Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 1363–1367

TETRAHEDRON: *ASYMMETRY*

An improved synthesis of enantiomerically pure CIP-AS, a potent and selective AMPA-kainate receptor agonist

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Received 27 April 2001; accepted 16 May 2001

Abstract—CIP-AS (−)-**2**, a chiral amino acid structurally related to glutamic acid, behaves as a potent agonist at the ionotropic AMPA-kainate receptors and was previously prepared in low overall yield through the 1,3-dipolar cycloaddition of ethoxycarbonylformonitrile oxide to *N*-BOC-3,4-didehydro-(*S*)-proline methyl ester (−)-**6**. Herein, we report an alternative strategy based on the cycloaddition of the same dipolarophile to *N*-(4-methoxybenzyl)-α-ethoxycarbonylnitrone **12**. The mixture of stereoisomeric 3-ethoxycarbonyl-*N*-(4-methoxybenzyl)isoxazolidinyl prolines **13** was then converted into the corresponding 3-ethoxycarbonyl-Δ^2-isoxazolinyl prolines by DDQ mediated oxidation. The new strategy yielded the precursor of CIP-AS in satisfactory overall yield and represents an improvement upon the existing procedure in terms of yield and efficiency.

1. Introduction

(*S*)-Glutamic acid **1** (Fig. 1), the main excitatory neurotransmitter in the mammalian central nervous system (CNS), is involved in important physiological processes such as neural plasticity, memory and learning.[1] Moreover, impairment of excitatory pathways seems to play a crucial role in the pathogenesis of a number of acute and chronic neurological disorders, e.g. stroke and brain injury, as well as chronic neurodegenerative pathologies, notably Huntington's, Parkinson's, and Alzheimer's diseases.[2] The effects of glutamic acid are mediated by a variety of presynaptic and postsynaptic neuronal receptors, which have been grouped in two families: the ionotropic (iGlu)[3,4] and metabotropic (mGlu)[5,6] receptors. The iGluRs are multimeric Glu-gated channels, which regulate the flux of cations (Na^+, K^+, and Ca^{2+}) across the synaptic membrane. Based on the activation by specific agonists, the iGluRs have been classified into *N*-methyl-D-aspartic acid (NMDA), (±)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid (AMPA), and kainic acid (KAIN) receptors. Conversely, the mGluRs belong to the superfamily of G protein-coupled receptors and regulate the activity of membrane enzymes such as phospholipase C or adenylate cyclase.[7–9]

The definition of the physiological and pharmacological relevance of the different excitatory amino acid receptors is strictly dependent on the availability of highly selective ligands. From this viewpoint, even though a number of agonists and antagonists selectively acting at AMPA or KAIN receptors have been reported,[2,10] there is still a demand for pharmacological tools capable to discriminate the different receptor subtypes.

In the recent past, we synthesized and tested a group of regioisomeric 3-carboxy(3-hydroxy)isoxazolinyl prolines,[11–13] designed as semi-rigid analogues of glutamic

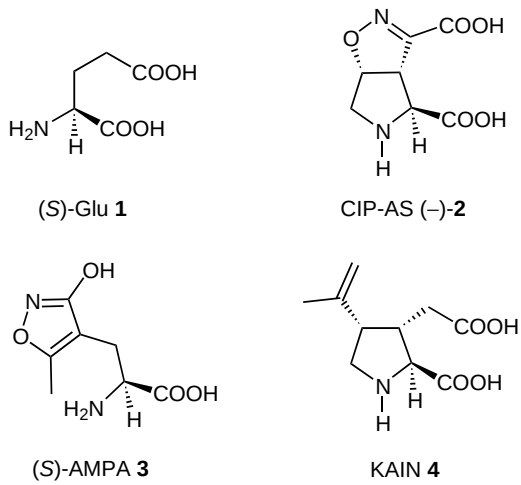

Figure 1.

