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Synthesis of New Bicyclic Analogues of Glutamic Acid

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Abstract: Regioisomeric 3-hydroxyisoxazolinyl prolines [HIP-A (±)-**6** and HIP-B (±)-**7**], which represent a restricted conformation of NMDA, AMPA, and kainic acid, potent and selective agonists at ionotropic glutamate receptors, have been prepared through two different strategies. In the first approach bromonitrile oxide was added to *N*-BOC-Δ^3-pyrroline or *N*-BOC-Δ^3-pyrrolin-2-one and the carboxylic group was subsequently appended. The alternative strategy is based on the cycloaddition of the same 1,3-dipole to *N*-BOC-3,4-didehydroproline methyl ester.

Keywords: bicyclic amino acids; glutamic acid analogues.

Introduction

It is widely accepted that (*S*)-glutamic acid (L-Glu, **1**) is the endogenous transmitter at a range of excitatory amino acid (EAA) receptors in the central nervous system (CNS). It plays a role of utmost importance in many physiological processes such as neural plasticity, memory, and learning.[1-3] An imbalance of excitatory pathways seems to be implicated in the pathogenesis of a number of acute and chronic neurological disorders, i.e., epilepsy, cerebral ischemia, stroke, hypoxia, and schizophrenia, as well as chronic neurodegenerative pathologies, i.e., neuropathic pain, amyotrophic lateral sclerosis, and Huntington's, Parkinson's, and Alzheimer's diseases.[4]

(*S*)-Glutamic acid activates two families of receptors: the ionotropic (iGlu)[5,6] and metabotropic (mGlu)[7,8] receptors. The iGluRs are multimeric Glu-gated channels which control the flux of cations (Na^+, K^+, and Ca^{2+}) across the postsynaptic membrane. They are responsible for the fast depolarization of postsynaptic cells.

At present there are three main classes of iGlu receptors named after the selective agonists that activate them: the NMDA (N-methyl-*D*-aspartic acid, **2**), AMPA [(±)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid, **3**], and KAIN (kainic acid, **4**) receptors (Figure 1). On the other hand, the mGluRs belong to

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the superfamily of G protein-coupled receptors and modulate the activity of phospholipase C (PLC) or adenylyl cyclase (AC). To date, eight distinct metabotropic glutamate ($mGlu_{1-8}$) receptors have been cloned and classified based on their amino acid sequence homology, signal transduction mechanism and pharmacology.[9-11] The eight mGlu receptors have been grouped into three subsets termed group I ($mGlu_{1,5}$) linked to PLC stimulation, group II ($mGlu_{2,3}$), and Group III ($mGlu_{4,6,7,8}$) both negatively coupled to adenylyl cyclase.

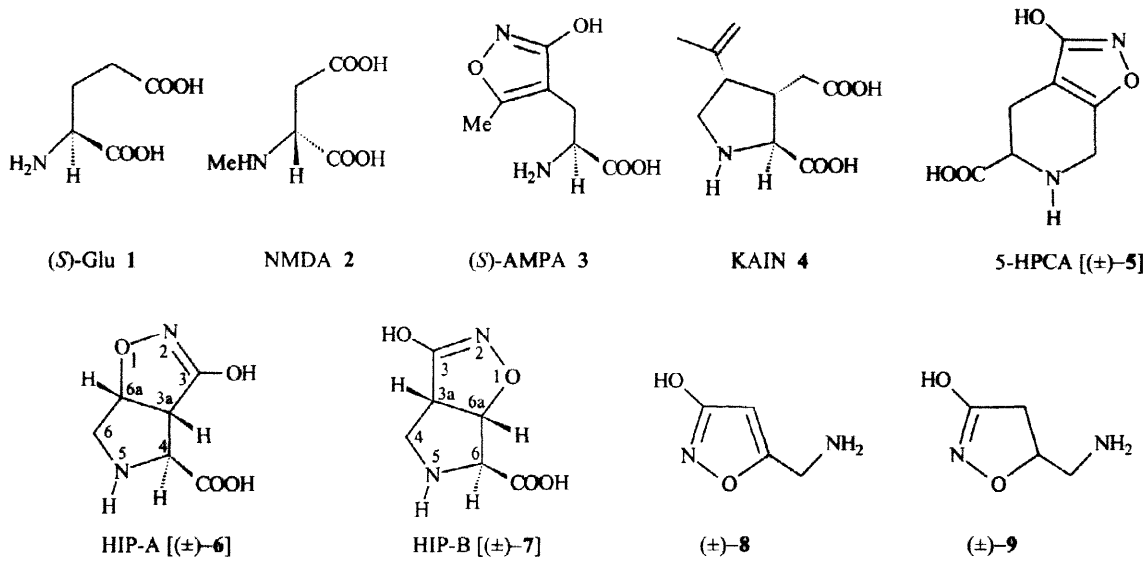


Figure 1

A prerequisite for the determination of the physiological role and pharmacological relevance of the subgroups of iGlu receptors as well as the subtypes of mGlu receptors is the availability of highly selective agonists and antagonists.[12] In previous studies a number of agonists and antagonists were developed, which allowed the pharmacological characterization of iGlu receptor subtypes, notably the AMPA receptors, and to delineate a relationship between structure and activity.[13-15] In this context the receptor-bound conformation of AMPA was deduced from the structure of the related conformationally restricted agonist, (R,S)-3-hydroxy-4,5,6,7-tetrahydro-isoxazolo[5,4-c]pyridine-5-carboxylic acid (5-HPCA, **5**) (Figure 1).[16]

