Efficient synthesis of kainic acid analogues

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Abstract
The present paper deals with an improved synthesis of two molecular hybrids of AMPA and KA, compounds **CIP-A** and **CIP-B**, and their transformation into **CIOP-A** and **CIOP-B**, the corresponding amido derivatives. Exploiting the continuous-flow technology, a significant improvement in the synthesis of the glutamate agonists **CIP-A** and **CIP-B** was accomplished, in terms of overall yield, time, and excess of ethyl chlorooximinoacetate. Moreover, we find out the HPLC conditions suitable to separate, at a preparative level, the three intermediates formed in the 1,3-dipolar cycloaddition step.

Keywords: 1,3-Dipolar cycloaddition, flow chemistry, preparative HPLC separation, glutamate receptor ligands, amino acids

Introduction

The acidic amino acid neurotransmitter L-glutamate (Glu) plays a pivotal role in the excitatory pathways of the mammalian central nervous system (CNS).[1,2] Once released from the presynaptic neurons into the glutamatergic synaptic cleft, Glu activates two main classes of receptors: G-protein-coupled metabotropic Glu receptors (mGluRs) and ligand-gated ionotropic Glu receptors (iGluRs). The iGluRs are the major players in the fast neuronal signaling and represent a potential therapeutic target for the treatment of a number of neurological and psychiatric disorders, i.e. chronic pain, stroke, epilepsy, drug addiction, schizophrenia, and Alzheimer, Huntington and Parkinson diseases.[3-7]

On the basis of the agonist selectivity, iGluRs have been subclassified into *N*-methyl-D-aspartate (NMDA) receptors, 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionate (AMPA) receptors, and kainate (KA) receptors.[1,2] The functional ion channel is composed of four subunits, which can assemble either homomerically or heteromerically. A total of seven NMDA

subunits (GluN1, GluN2A-D and GluN3A-B), four AMPA subunits (GluA1-4), and five KA subunits (GluK1-5) have been cloned and characterized.[2]

In the past, we have designed compounds **CIP-A**, **CIP-B**, **HIP-A**, and **HIP-B** (Figure 1) as molecular hybrids of AMPA and KA. In red is highlighted the structural part representing the skeleton of glutamic acid, homoglutamic acid (**CIP-B**) or aspartic acid (**HIP-A**).[8-11]

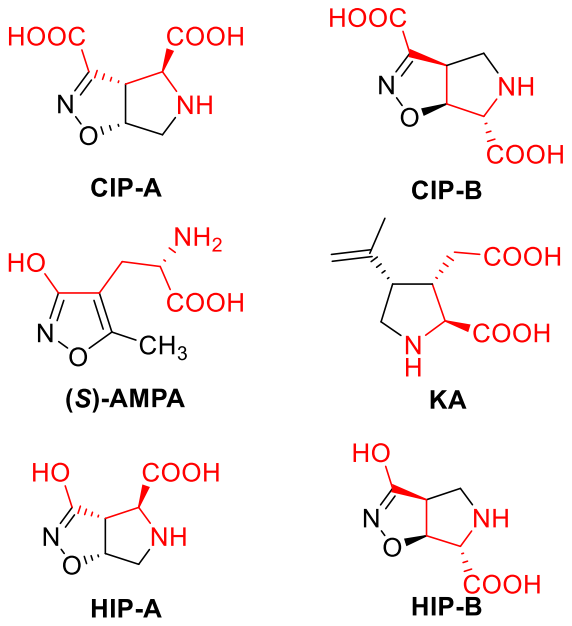


Figure 1. Structure of (*S*)-AMPA, KA and their molecular hybrids.