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0957-4166/01/$ - see front matter
PII: S0957-4166(01)00225-7

acid which incorporated structural elements common to AMPA and kainic acid. Among these compounds, 3a,5,6,6a-tetrahydro-4*H*-pyrrolo[3,4-*d*]isoxazole-3,4-dicarboxylic acid (CIP-A) emerged as a selective agonist at both AMPA and kainate receptors, displaying potent convulsant activity comparable to that of kainic acid.[11] Preparation of the two enantiomers of CIP-A revealed that CIP-AS[13,14] was the eutomer in all in vitro and in vivo assays. The structure of CIP-AS, (–)-**2**, depicted in Fig. 1 together with the eutomer of AMPA, (*S*)-AMPA, **3** and kainic acid **4**, evidence the stereochemical requirements at the AMPA-kainate receptors.

Since we were interested in the preparation of analogues of CIP-AS retaining the skeleton of the parent molecule, we needed a more effective synthesis of the model compound. The study presented herein deals with an alternative approach to CIP-AS, in which the dipolar cycloaddition based strategy was carried out with a different 1,3-dipole. Indeed, the replacement of a nitrile oxide with a nitrone produced an improvement in the overall yield combined with a significant reduction in the reaction time.

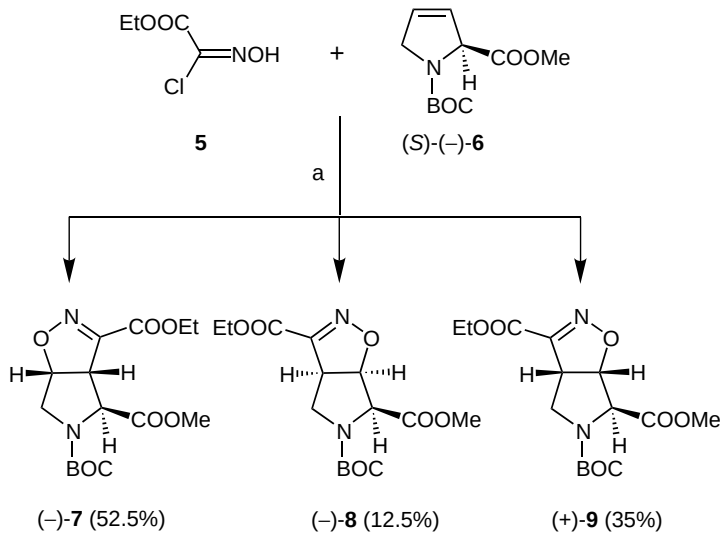
2. Results and discussion

The key step of the previously reported synthetic approach to CIP-AS was the 1,3-dipolar cycloaddition of ethoxycarbonylformonitrile oxide (ECFNO)[15] to *N*-BOC-3,4-didehydro-(*S*)-proline methyl ester (–)-**6**.[11,13] The nitrile oxide was generated in situ by treatment of ethyl 2-chloro-2-(hydroxyimino) acetate **5** with sodium hydrogen carbonate. The pericyclic reaction produced a mixture of three of the four possible stereoisomers in the relative amounts reported in Scheme 1. Column chromatography of the crude reaction mixture gave two fractions containing pure (+)-**9** and an inseparable mixture of (–)-**7** and (–)-**8**, respectively. Cycloadducts (–)-**7** and (–)-**8** were fully characterized through their separation as secondary amines followed by their conversion to *N*-BOC derivatives. Intermediate (–)-**7** was then converted into the target compound CIP-AS (–)-**2**, through standard transformations.[13]