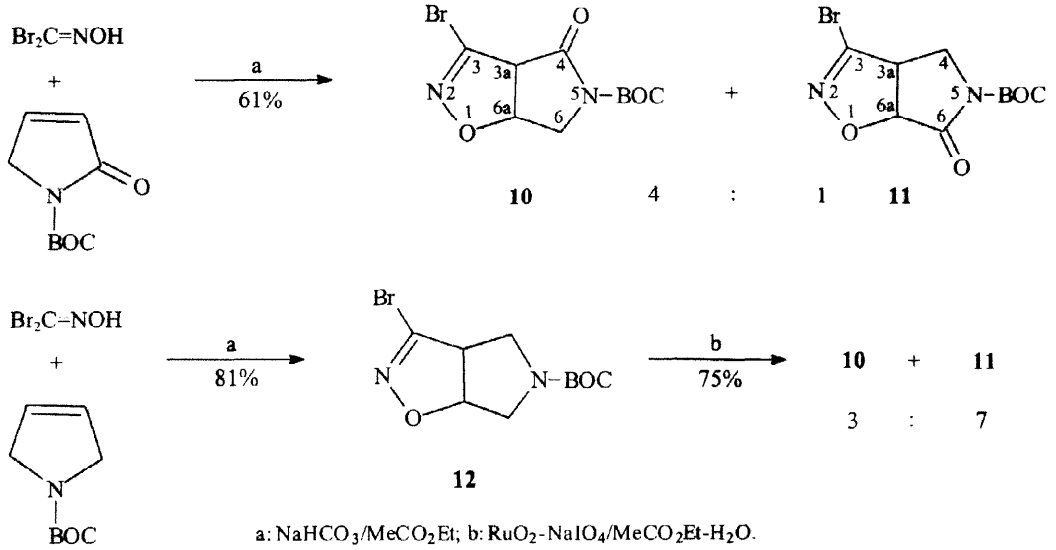
This paper deals with the synthesis of the bicyclic Glu analogues HIP-A {3a,5,6,6a-tetrahydro-4*H*-pyrrolo[3,4-*d*]isoxazole-3-hydroxy-4-carboxylic acid, (±)-**6**} and HIP-B {3a,5,6,6a-tetrahydro-4*H*-pyrrolo[3,4-*d*]isoxazole-3-hydroxy-6-carboxylic acid, (±)-**7**}, on the assumption that the 3-hydroxy-Δ^2-isoxazoline moiety of **6** and **7** could replace the 3-hydroxy-isoxazole nucleus which characterizes AMPA and related compounds. Such an analogy holds true in the GABA-ergic system where muscimol **8** and dihydromuscimol **9** behave both as agonists, with a comparable potency, of the endogenous neurotransmitter γ-aminobutyric acid.[17] The presence in both HIP-A and HIP-B of the pyrrolidino ring brings about a further resemblance to kainic acid **4**. In summary, HIP-A can be regarded as a restricted conformation of the lead

compound NMDA (**2**), whereas HIP-B mimics some conformations of AMPA (**3**) and kainic acid (**4**). If the structure of HIP-A and HIP-B represents the active conformation of the reference compounds, a selective activation of the corresponding iGlu receptors should be expected.

Chemistry

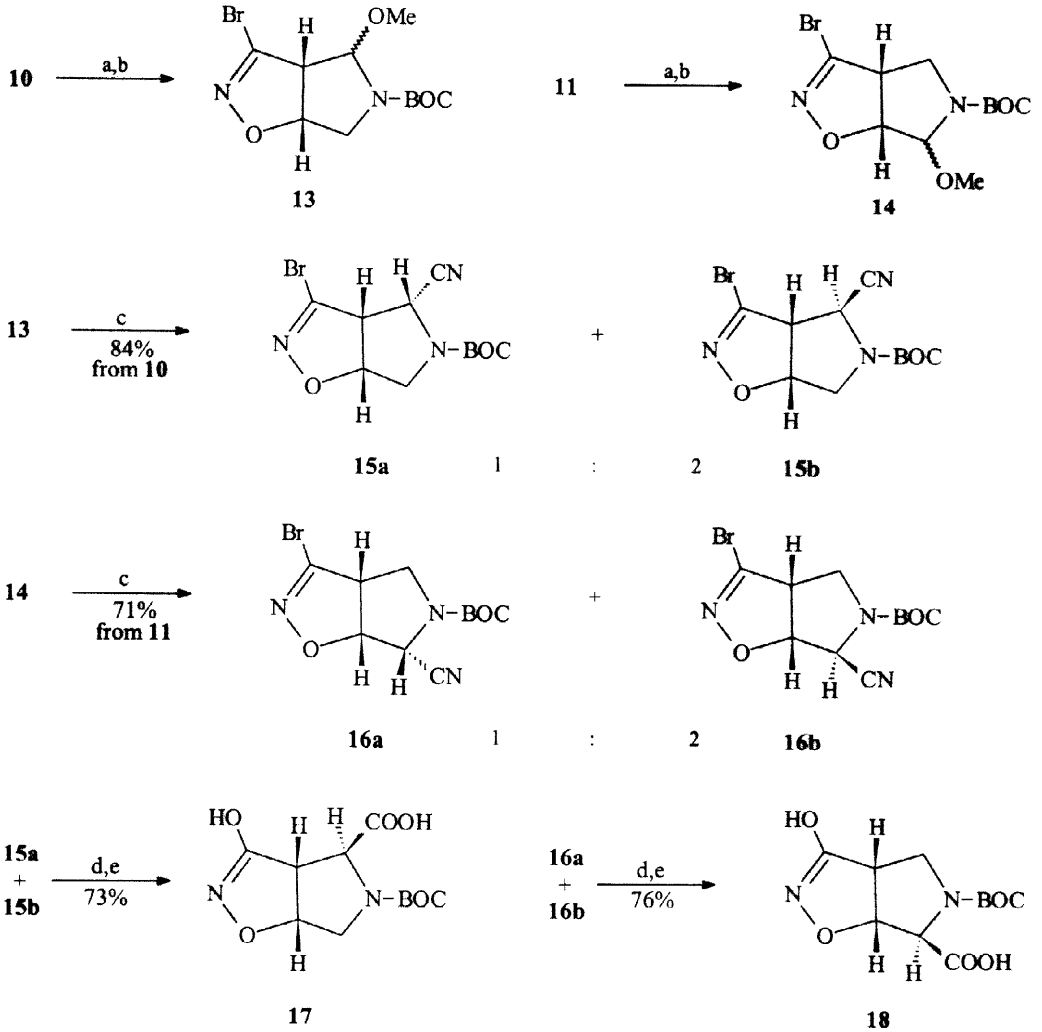
Target compounds (±)-**6** and (±)-**7** were initially prepared via cycloaddition of bromonitrile oxide, generated *in situ* by treatment of dibromoformaldoxime with a base, to *N*-BOC-Δ^3-pyrrolin-2-one.[18,19] As shown in Scheme 1, the pericyclic reaction produces a mixture of the two regioisomers **10** and **11** in a 4:1 ratio, which could be separated by column chromatography. This result parallels those previously reported for the cycloaddition of benzonitrile oxide to some α,β-unsaturated cycloalkenones where a mixture of regioisomers, with the prevalence of the 4-acyl derivative, was always observed.[20]