As expected, compound **CIP-A** turned out to be a potent agonist at both AMPA and KA receptors without any activity at NMDA receptors.[8,9] The eutomer of **CIP-A** was characterized by the S configuration at the amino acidic stereogenic center. On the contrary, both **HIP-A** and **HIP-B** turned out to be inactive at metabotropic and ionotropic glutamate receptors whereas they were provided with an interesting inhibitory activity at glutamate transporters.[12-15] In parallel, we carried out a detailed investigation on the structure-activity relationships of these rigidified aspartate-glutamate analogues to find out selective iGluR ligands. The bicyclic structure was either simplified into monocyclic derivatives or was conserved and the chain connecting the amino acidic moiety and the distal acidic group was elongated or shortened. In such a way we were able to modulate their pharmacological profile on passing from agonists to antagonists or to generate a remarkable selectivity for one of the three iGluRs.[16-30] In the planned derivatives, we always preserved the aminoacid moiety because the X-ray structural analyses of the iGluRs bilobular ligand-binding core in complex with agonists or antagonists evidenced the crucial role played by such a group.[31] In a recent paper,[32] it was described a series of derivatives generated by the incorporation of the structural elements of both kainic acid and neodysiherbaine A (**neoDH**), two naturally occurring pro-convulsant agents. Surprisingly, some of them, e.g.

derivative **IKM-159**, are characterized by the presence in their structure of the α-carboxylate amido group (Figure 2) and are provided with a potent AMPA-selective antagonistic activity.[32] On this ground, we designed the corresponding analogues of **CIP-A** and **CIP-B** in order to test their pharmacological profile and their selectivity versus AMPA receptor subtypes.

The present paper deals with an improved synthesis of **CIP-A** and **CIP-B** and their transformation into **CIOP-A** and **CIOP-B**, the corresponding amido derivatives (Figure 2).

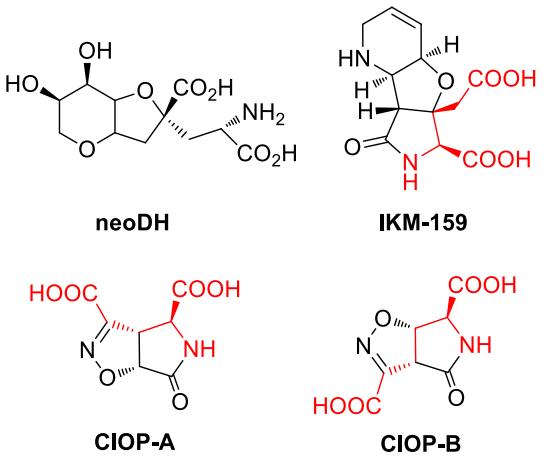


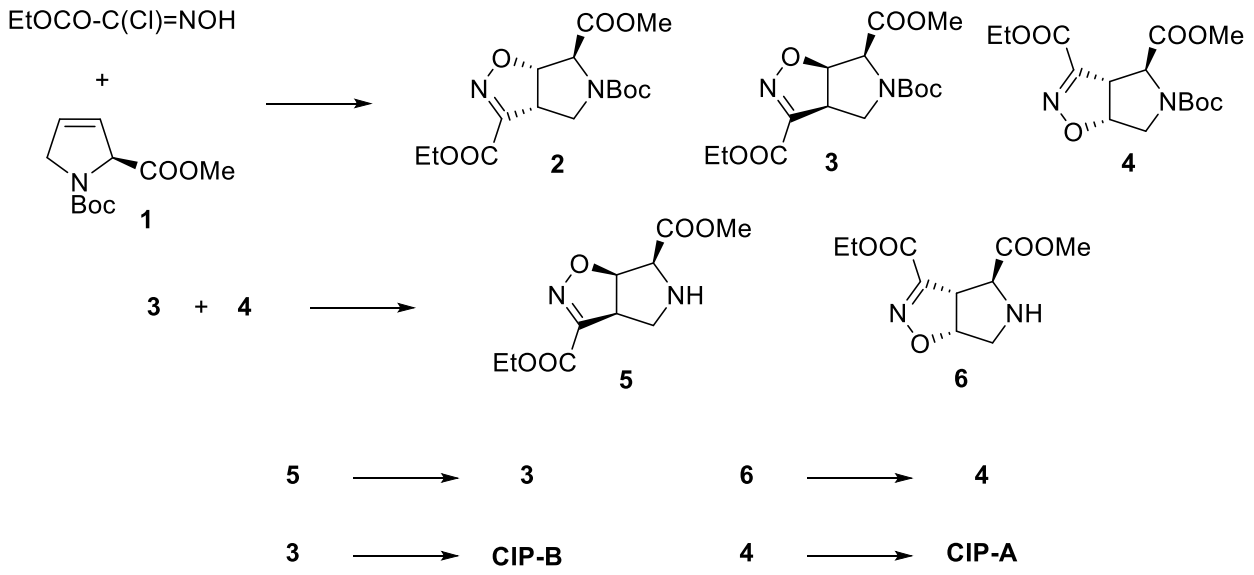
Figure 2. Structures of model and target compounds.

