), 5.91 (m, 1H), 5.35 (d, J = 17.7 Hz, 1H), 5.21 (d, J = 10.5 Hz, 1H), 5.96 (d, J = 10.8 Hz, 1H), 4.81 (br d, J = 17.3 Hz, 2H), 4.61 (br d, J = 15.2 Hz, 2H), 4.36 (br d, J = 14.7 Hz, 1H), 3.98 (br d, J = 15.9 Hz, 1H), 3.66 (s, 3H), 3.41 (d, J = 15.9 Hz, 1H), 3.12 (d, J = 15.9 Hz, 1H), 2.13 (s, 3H), 1.44 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, CD_3CN, 77 °C) δ 177.5, 173.0, 171.4, 137.7, 135.6, 133.4, 132.24, 130.2−126.5 (4C), 117.4, 115.5, 60.8, 54.8, 51.9, 50.8, 48.4, 37.2, 23.3, 14.2. HRMS (ESI) calcd for $C_{21}H_{26}N_2O_4$ 370.1893, found 370.1853.

Compound 17. Under N_2 atmosphere, 1,3-bis(2,4,6-trimethylphenyl)-2-(imidazolidinylidene)(dichlorophenylmethylene)-(tricyclohexylphosphine)ruthenium (Grubbs second generation catalyst) (32 mg, 0.04 mmol, 0.2 equiv) was added to a solution of **15** (69 mg, 0.19 mmol) in anhydrous toluene (6 mL). The reaction mixture was stirred at reflux for 4 h, then concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/MeOH 95:5), affording **17** (58 mg, 91% yield) as an oil. R_f (EtOAc/MeOH 9:1) 0.26. $[\alpha]^{25}_D$ −24.3 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.13 (4H), 5.70 (dt, J = 10.1, 3.2 Hz, 1H), 5.13 (dt, J = 10.1, 2.0 Hz, 1H), 4.68 (d, J = 14.2 Hz, 1H), 4.59 (d, J = 14.2 Hz, 1H), 4.26 (q, J = 7.1 Hz, 1H), 4.16 (br d, J = 15.4 Hz, 1H), 3.97 (br d, J = 16.1 Hz, 1H), 3.77 (s, 3H), 3.55 (d, J = 14.5 Hz, 1H), 2.88 (d, J = 14.5 Hz, 1H), 2.21 (s, 3H), 1.56 (d, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 169.1, 168.8, 134.0, 133.9, 127.9−126.6 (4C), 125.2, 120.4, 59.0, 55.5, 51.8, 48.0, 47.51, 40.5, 22.6, 12.9. HRMS (ESI) calcd for $C_{19}H_{22}N_2O_4$ 342.1580, found 342.1572.

Compound 18. At 0 °C, under N_2 atmosphere, **17** (57 mg, 0.17 mmol) was dissolved in a 8.0 M $MeNH_2$ solution in EtOH (5 mL). The reaction mixture was stirred for 4 h at rt, then evaporated in vacuo. The crude was purified by flash chromatography (EtOAc/MeOH 9:1), affording **18** (51 mg, 90% yield) as a foam. R_f (EtOAc/MeOH 85:15) 0.38. $[\alpha]^{25}_D$ −146.5 (*c* 1.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (br s, 1H), 7.37−7.13 (m, 4H), 5.73 (dt, J = 10.2, 3.2 Hz, 1H), 5.49 (q, J = 7.4 Hz, 1H), 5.13 (dt, J = 10.2, 2.0 Hz, 1H), 4.72 (d, J = 14.2 Hz, 1H), 4.63 (d, J = 14.2 Hz, 1H), 3.97 (dt, J = 18.0, 2.6, 1H), 3.75 (dt, J = 18.0, 2.6 Hz, 1H), 3.60 (d, J = 14.5, 1H), 2.90 (d, J = 14.5 Hz, 1H), 2.80 (d, J = 4.6 Hz, 3H), 2.24 (s, 3H), 1.47 (d, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.8, 168.5, 133.5, 133.5, 127.9−126.9 (4C), 125.4, 120.3, 59.2, 51.4, 48.3, 43.3, 40.5, 26.1, 22.9, 13.3. HRMS (EI) calcd for $C_{19}H_{23}N_3O_3$ 341.1739, found 341.1726.

Compound 2a. To a solution of **18** (30 mg, 0.09 mmol) in MeOH (2 mL) was added 10% Pd/C (6 mg) and the reaction was stirred at rt under H_2 atmosphere for 24 h. The suspension was then filtered through a pad of Celite and evaporated under reduced pressure to afford **2a** (30 mg, quantitative yield) as a white solid. R_f (AcOEt/MeOH 85:15) 0.38. $[\alpha]^{25}_D$ −42.4 (*c* 1.3,

$CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (br s, 1H), 7.39−7.19 (m, 4H), 5.48 (q, J = 7.0 Hz, 1H), 4.69 (d, J = 14.2 Hz, 1H), 4.59 (d, J = 14.2 Hz, 1H), 3.38 (d, J = 14.8 Hz, 1H), 3.33−3.20 (m, 2H), 3.02 (d, J = 14.8 Hz, 1H), 2.83 (d, J = 4.3 Hz, 3H), 2.25 (s, 3H), 2.10−1.97 (m, 1H), 1.90−1.74 (m, 2H), 1.51−1.44 (m, 1H), 1.41 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.1, 170.2, 134.7, 134.0, 128.5−126.2 (4C), 62.2, 51.7, 49.2, 41.5, 37.9, 31.3, 26.7, 23.7, 20.8, 13.8. HRMS (EI) calcd for $C_{19}H_{25}N_3O_3$ 343.1896, found 343.1859.

Supporting Information Available: General procedures and copy of NMR spectra for all compounds; VT NMR studies, DMSO-d_6 titrations, IR and crystallographic data for compound **2a**; computational details. This material is available free of charge via the Internet at http://pubs.acs.org.