Regioselective Preparation of Functionalized Isoxazoline Derivatives as Key Intermediates for the Synthesis of Selective *N*-Methyl-D-aspartate Receptor Antagonists

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Received 10 December 2010; revised 10 February 2011

Abstract: Two highly versatile isoxazoline derivatives, as key intermediates for the synthesis of differently functionalized subtype-selective *N*-methyl-D-aspartate receptor antagonists, were designed and synthesized. The synthetic strategy is based on an intramolecular nitrile oxide cycloaddition reaction which is a powerful method capable of controlling both the regio- and stereochemistry of the pericyclic reaction.

Key words: *N*-methyl-D-aspartate receptor antagonist, intramolecular nitrile oxide cycloaddition, regioselective, isoxazoline, amino acids

N-Methyl-D-aspartate (NMDA) receptors are subtypes of the family of ligand-gated ion channels activated by endogenous L-glutamate, the major excitatory neurotransmitter of the central nervous system. NMDA receptors mediate the slow component of glutamatergic excitatory synaptic currents. The entry of calcium (Ca^{2+}) ions into neurons, via this receptor channel, triggers a cascade of biochemical events that leads to physiologically relevant processes such as synaptic plasticity and synaptogenesis as well as pathological events, for example, excitotoxicity.[1,2]

NMDA receptor antagonists have been investigated for many years as therapeutic agents for the treatment of neurological disorders such as stroke, epilepsy, pain and Parkinson's disease. However, it has been discovered that the majority of drug candidates cause adverse psychotomimetic effects due to the blockade of normal neuronal function.[3]

The majority of NMDA receptors are comprised of two GluN1 and two GluN2 subunits. The GluN1 subunit, the sole member of the GluN1 subfamily, is essential for functional NMDA receptor channels and for their exit from the endoplasmic reticulum, whereas four members of the GluN2 subfamily (GluN2A–D) are the major determinants of functional diversity and synaptic localization.[4] Current efforts are devoted to the development of subtype-selective NMDA receptor antagonists that might retain therapeutic utility without the side effects associated with the non-selective examples.

In a previous paper, we disclosed two new competitive NMDA receptor antagonists, (–)-**1** and (+)-**2** (Figure 1), which were characterized by a slight preference for GluN2A and GluN2B versus GluN2C and GluN2D subtypes.[5] In order to improve their selectivity profile we planned to increase their molecular complexity, according to the general concept that new substituents could create additional interactions with the binding site of one receptor subunit, or determine a steric clash in another. A suitable position for further derivatization of our model compounds is C-4 of the isoxazoline ring.

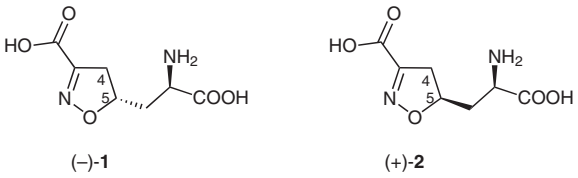
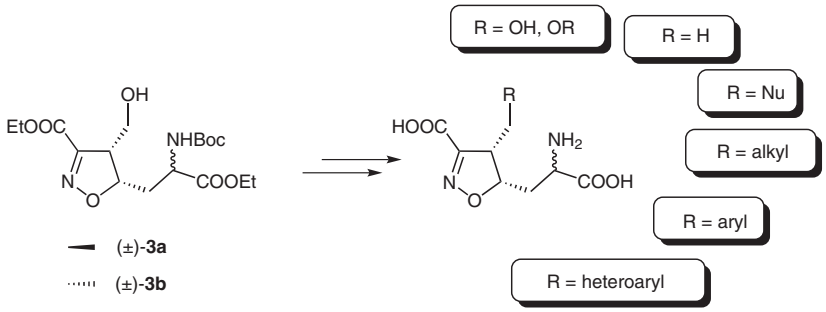


Figure 1 Structures of NMDA receptor antagonists (–)-**1** and (+)-**2**

Thus, we identified compounds (±)-**3a**,**b** as useful key intermediates that, employing conventional synthetic steps, could be easily transformed into a series of differently functionalized amino acid derivatives (R = OH, OR, Nu, alkyl, aryl, heteroaryl) (Scheme 1). Compounds endowed with a promising biological profile could eventually be resolved into pure enantiomers by chiral high-performance liquid chromatography (HPLC). As an alternative, enantiomerically pure target derivatives could be obtained directly after enzymatic resolution of racemic intermediates (±)-**3a** or (±)-**3b**, a strategy successfully applied to our model compounds.[5]

The synthesis of 4,5-disubstitued-4,5-dihydroisoxazole derivatives can be achieved via 1,3-dipolar cycloaddition of a nitrile oxide to the appropriate *cis*- or *trans*-1,2-disubstituted alkene.[6] The advantage of such a methodology is the complete translocation of the stereochemical features of the reagents into the final products. For example, by reacting a nitrile oxide with a *cis*-1,2-disubstituted alkene, only a *cis*-4,5-disubstituted 4,5-dihydroisoxazole is obtained.[7] However, in many cases this strategy lacks control of the regiochemistry, producing a mixture of the two