Frontier orbital theory provides an explanation of the results as previously reported.[20] The dominant interaction between the LUMO of the nitrile oxide and the HOMO of the dipolarophile favors the formation of **10**, the 4-carbonyl regioisomer. Alternatively, a mixture of **10** and **11**, in a reversed ratio of regioisomers (3:7), was obtained via RuO_4 oxidation[21,22] of intermediate **12**, in turn prepared through the cycloaddition of bromonitrile oxide to *N*-BOC-Δ^3-pyrroline[23] (Scheme 1).



Scheme 1

The two methodologies are thus complementary. The assignment of the structure to cycloadducts **10** and **11** relies on the multiplicity of H-6a, which appears as a double doublet of doublets in **10** and as a simple doublet in regioisomer **11**. Intermediates **10** and **11** were sequentially treated with DIBAL-H,[24] and a methanol solution of toluene-4-sulfonic acid[25] to produce methoxy derivatives **13** and **14** as a mixture of stereoisomers (Scheme 2).

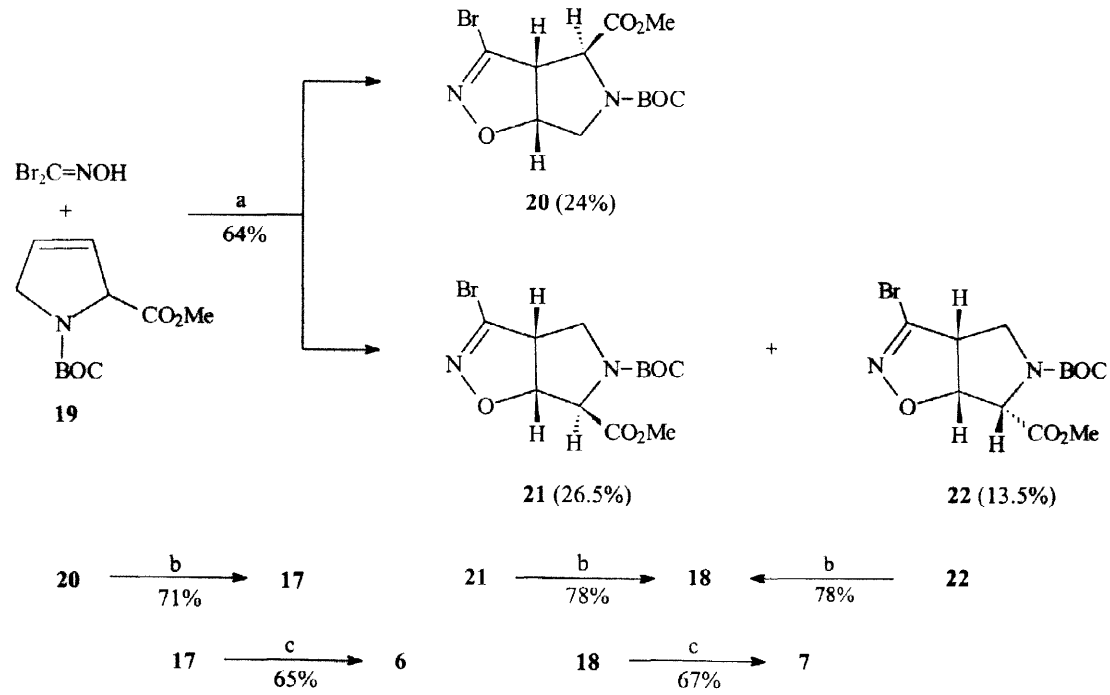


a: DIBAL-H/THF, −78 °C; b: p-TosOH/MeOH; c: Me_3SiCN, BF_3–Et_2O/CH_2Cl_2;
d: UHP–K_2CO_3/H_2O–acetone; e: 5 N NaOH/H_2O, 60 °C.