Results and Discussion

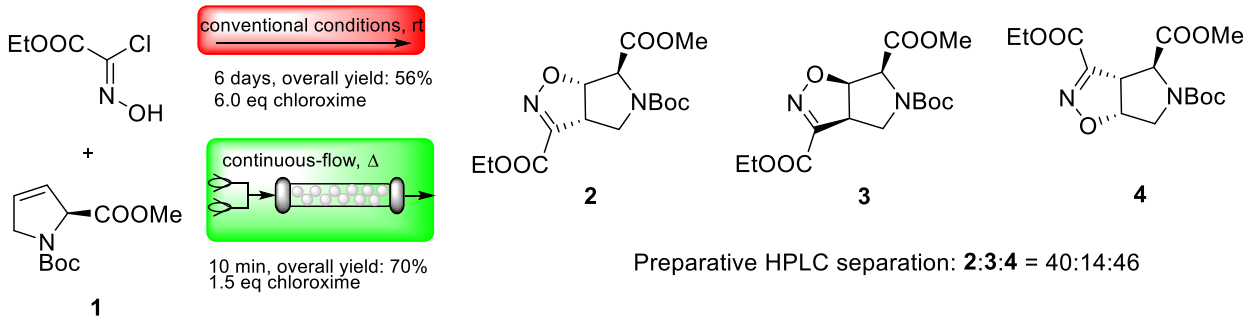
According to a literature report,[9] the synthesis of **CIP-A** and **CIP-B**, as pure enantiomers, has been accomplished as depicted in Scheme 1.

The major drawbacks associated to the described procedure[9] are: i) the low reactivity of dipolarophile **1** and ii) the failure to separate cycloadduct **3** from **4** by column chromatography. For such a reason, the mixture was transformed into the corresponding secondary amines **5** and **6** which were separated by column chromatography and then reconverted into the *N*-Boc derivatives **3** and **4**, respectively.

In order to overcome the first drawback, we decided to investigate the feasibility of the cycloaddition reaction with the flow technology by using a procedure previously applied to similar pericyclic reactions.[33,34] As shown in scheme 2, an ethyl acetate solution of *N*-Boc-3,4-dehydro-L-proline methyl ester **1**, prepared under flow conditions as previously reported,[35] and ethyl chlorooximinoacetate were mixed and delivered to a glass column filled with solid K_2CO_3 heated at 90 °C. The desired cycloadducts were obtained in good yield (70%) in only 10 min and a slight excess of chloroxime. This result represents a significant improvement over the above described conventional methodology because the reaction time was considerably reduced (from 6 days to 10 min) and the overall yield improved (from 56% to 70%).



Scheme 1. Previously reported synthesis of CIP-A and CIP-B.[9]

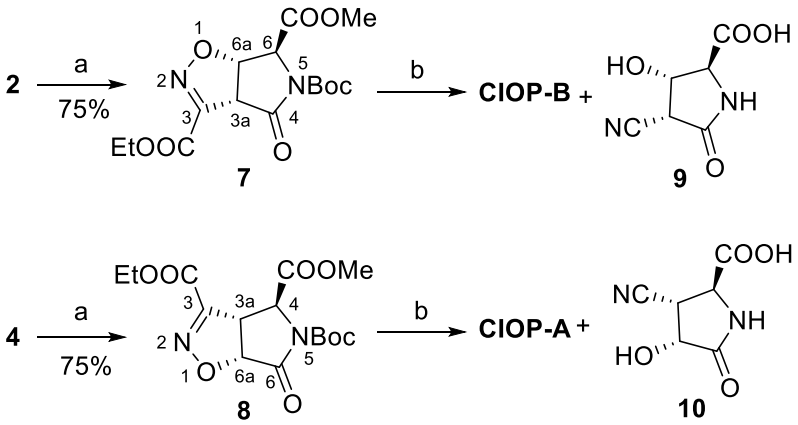


Scheme 2. The cycloaddition reaction in a continuous flow reactor.