SYNTHESIS 2011, No. 8, pp 1255–1260
Advanced online publication: 16.03.2011
DOI: 10.1055/s-0030-1258477; Art ID: Z52810SS

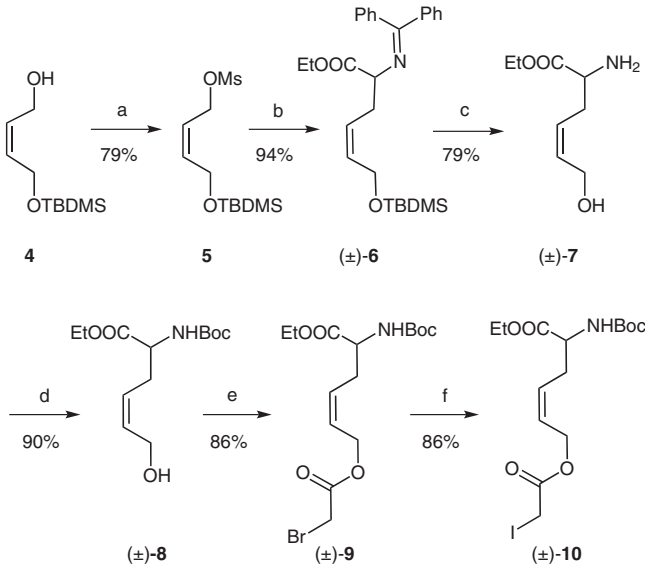


Scheme 1 Structures of key intermediates (±)-**3a**,**b** and conceivable functionalized amino acids

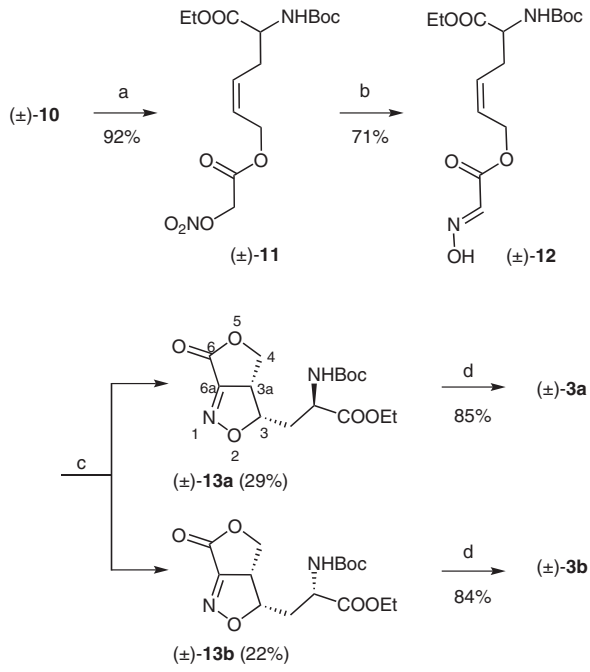
possible regioisomers.[8] Moreover, in the present case, the additional stereogenic center present in the α-amino acid moiety would produce a complex mixture of four stereoisomers, which would be difficult to separate by column chromatography. Therefore, we devised a regioselective synthesis of key intermediates (±)-**3a** and (±)-**3b** based on an intramolecular nitrile oxide cycloaddition (INOC) of unsaturated oxime (±)-**12**. The INOC methodology is a powerful tool, characterized by excellent control of both regio- and stereochemistry, which has been widely employed to prepare complex medium- and large-sized cyclic compounds.[9]

The synthetic route to obtain key intermediates (±)-**3a** and (±)-**3b** started from commercially available (2*Z*)-but-2-ene-1,4-diol, which was transformed into the corresponding monosilyl ether **4** using *tert*-butyldimethylsilyl chloride, following a literature procedure.[10] Subsequently, the second hydroxy group was converted into a mesylate using standard chemistry, and then submitted to a nucleophilic displacement reaction using the preformed anion of ethyl-*N*-(diphenylmethylene)glycinate,[11,12] yielding intermediate (±)-**6** (Scheme 2). Treatment of (±)-**6** with hydrochloric acid (1 M) induced cleavage of both the imino and silyl groups to yield amino alcohol (±)-**7** which was immediately transformed into the *tert*-butylcarbamate (±)-**8**. The hydroxy group was acylated with 2-bromoacetyl bromide in the presence of pyridine, and the resulting bromo derivative (±)-**9** was treated with sodium iodide in acetone to give the corresponding iodo derivative (±)-**10** (Scheme 1).

The iodo moiety was needed in order to accomplish efficiently its nucleophilic substitution by a nitrate anion to give (±)-**11**.[13] Intermediate (±)-**11** was then transformed into the corresponding oxime by reaction with hydroxylamine hydrochloride in dimethyl sulfoxide (Scheme 2).[14] The key step of this synthetic sequence is the one-pot chlorination and elimination of hydrogen chloride to generate the nitrile oxide in situ which then underwent an INOC reaction. This transformation was performed by adding dropwise a 3.5% solution of sodium hypochlorite (NaOCl) to a vigorously stirred solution of oxime (±)-**12** in dichloromethane (Scheme 3). The intramolecular cycloaddition reaction was very fast and resulted in forma-



Scheme 2 *Reagents and conditions*: (a) MsCl, Et_3N, CH_2Cl_2; (b) $(C_6H_5)_2C{=}NCH_2CO_2Et$, LDA, THF, –78 °C; (c) 1 M HCl, Et_2O; (d) Boc_2O, Et_3N, CH_2Cl_2; (e) $BrCH_2COBr$, py, CH_2Cl_2, 0 °C; (f) NaI, acetone.