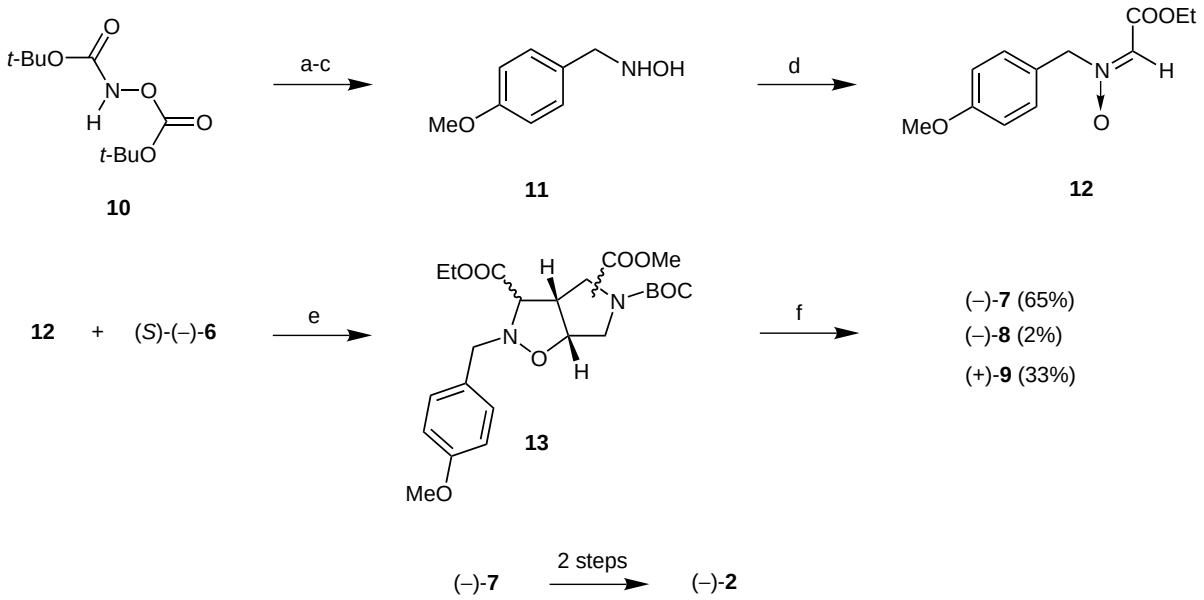
However, the procedure described above had a number of drawbacks. We found ethyl 2-chloro-2-(hydroxyimino) acetate[16] **5** to be a strong irritant, which caused severe skin allergies even when handled with care. Such a drawback is magnified by the need to employ a large excess of **5** (up to ten-fold) due to the poor reactivity of the dipolarophile. In practice, 40% of the unreacted dipolarophile was recovered even on prolonged reaction times of about 10 days.

As a consequence, we investigated an alternative strategy based on the cycloaddition of the same dipolarophile to a suitable nitrone. For such a purpose we prepared *N*-(4-methoxybenzyl)-α-ethoxycarbonylnitrone **12** (Scheme 2) by condensing *N*-(4-methoxybenzyl)hydroxylamine **11** with glyoxylic acid ethyl ester, a procedure widely applied in this field.[17,18] The desired trifluoroacetate of hydroxylamine **11** was prepared by reacting *N*,*O*-bis-*tert*-butoxycarbonylhydroxylamine **10**[19] with 4-methoxybenzyl chloride, followed by treatment with a 30% dichloromethane solution of TFA. In our hands, such a preparation of **11** proved more efficient than the reduction of anisaldoxime carried out with sodium cyanoborohydride in acidic methanol.[20,21] Glyoxylic acid ethyl ester, which was obtained by oxidation of (+)-diethyl L-tartrate with periodic acid,[22,23] was then condensed with **11** to afford in high yield *N*-(4-methoxybenzyl)-α-ethoxycarbonylnitrone **12** as a mixture of (*E*)- and (*Z*)-isomers. As deduced from comparative ^{1}H NMR data,[24] the (*E*)-isomer, represented in Scheme 2, slightly predominates over the (*Z*)-counterpart (1.3:1).

The cycloaddition reaction was performed in refluxing toluene with nitrone **12** and dipolarophile (–)-**6** in a 2:1 molar ratio. The reaction was completed over about 36 h, when the dipolarophile was shown to be completely



Scheme 1. (a) $NaHCO_3$, AcOEt, rt.