Scheme 2

The treatment of **13** and **14** with trimethylsilylcyanide under the acid catalysis of boron trifluoride ethyl etherate[25] produced cyano derivatives **15a,b** and **16a,b** as a mixture of stereoisomers through the intermediacy of an iminium ion.[25] The mixtures could be separated into single components by flash chromatography. Intermediates **15a**, **15b**, **16a**, and **16b** were characterized by ^{1}H NMR spectroscopy. The multiplicity of H-4 in derivatives **15** and H-6 in derivatives **16** is highly diagnostic in the assignment of the relative configuration to the related stereogenic center. As a matter of fact, cis derivatives **15a** and **16a** show the above-mentioned protons as a sharp doublet with a coupling constant of 9.3 and 7.6 Hz respectively, whereas the same protons appear as a broad singlet in trans derivatives **15b** and **16b**. Final derivatives **6** and **7** were prepared by using the mixtures of stereoisomers (**15a,b** and **16a,b**), since the alkaline treatment necessary to transform the cyano group into the carboxylic moiety causes the conversion of the cis isomers (**15a** and **16a**) into the more stable trans ones (**15b** and **16b**) (Scheme 2). Cyano derivatives **15** and **16** were transformed into the corresponding

amides by using the urea-hydrogen peroxide (UHP)[26] adduct which was subsequently reacted with a 5 N sodium hydroxide solution at 60 °C to yield acids **17** and **18** as pure stereoisomers.



a: $NaHCO_3/MeCO_2Et$; b: 1N NaOH/dioxane, 60°C; c: 30% $CF_3COOH–CH_2Cl_2$.

Scheme 3

The synthetic plan described in Schemes 1 and 2 is rather laborious and gives final derivatives in modest yield; nevertheless it permitted the unambiguous assignment of both regio– and stereochemistry to the products. Scheme 3 reports an alternative strategy which allowed the straightforward synthesis of amino acids **6** and **7**. The key step is represented by the 1,3-dipolar cycloaddition of bromonitrile oxide to racemic *N*-BOC-3,4-didehydroproline methyl ester **19**. The pericyclic reaction was rather unselective and yielded three out of the four possible stereoisomers in similar amounts. We were unable to detect the presence of the fourth stereoisomer, which is disfavored by the steric repulsion of the ester group of the dipolarophile with the substituent of the 1,3-dipole. The separation of the cycloadducts was rather laborious, since a column chromatography of the reaction mixture gave two fractions containing pure **22** and a mixture of **20** and **21**. Fortunately the mixture of **20** and **21** could be separated by fractional crystallization. The assignment of the structure to the cycloadducts is based on the ^1H NMR spectra. The ^1H NMR signals of cycloadducts **20**, **21**, and **22** were assigned by standard methods that rely on correlation through chemical bonds (COSY). As reported above, the coupling constant of proton H-6a (see Scheme 1 for numbering) is highly diagnostic in assigning the structure to the cycloadducts. Such a proton resonates as a doublet in cycloadduct **21**, as a double doublet in **22**, and as an eight-line signal (double doublet of doublets) in **20**. By reacting the three

cycloadducts with a 1 N sodium hydroxide/dioxane solution at 60 °C intermediates **17** and **18** were directly obtained. The hydrolysis of the ester group is accompanied by the concomitant nucleophilic substitution of the 3-bromo with the hydroxyl moiety. As previously observed, the alkaline treatment of cycloadducts **21** and **22** yielded the same intermediate **18**, since the hydrolysis of the cis derivative **22** is accompanied by an inversion of the chiral center at C-6. Final derivatives **6** and **7** were obtained by reacting **17** and **18** with a 30% solution of trifluoroacetic acid in dichloromethane.

In order to test the assumption that a 3-hydroxyisoxazoline is a good bioisostere of the 3-hydroxyisoxazole nucleus and both are bioisosteres of the carboxylate moiety, we measured the pKa values of **7** and AMPA, and their values were compared with those reported in the literature for AMPA, KAIN, and glutamic acid (GLU) (Table 1). We carried out the measurement on regioisomer **7** since it contains the same through-bond connection between the two acidic moieties of glutamic acid.

Table 1. pKa values of HIP-B, GLU, AMPA, and KAIN measured at 20 °C in water.

compd	pKa_1 (α-COOH)	pKa_2 (ω-COOH or OH)	pKa_3 (NH_3^+)
HIP-B	1.9	5.1	8.3
AMPA	2.3 (2.5)[27]	5.3 (4.8)[27]	9.7 (10.0)[27]
KAIN	2.1[28]	4.3[28]	10.1[28]
GLU	2.39[29]	4.21[29]	9.54[29]

The data of Table 1 put in evidence that whereas the value of pKa_1 is almost the same for all the four compounds, significant differences can be detected in the values of pKa_2 and pKa_3. In particular, the ω-carboxylic group of KAIN and GLU is at least ten times more acidic than the hydroxyl group of AMPA and HIP-B. Nevertheless, AMPA is a very potent GLU receptor agonist and is able to discriminate among different ionotropic GLU receptor subtypes. In this context, the close value of pKa_2 for HIP-B and AMPA could indicate that the 3-hydroxy-Δ^2-isoxazoline moiety can surrogate the acidity of the 3-hydroxyisoxazole nucleus in ligands behaving as analogs of glutamic acid.

In conclusion, this paper reports two different strategies for the synthesis of the two new amino acids, HIP-A (**6**) and HIP-B (**7**), which represent a restricted conformation of lead compounds NMDA (**2**) and AMPA-KAIN (**3** and **4**), respectively. The biological activity of the new compounds at iGlu receptors will be evaluated either by binding experiments and electrophysiological tests and the results will be reported in due course.