Scheme 3 *Reagents and conditions*: (a) $AgNO_3$, MeCN; (b) $NH_2OH{\cdot}HCl$, NaOAc, DMSO; (c) NaOCl (3.5%), CH_2Cl_2; (d) K_2CO_3, EtOH.

tion of the two diastereoisomers (±)-**13a** and (±)-**13b**, which could be separated easily by column chromatography.

Compound (±)-**13a** was successfully crystallized from acetonitrile, and its relative stereochemistry secured by single crystal X-ray analysis (Figure 2).

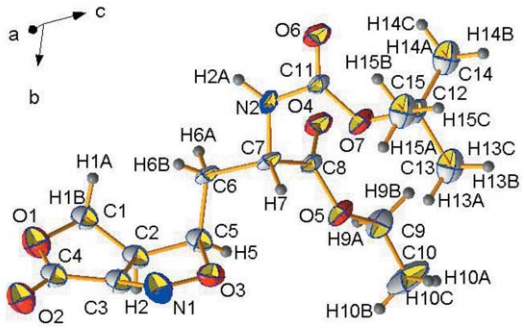


Figure 2 ORTEP view of the asymmetric unit of compound (±)-**13a**; thermal ellipsoids are drawn at the 25% probability level

Finally, the desired products (±)-**3a** and (±)-**3b** were obtained in good yield by ethanolysis of lactones (±)-**13a** and (±)-**13b**.

In conclusion, we have accomplished an efficient synthesis of the key intermediates (±)-**3a** and (±)-**3b** by applying an INOC synthetic strategy on the appropriate unsaturated oxime (±)-**12**. Isoxazolines (±)-**3a** and (±)-**3b** represent valuable synthetic tools and will be used in due course for the preparation of a series of analogues of (±)-**1** and (±)-**2**, in order to evaluate the influence of the substituent at the 4-position of the isoxazoline nucleus on the potency and NMDA subtype selectivity.

All reagents were purchased from Sigma. TLC analyses were performed on Fluka silica gel 60 F_{254} aluminum sheets; spots were made visual by spraying with a dilute alkaline $KMnO_4$ solution or with ninhydrin. Column chromatography was carried out using Nova Chimica silica gel LC-60A (60–200 μm). Melting points were determined using a model B 540 Büchi apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hz. Elemental analyses were obtained using a Thermo Finnigan Elemental Analyzer Flash EA 1112.

(2*Z*)-4-[(*tert*-Butyldimethylsilyl)oxy]but-2-en-1-yl Methanesulfonate (5)

A soln of alcohol **4** (7.1 g, 35.0 mmol) in CH_2Cl_2 (150 mL) was cooled to 0 °C, and Et_3N (5.3 mL, 38.5 mmol) and MsCl (3 mL, 38.5 mmol) were added dropwise. The mixture was stirred at r.t. for 3 h. H_2O was added (100 mL), and the aq layer was extracted with $CHCl_3$ (2 × 100 mL). The combined organic layer was dried over anhyd Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (cyclohexane–EtOAc, 4:1) to give the corresponding mesylate **5**.

Yellow oil; yield: 7.7 g (79%); R_f = 0.28 (cyclohexane–EtOAc, 9:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.10 (s, 6 H), 0.88 (s, 9 H), 3.00 (s, 3 H), 4.26–4.31 (m, 2 H), 4.86 (dd, *J* = 1.1, 6.8 Hz, 2 H), 5.56–5.66 (m, 1 H), 5.83 (ddt, *J* = 1.1, 5.5, 5.5 Hz, 1 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = –5.10, 18.48, 26.08, 38.33, 59.85, 65.82, 122.59, 136.14.

Anal. Calcd for $C_{11}H_{24}O_4SSi$: C, 47.11; H, 8.63. Found: C, 47.38; H, 8.89.

Ethyl (4*Z*)-6-[(*tert*-Butyldimethylsilyl)oxy]-2-[(diphenylmethylidene)amino]hex-4-enoate [(±)-6]

n-BuLi (15 mL, 24.0 mmol, 1.6 M in hexanes) and DMPU (7 mL, 58.0 mmol) were added dropwise to a soln of diisopropylamine (3.4 mL, 24 mmol) in anhyd THF (37 mL) at –78 °C. A soln of ethyl-*N*-(diphenylmethylene)glycinate (6.7 g, 26.4 mmol) in THF (36 mL) was added dropwise and the resulting mixture stirred at –78 °C for 3 h. A soln of mesylate **5** (6.7 g, 24.0 mmol) in anhyd THF (15 mL) was added and the mixture was stirred for a further 30 min at –78 °C and then warmed to r.t. After 30 min, a sat. soln of NH_4Cl (8 mL) was added and the mixture was extracted with Et_2O (3 × 50 mL). The combined organic layer was dried over anhyd Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography (cyclohexane–EtOAc, 98:2) to afford (±)-**6**.