Scheme 2. (a) 4-$MeOC_6H_4CH_2Cl$/DMF/K_2CO_3, 30°C; (b) TFA/CH_2Cl_2; (c) aqueous K_2CO_3, pH 10; (d) $CHOCO_2Et$, ether; (e) toluene, reflux; (f) DDQ/NEt_3/CH_2Cl_2–H_2O.

consumed by TLC analysis of the reaction mixture. Upon treatment of the resulting mixture of isomeric 3-ethoxycarbonyl-*N*-(4-methoxybenzyl)isoxazolidinyl prolines **13** (Scheme 2) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and catalytic triethylamine in water/dichloromethane (1:9),[25] oxidative cleavage of the *p*-methoxybenzyl group occurred readily. The bicyclic isoxazolines (–)-**7**, (–)-**8**, and (+)-**9** were obtained in 72% overall yield. The relative percentages of the three isomers, reported in Scheme 2, were established by HPLC analysis of the crude reaction mixture, in analogy to the related results reported in Scheme 1. It is worthy of note that the relative amount of (–)-**7**, the precursor of CIP-AS, is higher in the strategy we present than that of the reported nitrile oxide cycloaddition. Column chromatography of the crude product yielded pure (+)-**9** and (–)-**7** contaminated with low levels of (–)-**8**. The fraction containing (–)-**7**, owing to the negligible amount of (–)-**8**, was transformed into the final derivative (–)-**2**, and the contaminant was removed by recrystallization. The values of specific rotation match those previously reported.[13] In the case of CIP-AS the result was confirmed by chiral HPLC analysis.[13]

In conclusion, the synthesis of CIP-AS was achieved more efficiently by changing the nature of the 1,3-dipolar component of the cycloaddition reaction. The replacement of ECFNO with *N*-(4-methoxybenzyl)-α-ethoxycarbonylnitrone made it possible to perform the reaction in refluxing toluene, with a substantial increase in the rate and the yield of the cycloaddition step. These improvements, coupled with an effective oxidation step, make the new strategy suitable for the preparation of structural analogues of CIP-AS, even when employing very unreactive dipolarophiles.

3. Experimental

Melting points were determined in open capillaries using a Büchi B 540 apparatus and are uncorrected. Organic solvents were analytical grade. *N*-BOC-3,4-didehydro-(*S*)-proline methyl ester[13,26] **6**, *N*,*O*-bis(*tert*-butoxycarbonyl)hydroxylamine **10**[19] and glyoxylic acid ethyl ester[22] were prepared according to literature procedures. ^{1}H NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer: chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hz. HPLC analyses (see the appropriate paragraph) were carried out on a JASCO PU-980 instrument equipped with a UV detector or an evaporative light scattering PL-EMD 960 detector. Optical rotations were determined on a Perkin–Elmer 241 polarimeter 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. Microanalyses of new compounds agreed with theoretical values ±0.3%.

3.1. Preparation of *N*-(4-methoxybenzyl)-α-ethoxycarbonylnitrone 12

(A) To a solution of *N*,*O*-bis-*tert*-butoxycarbonylhydroxylamine **10** (14.93 g, 64 mmol) in *N*,*N*-dimethylformamide (80 mL) at 20°C was added potassium carbonate (11.06 g, 80 mmol) and the resultant suspen-

sion was heated to 30°C. The reaction mixture was treated with 4-methoxybenzyl chloride (9.65 mL, 71 mmol) dropwise over 30 min. After stirring the mixture overnight at 30°C, the solution was cooled to 20°C and diluted with water (80 mL) and ethyl acetate (100 mL). The ethyl acetate layer was separated, washed with water, dried over anhydrous sodium sulfate, and concentrated under vacuum. Column chromatography on silica gel of the crude reaction mixture (eluent: 5% ethyl acetate/petroleum ether) afforded the intermediate bis-(*tert*-BOC)hydroxylamine as a light yellow crystalline compound (17.64 g, 78%).

3.1.1. *N,O*-Bis(*tert*-butoxycarbonyl)-*N*-(4-methoxybenzyl)hydroxylamine. Colorless prisms; mp 74–75°C (from propan-2-ol); ^{1}H NMR ($CDCl_3$): δ 1.47 (s, 9, $C(CH_3)_3$), 1.52 (s, 9, $C(CH_3)_3$), 3.78 (s, 3, OCH_3), 4.67 (s, 2, CH_2), 6.82 (dd, 2, arom., $J=8.3$), 7.23 (dd, 2, arom., $J=8.3$). Anal. calcd for $C_{18}H_{27}NO_6$ (353.41): C, 61.17; H, 7.70; N, 3.96. Found: C, 61.33; H, 7.61; N, 3.88%.