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Experimental Section

Dibromoformaldoxime,[30] (±)-3,4-didehydroproline,[31] Δ^3-pyrroline,[23] and Δ^3-pyrrolin-2-one[18] were prepared according to literature procedures. The synthesis of the ester moiety and the protection of the secondary amine of (±)-3,4-dehydroproline as well as the transformation of the secondary amine group of Δ^3-pyrroline, and Δ^3-pyrrolin-2-one into the corresponding *N*-BOC derivatives was accomplished along standard methodologies. ^{1}H NMR and ^{13}C NMR spectra were recorded with a 300 MHz spectrometer in toluene d_8 at 80 °C or as $CDCl_3$, CF_3COOD, CD_3OD solutions at 20 °C; chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hz. IR spectra were recorded with a Perkin Elmer mod.1310 spectrophotometer. Potentiometric titrations were performed with the GLpKa apparatus (Sirius Analytical Instruments Ltd, Forrest Row, East Sussex, UK) equipped with a pH electrode, a temperature probe, an overhead stirrer and precision dispensers for automated distribution of the diluent (0.15 M KCl in water) and titrants (0.5 M HCl, 0.5 M KOH). Four separate 15 mL aqueous solutions were added to weighted samples (1-10 mg) and acidified to pH 1.8 with HCl. The solutions were then titrated with standardized KOH to pH 8. The titrations were conducted under nitrogen at 25.0±0.1 °C. The initial estimates of the pK_a values were obtained by difference plots (Bjerrum plots).[32] These values were then refined by a weighted nonlinear least-squares procedure. TLC was performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Melting points were determined with a capillary method on a Büchi B 540 apparatus and are uncorrected.

1,3-Dipolar cycloaddition of bromonitrile oxide to *N*-BOC-Δ^3-pyrrolin-2-one. To a solution of *N*-BOC-Δ^3-pyrrolin-2-one (7.6 g, 41.6 mmol) in ethyl acetate (80 mL) were added dibromoformaldoxime (8.4 g, 41.6 mmol) and $NaHCO_3$ (15 g). The mixture was vigorously stirred for 3 days, than a further amount of dibromoformaldoxime (4.2 g, 20.8 mmol) was added and the mixture was stirred for additional 2 days. The progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate 7:3). Water was added to the reaction mixture and the organic layer was separated and dried over anhydrous sodium sulfate. The crude material, obtained after evaporation of the solvent, was chromatographed on silica gel (eluant: petroleum ether/ethyl acetate 7:3) to give 6.1 g of cycloadduct **10**, and 1.6 g of **11**. Combined yield: 60.7%.

Compound **10** crystallized from ethanol as colorless prisms, mp 155 °C, dec.; [Found: C, 39.15; H, 4.37; N, 9.41. $C_{10}H_{13}BrN_2O_4$ requires C, 39.36; H, 4.29; N, 9.18 %]; R_F (petroleum ether/ ethyl acetate 3:2) 0.42; IR ν_{max} (Nujol) 1725, 1708, 1372, 1150, 890, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$) 1.52 (s, 9H); 4.03 (dd, 1H; J = 2.8 and 12.7); 4.14 (dd, 1H; J = 7.0 and 12.7); 4.21 (d, 1H; J = 9.6); 5.35 (ddd, 1H; J = 2.8, 7.0 and 9.6); ^{13}C NMR ($CDCl_3$) 28.9, 53.3, 62.0, 78.3, 85.5, 135.2, 150.0, 165.6.

Compound **11** crystallized from ethanol as colorless needles, mp 162-163 °C; [Found: C, 39.50; H, 4.20; N, 9.35. $C_{10}H_{13}BrN_2O_4$ requires C, 39.36; H, 4.29; N, 9.18 %]; R_F (petroleum ether/ ethyl acetate 3:2) 0.36; IR ν_{max} (Nujol) 1763, 1688, 1365, 1140, 861, 768 cm^{-1}; ^{1}H NMR (C_6D_6) 1.35 (s, 9H); 2.44 (ddd, 1H; J = 2.2, 7.6

and 10.5); 2.83 (dd, 1H; J = 7.6 and 11.8); 3.51 (dd, 1H; J = 2.2 and 11.8); 4.22 (d, 1H; J = 10.5); ^{13}C NMR ($CDCl_3$) 28.9, 47.2, 49.6, 82.7, 85.6, 141.1, 150.0, 168.7.

1,3-Dipolar cycloaddition of bromonitrile oxide to *N*-BOC-Δ^3-pyrroline. To a solution of *N*-BOC-Δ^3-pyrroline (10.0 g, 59.2 mmol) in ethyl acetate (100 mL) was added dibromoformaldoxime (24.0 g, 118.4 mmol) and $NaHCO_3$ (15 g). The mixture was vigorously stirred at room temperature until TLC (petroleum ether/ethyl acetate 7:3) indicated disappearance of the dipolarophile (3 days). After the above reported workup, a column chromatography of the residue on silica gel (eluant: petroleum ether/ethyl acetate 7:3) gave 13.9 g of cycloadduct **12** as a white solid. Yield: 80.7%.

Compound **12** crystallized from 2-propanol as colorless prisms, mp 50-52 °C; [Found: C, 41.52; H, 5.02; N, 9.91. $C_{10}H_{15}BrN_2O_3$ requires C, 41.25; H, 5.19; N, 9.62 %]; R_F (cyclohexane/ethyl acetate 7:3) 0.35; IR ν_{max} (Nujol) 1668, 1395, 1158, 1100 cm^{-1}; ^{1}H NMR ($CDCl_3$) 1.43 (s, 9H); 3.37 (dd, 1H; J = 6.1 and 13.5); 3.51 (dd, 1H; J = 4.4 and 13.5); 3.80-4.10 (m, 3H); 5.12 (bdd, 1H; J = 5.4 and 7.9); ^{13}C NMR ($CDCl_3$) 29.3, 49.3, 54.7, 58.1, 81.5, 85.7, 140.3, 154.9.