Colorless oil; yield: 10.2 g (94%); R_f = 0.38 (cyclohexane–EtOAc, 9:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = –0.01 (s, 3 H), 0.01 (s, 3 H), 0.84 (s, 9 H), 1.22 (t, *J* = 7.0 Hz, 3 H), 2.59–2.66 (m, 2 H), 4.02–4.26 (m, 5 H), 5.22–5.33 (m, 1 H), 5.50–5.59 (m, 1 H), 7.10–7.15 (m, 2 H), 7.20–7.45 (m, 6 H), 7.57–7.63 (m, 2 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = –4.91, 14.43, 18.57, 26.21, 32.11, 59.85, 61.20, 65.44, 125.75, 128.26, 128.72, 128.85, 129.08, 130.59, 132.83, 136.57, 139.65, 170.84, 171.96.

Anal. Calcd for $C_{27}H_{37}NO_3Si$: C, 71.80; H, 8.26; N, 3.10. Found: C, 71.68; H, 8.08; N, 3.00.

Ethyl (4*Z*)-2-[(*tert*-Butoxycarbonyl)amino]-6-hydroxyhex-4-enoate [(±)-8]

An aq soln of HCl (1 M, 83 mL) was added to a stirred soln of (±)-**6** (10.2 g, 22.6 mmol) in Et_2O (83 mL). The mixture was stirred at r.t. for 6 h. The organic layer was separated and the aq layer was made basic with K_2SO_4, and extracted with EtOAc (2 × 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford the free amine (±)-**7** (3.1 g, 18.1 mmol) as a yellow oil; R_f = 0.15 (EtOAc–MeOH, 9:1).

Et_3N (3.8 mL, 27.1 mmol) and Boc_2O (4.3 g, 19.9 mmol) were added to a soln of the amine (±)-**7** (3.1 g, 18.1 mmol) in CH_2Cl_2 (70 mL) at 0 °C. The mixture was stirred at r.t. for 12 h and then washed with aq HCl (1 M, 2 × 20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (cyclohexane–EtOAc, 3:2) to afford alcohol (±)-**8**.

Yellow oil; yield: 4.4 g (71% over two steps); R_f = 0.19 (cyclohexane–EtOAc, 7:3).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.24 (t, *J* = 7.1 Hz, 3 H), 1.39 (s, 9 H), 2.40 (br s, 1 H), 2.44–2.53 (m, 1 H), 2.53–2.65 (m, 1 H), 4.09–4.12 (m, 2 H), 4.16 (q, *J* = 7.1 Hz, 2 H), 4.26–4.34 (m, 1 H), 5.30 (br d, *J* = 7.5 Hz, 1 H), 5.36–5.48 (m, 1 H), 5.71–5.82 (m, 1 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.37, 26.47, 30.48, 53.31, 58.18, 61.70, 80.19, 126.17, 132.88, 155.52, 172.25.

Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.46; H, 8.59; N, 5.21.

Ethyl (4*Z*)-6-[(Bromoacetyl)oxy]-2-[(*tert*-butoxycarbonyl)amino]hex-4-enoate [(±)-9]

Pyridine (2.2 mL, 27.0 mmol) and 2-bromoacetyl bromide (1.6 mL, 18.9 mmol) were added dropwise to an ice-cold soln of (±)-**8** (3.7 g, 13.5 mmol) in CH_2Cl_2 (60 mL). The mixture was stirred at 0 °C for 30 min. Cold H_2O (30 mL) was added and the organic layer was separated. The aq layer was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers dried over anhyd Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (cyclohexane–EtOAc, 7:3) to afford bromide (±)-**9**.

Yellow oil; yield: 4.6 g (86%); R_f = 0.27 (cyclohexane–EtOAc, 85:15).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.25 (t, *J* = 7.1 Hz, 3 H), 1.40 (s, 9 H), 2.48–2.70 (m, 2 H), 3.82 (s, 2 H), 4.17 (q, *J* = 7.1 Hz, 2 H), 4.35 (dd, *J* = 7.1, 12.6 Hz, 1 H), 4.56–4.73 (m, 2 H), 5.18 (br d, *J* = 7.1 Hz, 1 H), 5.55–5.75 (m, 2 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.40, 25.96, 28.50, 30.76, 53.06, 61.76, 61.77, 80.17, 126.50, 129.96, 155.40, 167.26, 171.91.

Anal. Calcd for $C_{15}H_{24}BrNO_6$: C, 45.70; H, 6.14; N, 3.55. Found: C, 45.42; H, 5.89; N, 3.38.