The second drawback, due to the incapability to split with a silica gel column chromatography the mixture of cycloadducts **2**, **3**, and **4**, was explored by making use of a preparative HPLC. After a substantial number of attempts, we found out the conditions suitable to separate the cycloadducts at a preparative level. An excellent separation (see experimental section) was obtained with the amylose tris-(5-chloro-2-methyl-phenyl-carbamate) stationary phase, allowing us to collect a substantial amount of each stereoisomer. The cycloaddition step yielded intermediates **2**:**3**:**4** in the ratio 40:14:46.

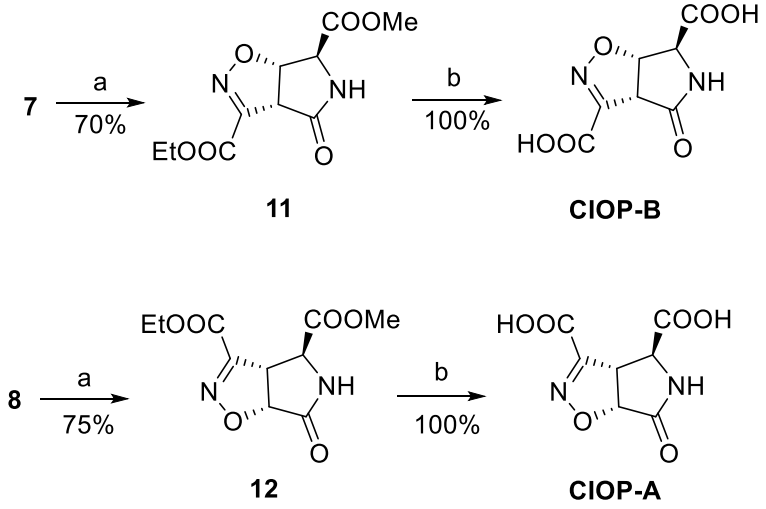
Cycloadducts **2** and **4** were then oxidized with a catalytic amount of hydrated ruthenium (IV) oxide and a 10% aqueous solution of sodium periodate in a biphasic system water/ethyl acetate to give intermediate **7** and **8**, respectively. Their stereochemistry was secured by ^{1}H NMR. As a matter of fact, derivative **7** shows proton 3a at 4.53 as a doublet (J = 9.3 Hz), whereas the same proton in derivative **8** resonates at 4.10 as a doublet of doublet (J = 1.3, 10.4 Hz). Intermediates **7**

and **8** were treated with an aqueous 6N HCl solution, according to the procedure described for the synthesis of **IKM 159** (Scheme 3).[36]



Scheme 3. Reagents and conditions: a: RuO_2*H_2O, $NaIO_4$, H_2O/AcOEt; b: HCl 6N, Δ.

Unfortunately, we obtained a 1:2 unsplitable mixture of **CIOP-B** and **9** or **CIOP-A** and **10**. The hydroxynitrile side-products **9** and **10** derived from the low stability of the isoxazoline ring under aqueous acidic conditions. Therefore, we decided to remove the nitrogen protecting group by treating derivatives **7** and **8** with an excess of trifluoroacetic acid; amides **11** and **12** were obtained in good yield (Scheme 4).



Scheme 4. Reagents and conditions: a: 30% TFA/CH_2Cl_2; b: K_2CO_3, H_2O.

Final compounds **CIOP-B** and **CIOP-A** were obtained, in quantitative yield, through the alkaline hydrolysis of the two ester groups under mild conditions, i.e. 2.2 equivalent of an aqueous K_2CO_3 solution. Using harsher basic condition, such as an aqueous NaOH solution, we observed a substantial degradation of the cycloadducts.