RuO_4 oxidation of cycloadduct 12. To a magnetically stirred 10% aqueous solution of $NaIO_4$ (9.45 g, 44.1 mmol) was added $RuO_2.xH_2O$ (78 mg). Such a mixture was immediately poured into a solution of cycloadduct **12** (3.1 g, 10.6 mmol) in ethyl acetate (100 mL) and the resulting suspension was stirred at room temperature until disappearance of the starting material. 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 (10 mL). The organic solution was dried over anhydrous sodium sulfate, the solvent removed under vacuum, and the residue was column chromatographed to yield 0.73 g of **10** and 1.71 g of **11**. Combined yield: 75%.

1,3-Dipolar cycloaddition of bromonitrile oxide to 19. To a solution of **19** (3.1 g, 13.7 mmol) in ethyl acetate (50 mL) was added dibromoformaldoxime (8.34 g, 41.1 mmol) and $NaHCO_3$ (15 g). The mixture was vigorously stirred for 3 days, than a further amount of dibromoformaldoxime (4.17 g, 20.5 mmol) was added and the mixture stirred for additional 3 days. The progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate 7:3). The crude material, obtained after the previously reported workup, was chromatographed on silica gel (eluant: petroleum ether/ethyl acetate 7:3) to give 0.40 g of unreacted olefin, 2.4 g of a mixture of cycloadducts **20** and **21** in a 1:1.1 ratio, and 0.64 g of **22** as a colorless solid. Combined yield: 64 %. The mixture of **20** and **21** was treated with hot 2-propanol to yield **21** as a colorless solid. Cycloadduct **20** was recovered almost pure from the mother liquor.

Compound **20**: yellowish oil; [Found: C, 41.65; H, 5.27; N, 7.75. $C_{12}H_{17}BrN_2O_5$ requires C, 41.28; H, 4.91; N, 8.02 %]; R_F (cyclohexane/ethyl acetate 7:3) 0.35; IR ν_{max} (neat) 1745, 1696, 1398, 1155, 897, 780 cm^{-1}; ^{1}H NMR ($CDCl_3$) 1.43 (s, 9H); 3.73 (dd, 1H; J = 5.4 and 13.4); 3.78 (s, 3H); 3.95 (bd, 1H; J = 13.4); 4.07 (d, 1H; J = 10.0); 4.67 (s, 1H); 5.28 (dd, 1H; J = 5.4 and 10.0). ^{13}C NMR ($CDCl_3$) 29.0, 53,7, 55.2, 62.6, 82.1, 84.8, 85.7, 138.4, 154.9, 171.2.

Compound **21** crystallized from 2-propanol as colorless prisms, mp 122-123 °C; [Found: C, 41.37; H, 5.11; N, 7.94. $C_{12}H_{17}BrN_2O_5$ requires C, 41.28; H, 4.91; N, 8.02 %]; R_F (cyclohexane/ethyl acetate 7:3) 0.35; IR ν_{max} (KBr disc) 1741, 1688, 1227, 1210, 876, 778 cm^{-1}; ^1H NMR (C_7D_8) 1.49 (s, 9H); 3.24 (ddd, 1H; J = 1.8, 7.8 and 8.4); 3.53 (s, 3H); 3.58 (dd, 1H; J = 7.8 and 11.8); 4.10 (dd, 1H; J = 1.8 and 11.8); 4.88 (s, 1H); 5.43 (d, 1H; J = 8.4). ^{13}C NMR (C_7D_8) 29.0, 50.3, 52.8, 57.9, 69.1, 81.6, 88.6, 140.4, 157.3, 170.8.

Compound **22** crystallized from 2-propanol as colorless prisms, mp 177-178 °C; [Found: C, 41.45; H, 5.17; N, 7.83. $C_{12}H_{17}BrN_2O_5$ requires C, 41.28; H, 4.91; N, 8.02 %]; R_F (cyclohexane/ethyl acetate 7:3) 0.28; IR ν_{max} (KBr disc) 1728, 1680, 1275, 1249, 1014, 859 cm^{-1}; ^1H NMR ($CDCl_3$) 1.43 (s, 9H); 3.76 (s, 3H); 3.78 (dd, 1H; J = 9.0 and 11.5); 3.84 (dd, 1H; J = 2.4 and 11.5); 4.13 (ddd, 1H; J = 2.4, 9.0 and 10.2); 4.70 (d, 1H; J = 8.5); 5.43 (dd, 1H; J = 8.5 and 10.2); ^{13}C NMR ($CDCl_3$) 29.2, 49.7, 53.4, 57.2, 66.1, 82.3, 85.1, 141.4, 154.4, 169.5.

Synthesis of 3-Bromo-4-cyano-5-tert-butoxycarbonyl-3a,5,6,6a-tetrahydro-4*H*-pyrrolo[3,4-*d*]isoxazoles 15a and 15b. A. To a magnetically stirred solution of pyrrolidinone **10** (0.65 g, 2.1 mmol) in dry THF (30 mL) was added dropwise a solution of DIBAL-H in hexane (1 M, 3 mL). The reaction mixture was stirred at -78 °C for 2 h, then treated with a saturated aqueous solution of ammonium chloride (10 mL). The mixture was brought to room temperature, the THF removed under vacuum and the aqueous layer extracted with dichloromethane (3x10 mL). The organic extracts were pooled, dried over sodium sulfate, and concentrated under vacuum to give 615 mg (94 % yield) of the crude product, which was not further characterized but directly used in the following step.

B. A solution of p-toluenesulfonic acid (50 mg) in methanol (50 mL) was added to the above prepared compound (615 mg) and stirred at room temperature until disappearance of the starting material (2 h). Methanol was removed under vacuum, the residue was treated with an aqueous solution of sodium carbonate (10%, 10 mL) and extracted with dichloromethane (2x10 mL). The organic extracts were pooled, dried over sodium sulfate, and concentrated under vacuum to give α-methoxy carbamate **13** in quantitative yield. ^1H NMR spectrum of the crude product showed the presence of two stereoisomers in a 1:1 ratio. Such an intermediate was not further characterized but directly used in the following step.