(B) To an ice-cooled solution of the previous intermediate (14.14 g, 40 mmol) in dichloromethane (150 mL) was added a solution of trifluoroacetic acid (30% in dichloromethane, 52 mL). After stirring the mixture for 4 h at rt, the volatiles were removed at reduced pressure and the residue was crystallized from di-*iso*-propyl ether to give the desired salt (8.66 g, 81%).

3.1.2. *N*-(4-Methoxybenzyl)hydroxylammonium trifluoroacetate. Colorless prisms; mp 113.5–115°C (from di-*iso*-propyl ether); ^{1}H NMR (D_2O): δ 3.61 (s, 3, OCH_3), 4.17 (s, 2, CH_2), 6.82 (dd, 2, arom., $J=8.5$), 7.23 (dd, 2, arom., $J=8.5$). Anal. calcd for $C_{20}H_{12}F_3NO_4$ (267.20): C, 44.95; H, 4.53; N, 5.24. Found: C, 44.78; H, 4.72; N, 5.30%.

(C) The above reported trifluoroacetate (8.0 g, 30 mmol) was dissolved in water (80 mL) and the pH of the solution adjusted to 10 by addition of solid K_2CO_3. After extraction with ethyl acetate (4×50 mL), the combined organic layers were dried and concentrated. To a solution of the resulting free hydroxylamine **11** (3.52 g, 23 mmol) in diethyl ether (80 mL) was added dropwise over 15 min a solution of crude glyoxylic acid ethyl ester[22] (4.70 g, 46 mmol) in diethyl ether (30 mL). After stirring the mixture at rt for 2 h, the precipitate was dissolved by addition of ethyl acetate (100 mL). Water (100 mL) was then added, the organic layer was separated, and the aqueous phase was extracted with ethyl acetate (3×50 mL). The pooled organic extracts were dried and concentrated, leaving a yellow residue (4.42 g, 81%), which was purified by recrystallization.

3.1.3. *N*-(4-Methoxybenzyl)-α-ethoxycarbonylnitrone 12. Colorless prisms; mp 109–111°C (from propan-2-ol); ^{1}H NMR ($CDCl_3$) of (*E*)-isomer: δ 1.32 (t, 3, CH_2CH_3, $J=7.0$), 3.79 (s, 3, OCH_3), 4.26 (q, 2, CH_2CH_3, $J=7.0$), 5.62 (s, 2, CH_2Ar), 6.87 (d, 2, $J=8.7$), 7.15 (s, 1, N=CH), 7.47 (d, 2, $J=8.7$); ^{1}H NMR ($CDCl_3$) of (*Z*)-isomer: δ 1.27 (t, 3, CH_2CH_3, $J=7.0$), 3.82 (s, 3, OCH_3), 4.22 (q, 2, CH_2CH_3, $J=7.0$), 4.91 (s, 2, CH_2Ar), 6.92 (d, 2, $J=8.7$), 7.00 (s, 1, N=CH), 7.34 (d, 2, $J=8.7$). Anal. calcd for $C_{12}H_{15}NO_4$ (237.25): C, 60.75; H, 6.37; N, 5.90. Found: C, 60.97; H, 6.14; N, 5.72%.

3.2. Synthesis of CIP-AS (–)-2

(A) A solution of nitrone **12** (1.47 g, 6.2 mmol) and methyl *N*-BOC-3,4-didehydro-(*S*)-proline methyl ester **6** (1.41 g, 6.2 mmol) in toluene (50 mL) was stirred under reflux under nitrogen for 12 h. After TLC monitoring (eluent: 40% ethyl acetate/petroleum ether), further **12** (1.47 g, 6.2 mmol) was added and heating was continued for an additional 24 h. TLC analysis indicated the disappearance of the starting dipolarophile. The solvent was removed at reduced pressure and the crude reaction mixture was purified by column chromatography (eluent: 25% ethyl acetate/petroleum ether), producing a mixture of stereoisomeric 3-ethoxycarbonyl-*N*-(4-methoxybenzyl)isoxazolidinyl prolines **13** (2.80 g, 94% yield).