Conclusions

We have accomplished a significant improvement in the synthesis of glutamate agonists **CIP-A** and **CIP-B** in terms of overall yield, time, and excess of ethyl chlorooximinoacetate. Furthermore, we find out the HPLC conditions suitable to separate, at a preparative level, the three cycloadducts **2**, **3**, and **4**. In such a way, we avoided the lengthy procedure based on the transformation of the unsplittable mixture of derivative **3** and **4** into the corresponding secondary amines **5** and **6**, their separation by a silica gel column chromatography and then their reconvertion into the *N*-Boc derivatives **3** and **4**. Furthermore, the two regioisomers **2** and **4** were transformed into final derivatives **CIOP-B** and **CIOP-A**, respectively, via an oxidation step, removal of the *N*-Boc protecting group and a careful hydrolysis of the two ester groups. The biological results of the novel derivatives **CIOP-A** and **CIOP-B** will challenge the involvement of the amino acid moiety in determining the binding to the active site of the ionotropic glutamate receptors. These data will be reported in due course.

Experimental Section

General. All reagents were purchased from Sigma. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts (δ) are expressed in ppm, and coupling constants *(J*) are expressed in Hz. Rotary power determinations were carried out using a Jasco P-1010 spectropolarimeter, coupled with a Haake N3-B thermostat. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution or ninhydrin. MS analyses were performed on a Varian 320-MS triple quadrupole mass spectrometer with ESI source. Microanalyses (C, H, N) of new compounds were within ±0.4% of theoretical values. HPLC analyses were performed with a Jasco PU-980 pump equipped with a UV–vis detector Jasco UV-975 (wavelength: 220 nm) and Phenomenex Lux Amylose-2 column (4.6 × 150 mm). Preparative HPLC was performed with a 1525 Extended Flow Binary HPLC Pump, equipped with a Waters 2489 UV-vis detector and a Phenomenex Lux Amylose-2 column (21.2 × 250 mm) at a flow rate of 15 mL/min. The continuous-flow cycloaddition was performed using a R2+/R4 flow reactor, commercially available from Vapourtec equipped with Omnifit glass column.

(3a*R*,6*R*,6a*S*)-5-*tert*-Butyl 3-ethyl 6-methyl 3a,4,6,6a-tetrahydropyrrolo[3,4-*d*]isoxazole-3,5,6-tricarboxylate (2), (3a*S*,6*S*,6a*R*)-5-*tert*-butyl 3-ethyl 6-methyl 3a,4,6,6a-tetrahydropyrrolo[3,4-*d*]isoxazole-3,5,6-tricarboxylate (3), and (3a*S*,4*S*,6a*R*)-5-*tert*-butyl 3-ethyl 4-methyl 3a,4,6,6a-tetrahydropyrrolo[3,4-*d*]isoxazole-3,4,5-tricarboxylate (4). A 0.25 M solution of methyl *N*-Boc-3,4-dehydro-L-proline methyl ester (1.0 mmol) in EtOAc (4 mL) and a 0.37 M solution of ethyl chlorooximinoacetate (1.5 mmol) in EtOAc (4 mL) were

prepared. The two reactant streams were mixed using a simple T-piece and delivered to a glass column (6.6 mm i.d.× 100 mm length) filled with K_2CO_3 (4.0 mmol, 540 mg) heated at 90 °C at a total flow rate of 0.1mL min^{-1}, equating to a residence time of about 10 min. A 100 psi backpressure regulator was applied to the system. The solvent was evaporated, and the crude material was purified by silica gel column chromatography (cyclohexane–EtOAc 7:3) to yield the mixture of cycloadducts **2**, **3**, **4** in 70% overall yield. Analytical HPLC condition: wavelength, 220 nm; eluent, *n*-hexane–2-propanol (8:2). Retention times: **4**, 7.51 min.; **2**, 13.01 min.; **3**, 16.77 min.