C. To a solution of α-methoxy carbamate **13** (635 mg, 1.98 mmol) in dichloromethane (15 mL) at -40 °C under nitrogen were added trimethylsilyl cyanide (1.06 mL, 7.92 mmol) and boron trifluoride ethyl etherate (0.5 mL). The progress of the reaction was monitored by TLC (eluant: cyclohexane/ethyl acetate 4:1). After 30 min, a solution of aqueous sodium carbonate (10%, 10 mL) was added before extraction with dichloromethane (3x10 mL). The crude product, obtained by usual workup, was purified by flash column chromatography on silica gel (eluant: petroleum ether/ethyl acetate 4:1) to afford diastereomers **15a** (178 mg) and **15b** (381 mg). Combined yield: 84% (from **10**). Compound **15a** crystallized from ethyl acetate as colorless prisms, mp 140-142 °C; [Found: C, 41.97; H, 4.70; N, 13.02. $C_{11}H_{14}BrN_3O_3$ requires C, 41.79; H,

4.46; N, 13.29 %]; R_F (cyclohexane/ethyl acetate 3:2) 0.42; IR ν_{max} (KBr disc) 2230, 1692, 1382, 1329, 1240, 1175, 897, 772 cm^{-1}; ^1H NMR ($CDCl_3$) 1.50 (s, 9); 3.82 (dd, 1H; J = 2.7 and 13.1); 4.07 (dd, 1H; J = 6.6 and 13.1);); 4.18 (dd, 1H; J = 9.1, and 9.3); 4.95 (d, 1H; J = 9.3); 5.38 (ddd, 1H; J = 2.8, 6.6 and 9.1); ^{13}C NMR ($CDCl_3$) 29.1, 51.3, 52.8, 60.4, 83.9, 86.9, 115.5, 136.9, 153.2.

Compound **15b** crystallized from cyclohexane-ethyl acetate as colorless prisms, mp 147-150 °C; [Found: C, 41.85; H, 4.48; N, 13.11. $C_{11}H_{14}BrN_3O_3$ requires C, 41.79; H, 4.46; N, 13.29 %]; R_F (cyclohexane/ethyl acetate 3:2) 0.87; IR ν_{max} (KBr disc) 2240, 1681, 1390, 1227, 1172, 879, 774 cm^{-1}; ^1H NMR ($CDCl_3$) 1.43 (s, 9H); 3.64 (dd, 1H; J = 5.2 and 13.2); 4.15 (bd, 1H; J = 13.2);); 4.20 (d, 1H; J = 9.3); 4.91 (s, 1H); 5.39 (dd, 1H; J = 5.2 and 9.3); ^{13}C NMR ($CDCl_3$) 29.1, 51.2, 54.5, 63.5, 84.0, 85.4, 117.1, 136.0, 163.1.

Synthesis of 3-Bromo-5-tert-butoxycarbonyl-6-cyano-3a,5,6,6a-tetrahydro-4*H*-pyrrolo[3,4-*d*]isoxazoles 16a and 16b. The reaction sequence described above for **10** was also applied to cycloadduct **11** to give cyano derivatives **16a** and **16b** with a combined yield of 71% (from **11**).

Compound **16a** crystallized from ethyl acetate as colorless prisms, mp 130-132 °C; [Found: C, 42.07; H, 4.27; N, 13.41. $C_{11}H_{14}BrN_3O_3$ requires C, 41.79; H, 4.46; N, 13.29 %]; R_F (cyclohexane/ethyl acetate 3:2) 0.28; IR ν_{max} (KBr disc) 2235 cm^{-1}; ^1H NMR ($CDCl_3$) 1.48 (s, 9H); 3.77 (d, 2H; J = 6.3); 4.17 (ddd, 1H; J = 6.3, 6.3 and 9.4); 4.92 (d, 1H; J = 7.6); 5.41 (dd, 1H; J = 7.6 and 9.4).

Compound **16b** crystallized from cyclohexane-ethyl acetate as colorless prisms, mp 126-128 °C; [Found: C, 41.95; H, 4.69; N, 12.98. $C_{11}H_{14}BrN_3O_3$ requires C, 41.79; H, 4.46; N, 13.29 %]; R_F (cyclohexane/ethyl acetate 3:2) 0.67; IR ν_{max} (KBr disc) 2235 cm^{-1}; ^1H NMR ($CDCl_3$) 1.50 (s, 9H); 3.58 (dd, 1H; J = 7.6 and 12.7); 4.05 (dd, 1H; J = 2.1 and 12.7); 4.12 (ddd, 1H; J = 2.1, 7.6 and 8.3); 4.88 (s, 1H); 5.42 (d, 1H; J = 8.3).

Synthesis of 3-Hydroxy-5-tert-butoxycarbonyl-3a,5,6,6a-tetrahydro-4*H*-pyrrolo[3,4-*d*] isoxazole-4-carboxylic acid 17 from 15a,b. A. Urea-hydrogen peroxide adduct (UHP) (0.75 g, 7.97 mmol) and potassium carbonate (22 mg, 0.158 mmol) were added to a solution of cyano derivatives **15a-15b** (0.5 g, 1.58 mmol) in acetone-water (1:1, 10 mL). The mixture was magnetically stirred at room temperature overnight. Acetone was evaporated under vacuum and the aqueous phase was thoroughly extracted with dichloromethane (3x5 mL). The extracts were dried over anhydrous sodium sulfate and concentrated at reduced pressure. The residue was not further characterized but directly submitted to the following reaction.