(B) To the mixture of stereoisomers **13** (2.80 g, 5.8 mmol) and triethylamine (90 μL) in dichloromethane/water (50 mL/5 mL) was added portionwise DDQ (3.40 g, 15 mmol). After stirring at rt for about 3 h (TLC monitoring), aqueous sodium bicarbonate solution (10%, 50 mL) was added to the red–brown mixture. The organic phase was separated and the aqueous layer was extracted with dichloromethane (3×30 mL). After the standard work up, the crude material was subjected to column chromatography over silica gel (eluent: 30% ethyl acetate/petroleum ether) to give pure (+)-**9** (0.51 g) and (–)-**7** (1.02 g) contaminated with trace amounts of (–)-**8** (77% overall yield). For analytical purposes the removal of (–)-**8** from (–)-**7** was achieved by the procedure previously described.[13] The ^{1}H NMR data of the isomers were superimposable to those previously reported.[13]

Isomer (–)-**7**: colorless oil; $[\alpha]_D^{20}=-153.2$ (*c* 1, $CHCl_3$) [lit.,[13] $[\alpha]_D^{20}=-151.06$ (*c* 1.042, $CHCl_3$)].

Isomer (+)-**9**: colorless prisms (mp 78–80°C from di-*iso*-propyl ether); $[\alpha]_D^{20}=+78.0$ (*c* 1, $CHCl_3$) [lit.,[13] $[\alpha]_D^{20}=+77.6$ (*c* 1, $CHCl_3$)].

(C) Δ^2-Isoxazoline (–)-**7** was transformed into CIP-AS by the literature procedure.[13] The ^{1}H NMR spectrum of (–)-**2** was identical to that previously reported.[13]

Amino acid (–)-**2**: colorless prisms (mp 195–225°C dec., from water–methanol); $[\alpha]_D^{20}=-53.9$ (*c* 0.110, H_2O) [lit.,[13] $[\alpha]_D^{20}=-53.7$ (*c* 0.108, H_2O)].

3.3. HPLC analysis

(A) A sample of the crude cycloaddition mixture of (*S*)-(–)-**6** to ECFNO[13] was submitted to HPLC analysis on a LiChrospher Si 60 column (4.0×125 mm). The column was eluted with 2% propan-2-ol-petroleum ether at a 1 mL/min flow rate, UV detection at $\lambda=254$ nm. As reported in Scheme 1, the relative amounts of isomeric Δ^2-isoxazolines (–)-**7**, (–)-**8**, and (+)-**9** were 52.5, 12.5, and 35%, respectively. Retention times

(min): (+)-**9** (20.0), (−)-**7** (28.3), (−)-**8** (37.9). A sample from the above reported oxidation of isoxazolidines **13** was analyzed using the same experimental protocol. The relative amounts of the three cycloadducts (see Scheme 2) are indicated in parentheses: (−)-**7** (65%), (−)-**8** (2%), (+)-**9** (33%).

(B) A sample of CIP-AS (−)-**2** was analyzed on a column (4.0×250 mm) of spherical silica Si 100, 5 μm, containing Teicoplanin as the chiral selector. The column was eluted at 25°C with 20 mM NH_4OAc/acetonitrile–water 4:1 (v/v) at a 1 mL/min flow rate, detection with a PL-EMD 960 detector operating at 80°C with an air flow of 6.0 L/min. Amino acid (−)-**2**: $k_{1'}=3.40$, $\alpha=1.73$; enantiomeric excess (e.e.) was >99.9%.

Acknowledgements

This research was financially supported by MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Rome). Thanks are due to Professors Carlo De Micheli and Marco De Amici for their encouragement and helpful discussion.

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