Ethyl (4*Z*)-2-[(*tert*-Butoxycarbonyl)amino]-6-[(iodoacetyl)oxy]hex-4-enoate [(±)-10]

NaI (4.0 g, 27.0 mmol) was added to a stirred soln of compound (±)-**9** (4.3 g, 10.8 mmol) in acetone (60 mL). The mixture was stirred at r.t. for 1 h. The resulting solid was removed by filtration and the filtrate was concentrated under vacuum, redissolved in EtOAc (120 mL) and washed with 10% aq $Na_2S_2O_3$ soln (60 mL), dried over anhyd Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (cyclohexane–EtOAc, 85:15) to afford iodide (±)-**10**.

Yellow oil; yield: 4.1 g (86%); R_f = 0.27 (cyclohexane–EtOAc, 85:15).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26 (t, *J* = 7.1 Hz, 3 H), 1.40 (s, 9 H), 2.50–2.70 (m, 2 H), 3.67 (s, 2 H), 4.18 (q, *J* = 7.1 Hz, 2 H), 4.30–4.40 (m, 1 H), 4.56–4.70 (m, 2 H), 5.16 (br d, *J* = 7.0 Hz, 1 H), 5.55–5.74 (m, 2 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = –5.44, 14.42, 28.53, 30.78, 53.10, 61.63, 61.76, 80.17, 126.57, 129.77, 155.42, 168.79, 171.93.

Anal. Calcd for $C_{15}H_{24}INO_6$: C, 40.83; H, 5.48; N, 3.17. Found: C, 41.08; H, 5.62; N, 3.24.

Ethyl (4*Z*)-2-[(*tert*-Butoxycarbonyl)amino]-6-[(nitrooxy)acetoxy]hex-4-enoate [(±)-11]

$AgNO_3$ (3.1 g, 18.5 mmol) was added to a soln of iodide (±)-**10** (4.1 g, 9.3 mmol) in MeCN (50 mL) in the dark under an N_2 atm. The mixture was stirred for 12 h at r.t. Et_2O (80 mL) was added, and the solid removed by filtration. The soln was concentrated under reduced pressure and the residue purified by column chromatography (cyclohexane–EtOAc, 4:1) to afford compound (±)-**11**.

Yellow oil; yield: 3.2 g (92%); R_f = 0.20 (cyclohexane–EtOAc, 4:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28 (t, *J* = 7.1 Hz, 3 H), 1.44 (s, 9 H), 2.50–2.74 (m, 2 H), 4.20 (q, *J* = 7.1 Hz, 2 H), 4.37 (dd, *J* = 6.6, 13.2 Hz, 1 H), 4.64–4.81 (m, 2 H), 4.92 (s, 2 H), 5.20 (br d, *J* = 7.4 Hz, 1 H), 5.60–5.76 (m, 2 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.34, 28.45, 30.73, 53.07, 61.52, 61.77, 67.25, 80.16, 126.12, 130.31, 155.38, 165.93, 171.85.

Anal. Calcd for $C_{15}H_{24}N_2O_9$: C, 47.87; H, 6.43; N, 7.44. Found: C, 48.00; H, 6.56; N, 7.60.

Ethyl (4*Z*)-2-[(*tert*-Butoxycarbonyl)amino]-6-[(2*E*)-2-(hydroxyimino)acetoxy]hex-4-enoate [(±)-12]

NH_2OH·HCl (0.88 g, 12.7 mmol) and NaOAc (2.1 g, 25.5 mmol) were added to a soln of compound (±)-**11** (3.2 g, 8.5 mmol) in DMSO (50 mL). The mixture was stirred for 2 h at r.t., and then brine (25 mL) was added. The mixture was extracted with Et_2O (3 × 15 mL), and the combined organic layer dried over anhyd Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (cyclohexane–EtOAc, 1:1) to give oxime (±)-**12**.

Yellow oil; yield: 2.1 g (71%); R_f = 0.23 (cyclohexane–EtOAc, 75:25).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.25 (t, *J* = 7.1 Hz, 3 H), 1.40 (s, 9 H), 2.49–2.68 (m, 2 H), 4.15 (q, *J* = 7.1 Hz, 2 H), 4.34–4.40 (m, 1 H), 4.65–4.80 (m, 2 H), 5.30 (br d, *J* = 7.3 Hz, 1 H), 5.58–5.70 (m, 2 H), 7.48 (br s, 1 H), 7.50 (s, 1 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.34, 28.43, 30.76, 53.12, 60.89, 61.85, 80.38, 126.40, 129.84, 141.43, 155.70, 162.31, 172.23.

Anal. Calcd for $C_{15}H_{24}N_2O_7$: C, 52.32; H, 7.02; N, 8.13. Found: C, 51.99; H, 6.89; N, 8.00.

Ethyl (2*R)-2-[(*tert*-Butoxycarbonyl)amino]-3-[(3*S**,3a*R**)-6-oxo-3a,4-dihydro-3*H*,6*H*-furo[3,4-*c*]isoxazol-3-yl]propanoate [(±)-13a] and Ethyl (2*S**)-2-[(*tert*-Butoxycarbonyl)amino]-3-[(3*S**,3a*R**)-6-oxo-3a,4-dihydro-3*H*,6*H*-furo[3,4-*c*]isoxazol-3-yl]propanoate [(±)-13b]**

A soln of NaOCl (3.5%, 7 mL) was added dropwise to a soln of the oxime (±)-**12** (2.1 g, 6.1 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred for 30 min at r.t., and the organic layer was separated, washed with H_2O (3 × 10 mL) and dried over anhyd Na_2SO_4. The solvent was evaporated under vacuum and the crude material purified by flash chromatography (cyclohexane–EtOAc, 7:3) to give compounds (±)-**13a** and (±)-**13b** as white solids in 51% combined yield.