(3a*R*,6*S*,6a*S*)-5-*tert*-Butyl 3-ethyl 6-methyl 3a,4,6,6a-tetrahydro-4-oxopyrrolo[3,4-*d*]-isoxazole-3,5,6-tricarboxylate (7). To a magnetically stirred 10% aqueous solution of $NaIO_4$ (2.38 g, 11.4 mmol) was added RuO_2*H_2O (21 mg). The mixture was immediately poured into a solution of cycloadduct **2** (960 mg, 2.84 mmol) in EtOAc (27 mL) and the resulting suspension was vigorously stirred at room temperature for 24 h. The black solid was removed by filtration under vacuum through a short Celite pad, and the organic layer was separated and treated with 2-propanol (2 mL). The organic solution was dried over anhydrous Na_2SO_4, the solvent removed under vacuum, and the residue was column chromatographed (cyclohexane–EtOAc 7:3) to give 130 mg of the desired product **7** and 560 mg of the unreacted starting material **2**. Yield based on recovered starting material: 75%. Colorless oil; $[\alpha]_D^{20}$ − 27.3 (*c* 0.56, $CHCl_3$); R_f 0.25 (Cyclohexane–EtOAc, 7:3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (t, 3H, *J* 7.0); 1.45 (s, 9H); 3.82 (s, 3H); 4.36 (q, 2H, *J* 7.0); 4.53 (d, 1H, *J* 9.3); 4.91 (s, 1H;); 5.23 (d, 1H, *J* 9.3). ^{13}C NMR (75 MHz, $CDCl_3$): δ14.2, 28.0, 53.6, 55.2, 63.1, 64.7, 82.0, 85.4, 148.7, 149.3, 158.5, 165.0, 168.8. MS: 357.1 $[M+H]^+$. Anal. Calcd for $C_{15}H_{20}N_2O_8$: C, 50.24; H, 5.79; N, 7.86. Found: C 50.37, H 5.76, N 7.75.

(3a*S*,4*S*,6a*R*)-5-*tert*-Butyl 3-ethyl 4-methyl 3a,4,6,6a-tetrahydro-6-oxopyrrolo[3,4-*d*]-isoxazole-3,4,5-tricarboxylate (8). To a magnetically stirred 10% aqueous solution of $NaIO_4$ (2.43 g, 11.4 mmol) was added RuO_2*H_2O (21 mg). The mixture was immediately poured into a solution of cycloadduct **4** (970 mg, 2.84 mmol) in EtOAc (27 mL) and the resulting suspension was vigorously stirred at room temperature for 24 h. The black solid was removed by filtration under vacuum through a short Celite pad, and the organic layer was separated and treated with 2-propanol (2 mL). The organic solution was dried over anhydrous Na_2SO_4, the solvent removed under vacuum, and the residue was column chromatographed (cyclohexane–EtOAc 7:3) to give 140 mg of the desired product **8** and 590 mg of the unreacted starting material **4**. Yield based on recovered starting material: 75%. Colorless oil; $[\alpha]_D^{20}$ − 176.0 (*c* 0.56, $CHCl_3$); R_f 0.25 (Cyclohexane–EtOAc, 7:3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (t, 3H, *J* 7.0); 1.45 (s, 9H); 3.82 (s, 3H); 4.10 (dd, 1H, *J* 1.3, 10.4); 4.33−4.44 (m, 2H); 4.90 (d, 1H, *J* 1.3); 5.37 (d, 1H, *J* 10.4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 28.0, 47.4, 53.6, 59.8, 63.2, 83.0, 85.5, 148.7, 150.2, 159.3, 166.8, 170.4. MS: 357.1 $[M+H]^+$. Anal. Calcd for $C_{15}H_{20}N_2O_8$: C, 50.24; H, 5.79; N, 7.86. Found: C 50.33, H 5.78, N 7.72.

(3a*R*,6*S*,6a*S*)-3-Ethyl 6-methyl 4,5,6,6a-tetrahydro-4-oxo-3a*H*-pyrrolo[3,4-*d*]isoxazole-3,6-dicarboxylate (11). Compound **7** (130 mg, 0.37 mmol) was treated with 1 mL of a 30% CH_2Cl_2

solution of trifluoroacetic acid at 0 °C. The solution was stirred at room temperature for 4 h. The volatiles were removed under vacuum and the residue was purified by flash chromatography to yield 65 mg (70% yield) of the desired product **11** as a colorless oil that was immediately used in the next synthetic step. R_f 0.6 (EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): 1.40 (t, 3H, *J* 7.0); 3.82 (s, 3H); 4.40 (q, 1H, *J* 7.0); 4.40 (d, 1H, *J* 9.3); 4.50 (s, 1H); 5.60 (d, 1H, *J* 9.3); 6.82 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3, 53.6, 55.3, 63.2, 64.9, 85.4, 149.4, 163.9, 165.0, 168.9. MS: 257.1 $[M+H]^+$. Anal. Calcd for $C_{10}H_{12}N_2O_6$: C, 46.88; H, 4.72; N, 10.93. Found: C 47.00, H 4.53, N 10.80.