B. A suspension of the above prepared amide (0.50 g, 1.5 mmol) in 5 N sodium hydroxide (10 mL) was magnetically stirred at 60 °C until disappearance of the starting material (3 h). The progress of the reaction was monitored by TLC (eluant: 2% acetic acid in chloroform/methanol 4:1). The aqueous solution was extracted with ethyl acetate (2x5 mL) then made acidic with 2 N HCl and newly extracted with ethyl acetate (4x5 mL). The organic extracts were pooled and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to leave 0.28 g (73% yield) of **17** as a colorless solid. Compound **17**: decomposes at a temperature >190 °C; [Found: C, 48.90; H, 5.65; N, 9.95. $C_{11}H_{16}N_2O_6$ requires C, 48.53; H, 5.92; N, 10.29%];

R_F (2% acetic acid in chloroform-methanol 4:1) 0.33; ^{1}H NMR (CD_3OD) 1.48 (s, 9H); 3.63-3.90 (m, 3H) 4.70 (bs, 1); 5.32 (m, 1H).

Transformation of cycloadduct 20 into carboxylic acid 17. To a solution of cycloadduct **20** (0.45 g, 1.29 mmol) in dioxane (9 mL)was added an aqueous solution of 1 N sodium hydroxide (3.0 mL) and the mixture was magnetically stirred at 60 °C until disappearance of the starting material (3 h). The mixture was extracted with ethyl acetate (2x5 mL) and the aqueous layer made acidic with 2 N HCl and newly extracted with ethyl acetate (4x5 mL). The organic extracts were pooled and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to leave 0.25 g (71% yield) of **17** as a colorless solid.

Synthesis of 3-Hydroxy-5-tert-butoxycarbonyl-3a,5,6,6a-tetrahydro-4*H*-pyrrolo [3,4-*d*] isoxazole-6-carboxylic acid 18. The above reported reaction sequence was also applied to the mixture of cyano derivatives **16a** and **16b** to yield acid **18** in 76% yield.

Compound **18**: decomposes at a temperature >185°C; [Found: C, 48.82; H, 5.71; N, 10.05. $C_{11}H_{16}N_2O_6$ requires C, 48.53; H, 5.92; N, 10.29 %]; R_F (2% acetic acid in chloroform-methanol 4:1) 0.29; ^{1}H NMR (CD_3OD) 1.47 (s, 9H); 3.47 (bdd, 1H; J = 8.5 and 9.3); 3.73 (dd, 1H; J = 9.3 and 10.3); 3.98 (bd, 1H; J = 10.3); 4.41 (bs, 1H); 5.24 (bd, 1H; J = 8.5).

Transformation of cycloadduct 21 (or 22) into carboxylic acid 18. To a solution of cycloadduct **21** (or **22**) (0.346 g, 0.99 mmol) in dioxane (7 mL) was added an aqueous solution of 1 N sodium hydroxide (2.5 mL) and the mixture was magnetically stirred at 60 °C until disappearance of the starting material (3 h). The mixture was extracted with ethyl acetate (2x5 mL) and the aqueous layer made acidic with 2 N HCl and newly extracted with ethyl acetate (4x5 mL). The organic extracts were pooled and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to leave 0.21 g (78% yield) of **18** as a colorless solid.

Synthesis of 3-Hydroxy-3a,5,6,6a-tetrahydro-4*H*-pyrrolo [3,4-*d*]isoxazole-4-carboxylic acid 6. Intermediate **17** (0.28 g, 1.03 mmol) was treated with a 30% dichloromethane solution of trifluoroacetic acid (10 mL) at 0 °C. The reaction mixture was stirred at room temperature until disappearance of the starting material (2 h). The volatiles were removed under vacuum and the residue was taken up with methanol and filtered under vacuum to give 0.122 g (65 % yield) of **6** as colorless prisms.

Compound **6**: decomposes at a temperature >190 °C; [Found: C, 41.57; H, 4.69; N, 15.97. $C_6H_8N_2O_4$ requires C, 41.86; H, 4.68; N, 16.27 %]; R_F (butanol/H_2O/acetic acid 60:25:15) 0.34; ^{1}H NMR (CF_3COOD): 3.97 (dd, 1H; J = 7.5, and 14.4); 4.13 (d, 1H; J = 14.4); 4.38 (dd, 1H; J = 2.9 and 7.9); 5.33 (d, 1H; J = 2.9); 5.60 (dd, 1H; J = 7.5 and 7.9); ^{13}C NMR (CF_3COOD): 53.4, 55.5, 65.0, 84.8, 171.3, 172.0.

Synthesis of 3-Hydroxy-3a,5,6,6a-tetrahydro-4*H*-pyrrolo [3,4-*d*]isoxazole-6-carboxylic acid 7. The above-reported procedure carried out on intermediate **18** and gave final derivative **7** in 67% yield.

Compound **7**: decomposes at a temperature >175 °C; [Found: C, 41.61; H, 4.55; N, 16.02. $C_6H_8N_2O_4$ requires C, 41.86; H, 4.68; N, 16.27 %]; R_F (butanol/H_2O/acetic acid 60:25:15) 0.37; ^{1}H NMR (CF_3COOD):

4.09 (dd, 1H; J= 8.4, and 12.1); 4.23 (dd, 1H; J = 8.4 and 8.4); 4.37 (d, 1H; J = 12.1); 5.17 (d, 1H; J = 1.7); 5.82 (dd, 1H; J = 1.7 and 8.4); ^{13}C NMR (CF_3COOD): 51.1, 52.3, 68.7, 86.7, 171.2, 172.3.

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