Compound (±)-13a

Yield: 0.60 g (29%); crystallized as colorless prisms from EtOAc–hexane (1:1); mp 144–146 °C; R_f = 0.39 (cyclohexane–EtOAc, 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (t, *J* = 7.1 Hz, 3 H), 1.44 (s, 9 H), 2.17 (dd, *J* = 6.9, 6.9 Hz, 2 H), 4.20 (dd, *J* = 5.8, 12.2 Hz, 1 H), 4.26 (q, *J* = 7.1 Hz, 2 H), 4.42–4.60 (m, 2 H), 4.65–4.75 (m, 1 H), 5.20 (ddd, *J* = 7.7, 7.7, 10.2 Hz, 1 H), 5.42 (br s, 1 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.33, 28.50, 32.70, 50.59, 51.51, 62.56, 66.35, 80.79, 84.78, 155.50, 155.73, 158.40, 171.10.

Anal. Calcd for $C_{15}H_{22}N_2O_7$: C, 52.63; H, 6.48; N, 8.18. Found: C, 52.68; H, 6.56; N, 7.93.

Compound (±)-13b

Yield: 0.46 g (22%); crystallized as colorless prisms from EtOAc–hexane (1:1); mp 46–48 °C; R_f = 0.29 (cyclohexane–EtOAc, 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26 (t, *J* = 7.1 Hz, 3 H), 1.40 (s, 9 H), 1.70–1.90 (m, 1 H), 2.26 (ddd, *J* = 4.1, 10.2, 14.3 Hz, 1 H), 4.12–4.28 (m, 2 H), 4.34 (ddd, *J* = 4.1, 9.1, 9.1 Hz, 1 H), 4.40–4.46 (m, 1 H), 4.48–4.64 (m, 2 H), 5.22 (ddd, *J* = 3.8, 10.2, 10.2 Hz, 1 H), 5.34 (br d, *J* = 9.1 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.36, 28.49, 33.14, 51.08, 51.21, 62.33, 66.20, 80.85, 85.21, 155.50, 155.70, 158.27, 171.56.

Anal. Calcd for $C_{15}H_{22}N_2O_7$: C, 52.63; H, 6.48; N, 8.18. Found: C, 52.89; H, 6.57; N, 7.89.

Ethyl (4*R,5*S**)-5-{(2*R**)-3-Ethoxy-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl}-4-(hydroxymethyl)-4,5-dihydroisoxazole-3-carboxylate [(±)-3a]**

Compound (±)-**13a** (600 mg, 1.8 mmol) was dissolved in EtOH (30 mL) and K_2CO_3 (248 mg, 1.8 mmol) was added. The mixture was stirred at r.t. for 1 h. The solvent was evaporated, Et_2O (30 mL) was added and the solid was removed by filtration. The solvent was removed under vacuum and the crude material was purified by column chromatography (cyclohexane–EtOAc, 1:1) to give isoxazole (±)-**3a**.

Colorless oil; yield: 594 mg (85%); R_f = 0.35 (cyclohexane–EtOAc, 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26 (t, *J* = 7.2 Hz, 3 H), 1.35 (t, *J* = 7.2 Hz, 3 H), 1.40 (s, 9 H), 2.27 (ddd, *J* = 6.9, 9.2, 15.0 Hz, 1 H), 2.38–2.50 (m, 1 H), 2.84 (br s, 1 H), 3.58 (ddd, *J* = 4.7, 4.7, 9.2 Hz, 1 H), 3.80–3.96 (m, 2 H), 4.20 (q, *J* = 7.2 Hz, 2 H), 4.32 (q, *J* = 7.2 Hz, 2 H), 4.39–4.47 (m, 1 H), 4.82 (ddd, *J* = 4.7, 9.2, 9.2 Hz, 1 H), 5.42 (br d, *J* = 6.9 Hz, 1 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.28, 28.50, 29.88, 31.44, 51.19, 51.77, 58.33, 62.07, 62.55, 80.39, 83.04, 153.15, 155.70, 161.58, 171.94.

Anal. Calcd for $C_{17}H_{28}N_2O_8$: C, 52.57; H, 7.27; N, 7.21. Found: C, 52.62; H, 7.32; N, 7.01.

Ethyl (4*R,5*S**)-5-{(2*S**)-3-Ethoxy-2-[(*tert*-butoxycarbonyl)amino]-3-oxopropyl}-4-(hydroxymethyl)-4,5-dihydroisoxazole-3-carboxylate [(±)-3b]**

Compound (±)-**13b** (460 mg, 1.3 mmol) was dissolved in EtOH (15 mL) and K_2CO_3 (180 mg, 1.3 mmol) was added. The mixture was stirred at r.t. for 1 h. The solvent was evaporated, Et_2O (15 mL) was added and the solid was removed by filtration. The solvent was removed under vacuum and the crude material was purified by column chromatography (cyclohexane–EtOAc, 1:1) to afford isoxazole (±)-**3b**.