(3a*S*,4*S*,6a*R*)-3-Ethyl 4-methyl 4,5,6,6a-tetrahydro-6-oxo-3a*H*-pyrrolo[3,4-*d*]isoxazole-3,4-dicarboxylate (12). Compound **8** (140 mg, 0.40 mmol) was treated with 1 mL of a 30% CH_2Cl_2 solution of trifluoroacetic acid at 0 °C. The solution was stirred at room temperature for 4 h. The volatiles were removed under vacuum and the residue was purified by flash chromatography to yield 75 mg (75% yield) of the desired product **12** as a colorless oil that was immediately used in the next synthetic step. R_f 0.6 (EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (t, 3H, *J* 7.3); 3.82 (s, 3H); 4.35-4.44 (m, 2H); 4.46 (dd, 1H, *J* 1.2, 10.2); 4.55 (d, 1H, *J* 1.2); 5.32 (d, 1H, *J* 10.2); 7.50 (bs, 1H).^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 47.5, 53.7, 59.7, 63.3, 85.6, 150.3, 163.5, 166.9, 170.5. MS: 257.1 $[M+H]^+$. Anal. Calcd for $C_{10}H_{12}N_2O_6$: C, 46.88; H, 4.72; N, 10.93. Found: C 47.08, H 4.50, N 10.78.

(3a*R*,6*S*,6a*S*)-4,5,6,6a-Tetrahydro-4-oxo-3a*H*-pyrrolo[3,4-*d*]isoxazole-3,6-dicarboxylic acid (CIOP-B). Compound **9** (60 mg, 0.23 mmol) was suspended in bi-distilled water and K_2CO_3 (71 mg, 0.52) was added. The reaction mixture was stirred at room temperature for 16 h. The mixture was made acidic (pH 2) with 1N aqueous HCl and the solvent was removed under reduced pressure. The residue was taken up with acetone and filtered. The solvent was removed under reduced pressure to give **CIOP-B** (50 mg, 0.23 mmol) as a white solid.$[\alpha]_D^{20}$ + 2.0 (*c* 0.41, H_2O). ^{1}H NMR (300 MHz, D_2O): δ 4.38 (d, 1H, *J* 1.1); 4.42 (d, 1H, *J* 9.1); 5.47 (dd, 1H, *J* 1.1, 9.1). ^{13}C NMR (75 MHz, D_2O): δ 55.5, 63.7, 86.7, 153.1, 163.9, 173.1, 174.4. MS: 212.9 $[M-H]^-$. Anal. Calcd for $C_7H_6N_2O_6$: C, 39.26; H, 2.82; N, 13.08. Found: C, 39.06; H, 2.87; N, 13.01.

(3a*S*,4*S*,6a*R*)-4,5,6,6a-Tetrahydro-6-oxo-3a*H*-pyrrolo[3,4-*d*]isoxazole-3,4-dicarboxylic acid (CIOP-A). Compound **10** (70 mg, 0.27 mmol) was suspended in bi-distilled water and K_2CO_3 (83 mg, 0.60) was added. The reaction mixture was stirred at room temperature for 16 h. The mixture was made acidic (pH 2) with 1N aqueous HCl and the solvent was removed under reduced pressure. The residue was taken up with acetone and filtered. The solvent was removed under reduced pressure to give compound **CIOP-A** (58 mg, 0.27 mmol) as a white solid.$[\alpha]_D^{20}$ – 189.8 (*c* 0.42, H_2O). ^{1}H NMR (300 MHz, D_2O): δ 4.42 (dd, 1H, *J* 1.5, 9.7); 4.53 (d, 1H, *J* 1.5); 5.33 (d, 1H, *J* 9.7). ^{13}C NMR (75 MHz, D_2O): δ 51.5, 58.0, 82.5, 155.1, 163.2, 174.1, 174.6. MS: 212.8 $[M-H]^-$. Anal. Calcd for $C_7H_6N_2O_6$: C, 39.26; H, 2.82; N, 13.08. Found: C, 39.64; H, 2.95; N, 12.86.

Acknowledgements

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