Colorless oil; yield: 424 mg (84%); R_f = 0.35 (cyclohexane–EtOAc, 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26 (t, *J* = 7.0 Hz, 3 H), 1.36 (t, *J* = 7.0 Hz, 3 H), 1.41 (s, 9 H), 2.22–2.40 (m, 2 H), 3.06 (br s, 1 H), 3.58 (ddd, *J* = 5.3, 5.3, 9.9 Hz, 1 H), 3.80–3.95 (m, 2 H), 4.18 (q, *J* = 7.0 Hz, 2 H), 4.34 (q, *J* = 7.0 Hz, 2 H), 4.42–4.50 (m, 1 H), 4.82 (ddd, *J* = 5.0, 9.9, 9.9 Hz, 1 H), 5.50 (br d, *J* = 8.5 Hz, 1 H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.27, 28.50, 30.53, 31.22, 51.21, 51.88, 58.10, 61.95, 62.62, 80.44, 83.45, 153.18, 155.82, 161.48, 172.17.

Anal. Calcd for $C_{17}H_{28}N_2O_8$: C, 52.57; H, 7.27; N, 7.21. Found: C, 52.63; H, 7.33; N, 6.99.

Ethyl (2*R)-2-[(*tert*-Butoxycarbonyl)amino]-3-[(3*S**,3a*R**)-6-oxo-3a,4-dihydro-3*H*,6*H*-furo[3,4-*c*]isoxazol-3-yl]propanoate [(±)-13a]; X-ray Analysis**

Crystals of the title compound were grown overnight by slow evaporation at 4 °C from a drop of MeCN covered by an inert oil. After testing several specimens, a very thin needle (0.225 × 0.025 × 0.025 mm^3) was eventually judged of suitable quality for single-crystal X-ray diffraction analysis.

Crystal data: $C_{15}H_{22}N_2O_7$, formula weight = 342.3, space group: $P\bar{1}$ (No 2), unit cell (Å, deg, $Å^3$): a = 6.033 (4), b = 12.131 (8), c = 13.610 (8), α = 114.76 (2), β = 99.73 (2), γ = 94.09 (2), V = 880.2 (10), Z = 2, Dc = 1.292 g cm^{-3}, F_{000} = 364, μ = 0.103 mm^{-1}.

Data Collection and Reduction

A total of 5692 reflections within an entire sphere [$(\sin\theta/\lambda)_{max}$ = 0.36 $Å^{-1}$], corresponding to 674 independent data, were collected using a graphite-monochromated MoK_α radiation source (λ = 0.71073 Å) on a Bruker AXS Smart Apex diffractometer, equipped with an Apex II CCD detector. Integration of the diffraction frames was performed using the APEX2 program suite [Bruker (2010); APEX2, AXScale and SAINT; Bruker AXS Inc., Madison, Wisconsin, USA], while the SADABS and XPREP programs were employed to analyze, scale and merge multiple measurements within the raw dataset.

Structure Solution and Refinement

The structure was solved by dual-space direct methods available within the APEX2 program package. Some attempts were also made to solve and refine the structure as *P*1, as we judged the E^2 statistics as not conclusive to assess the presence of the inversion center because of the large amount of individual very weak measurements. These attempts, however, were all unsuccessful. Careful analysis of the heavy atom positions of the symmetry-independent molecules in *P*1 symmetry revealed that, in fact, inversion centers existed in the unit cell. Hence the $P\bar{1}$ space group was eventually chosen as being the most reasonable. Due to the small dimensions of the crystals and limited scattering power of the substance, the final precision of the refined thermal and geometrical parameters was quite low. Such problems however, did not prevent us from reliably assessing the relative orientation of the two stereogenic centers. Final least-squares agreement factors (SHELX)[15]: $R(F)$ = 0.0927 (for 433 independent reflections with $I > 2\sigma(I)$), $wR(F^2)$ = 0.2034 (all independent data), goodness-of-fit = 1.062, residual Fourier density in the unit cell: $\Delta\rho_{max}$ = +0.24/–0.21 e/$Å^3$. Selected bond lengths (Å) and angles (°): N1–O3 1.39(2), N1–C3 1.29(3), O3–C5 1.49(2), N2–C7 1.47(2), N2–C11 1.38(3), C3–N1–O3 106(2), N1–O3–C5 110(2), C7–N2–C11 117(2).

CCDC 802274 contains the supplementary crystallographic data for the crystal structure in this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgment

Financial support from the Italian Ministry of Education (MIUR) and University of Milan are gratefully acknowledged.

References

(1) Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. *Pharmacol. Rev.* **1999**, *51*, 7.
(2) Carroll, R. C.; Zukin, R. S. *Trends Neurosci.* **2002**, *25*, 571.
(3) Chen, H.-S. V.; Lipton, S. A. *J. Neurochem.* **2006**, *97*, 1611.
(4) Paoletti, P.; Neyton, J. *Curr. Opin. Pharmacol.* **2007**, *7*, 39.
(5) Conti, P.; De Amici, M.; Grazioso, G.; Roda, G.; Pinto, A.; Hansen, K. B.; Nielsen, B.; Madsen, U.; Bräuner-Osborne, H.; Egebjerg, J.; Vestri, V.; Pellegrini-Giampietro, D. E.; Sibille, P.; Acher, F. C.; De Micheli, C. *J. Med. Chem.* **2005**, *48*, 6315.
(6) (a) Karlsson, S.; Högberg, H.-E. *Org. Prep. Proced. Int.* **2001**, *33*, 103. (b) Easton, C. J.; Hughes, C. M. M.; Savage, G. P.; Simpson, G. W. *Adv. Heterocycl. Chem.* **1994**, *60*, 261. (c) Gallos, J. K.; Koumbis, A. E. *Curr. Org. Chem.* **2003**, *7*, 397.
(7) (a) Bianchi, G.; Gandolfi, R. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; John Wiley & Sons: New York, **1984**, 477. (b) Yoshida, Y.; Ukaji, Y.; Fujinami, S.; Inomata, K. *Chem. Lett.* **1998**, *10*, 1023. (c) Askri, M.; Jgham, N.; Rammah, M.; Ciamala, K.; Monnier-Jobe, K.; Vebrel, J. *Heterocycles* **2007**, *71*, 289.
(8) (a) Caramella, P.; Grunanger, P. *Nitrile Oxides and Imines,* In *1,3-Dipolar Cycloaddition Chemistry*, Vol. 1; Padwa, A.,

Ed.; John Wiley & Sons: New York, **1984**, Chap. 3, 291–392. (b) Conti, P.; De Amici, M.; Pinto, A.; Tamborini, L.; Grazioso, G.; Frølund, B.; Nielsen, B.; Thomsen, C.; Ebert, B.; De Micheli, C. *Eur. J. Org. Chem.* **2006**, 5533. (c) Faita, G.; Mella, M.; Mortoni, A.; Paio, A.; Quadrelli, P.; Seneci, P. *Eur. J. Org. Chem.* **2002**, 1175. (d) Trogu, E.; De Sarlo, F.; Machetti, F. *Chem. Eur. J.* **2009**, *15*, 7940.

(9) (a) Ramachandiran, K.; Karthikeyan, K.; Muralidharan, D.; Perumal, P. T. *Tetrahedron Lett.* **2010**, *51*, 3006. (b) Chapelon, A.-S.; Giorgi, M.; Ollivier, C.; Santelli, M. *Synth. Commun.* **2010**, *40*, 87. (c) Shing, T. K. M.; So, K. H.; Kwok, W. S. *Org. Lett.* **2009**, *11*, 5070. (d) Kamimura, A.; Yoshida, T.; Uno, H. *Tetrahedron* **2008**, *64*, 11081. (e) Vaidya, V. V.; Wankhede, K. S.; Salunkhe, M. M.; Trivedi, G. K. *Synth. Commun.* **2008**, *38*, 2392. (f) Nair, V.; Suja, T. D. *Tetrahedron* **2007**, *63*, 12247. (g) Pellissier, H. *Tetrahedron* **2007**, *63*, 3235. (h) Shing, T. K. M.; Wong, W. F.; Cheng, H. M.; Kwok, W. S.; So, K. H. *Org. Lett.* **2007**, *9*, 753. (i) Xu, J.; Wang, J.; Ellis, E. D.; Hamme, A. T. II *Synthesis* **2006**, 3815. (j) Shing, T. K. M.; Zhong, Y.-L. *Synlett* **2006**, 1205. (k) Park, K.-H.; Marshall, W. J. *Tetrahedron Lett.* **2004**, *45*, 4931. (l) Akritopoulou-Zanze, I.; Gracias, V.; Moore, J. D.; Djuric, S. W. *Tetrahedron Lett.* **2004**, *45*, 3421. (m) Yip, C.; Handerson, S.; Tranmer, G. K.; Tam, W. *J. Org. Chem.* **2001**, *66*, 276. (n) Yip, C.; Handerson, S.; Jordan, R.; Tam, W. *Org. Lett.* **1999**, *1*, 791.

(10) Mauleon, P.; Krinsky, J. L.; Toste, F. D. *J. Am. Chem. Soc.* **2009**, *131*, 4513.

(11) O'Donnell, M. J.; Polt, R. L. *J. Org. Chem.* **1982**, *47*, 2663.

(12) Bachi, M. D.; Balanov, A.; Bar-Ner, N. *J. Org. Chem.* **1994**, *59*, 7752.

(13) Tschaen, D.; Whittle, R.; Weinreb, S. *J. Org. Chem.* **1986**, *51*, 2604.

(14) Hassner, A.; Murthy, K. S. K.; Padwa, A.; Chiacchio, U.; Dean, D. C.; Schoffstall, A. M. *J. Org. Chem.* **1989**, *54*, 5277.

(15) Sheldrick, G. M. *Acta Crystallogr. A* **2008**, *64*, 112.