An Expedient Synthesis of (±)-2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic Acid Hydrobromide *via* a 3-Bromoisoxazole Intermediate

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The excitatory, amino acid ±2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid hydrobromide was prepared in gram quantities in an 3.3% overall yield from methylbut 2-ynoate. The key step was the facile preparation of methyl 3-bromo-5-methylisoxazole-4-carboxylate.

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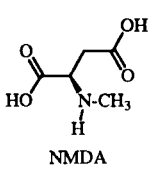
The pharmacology of excitatory amino-acids and the potential therapeutic applications of drugs which interact with their receptors has generated much research interest in recent years [1,2]. One particular area has focused on glutamic acid and specifically on a subgroup of receptors for this amino acid characterized as non-*N*-methyl-D-aspartic acid receptors [Figure 1]. A bioisostere of glutamic acid which has been used to characterize this subgroup of receptors is 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid and therefore efficient methods for its preparation are of interest.

Several previous syntheses of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid hydrobromide have been reported, however, several draw-backs are apparent. The schemes provided by Hansen and Krogsgaard-Larsen [3] as well as by Honore and Lauridsen [4] are low yield, small scale processes. That described by Bergtrup and Slok [5] is more efficient, however, it utilizes an intermediate that is not commercially available and the steps are sensitive to reaction conditions.



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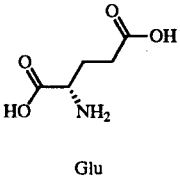

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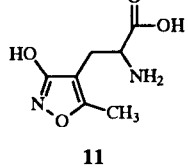

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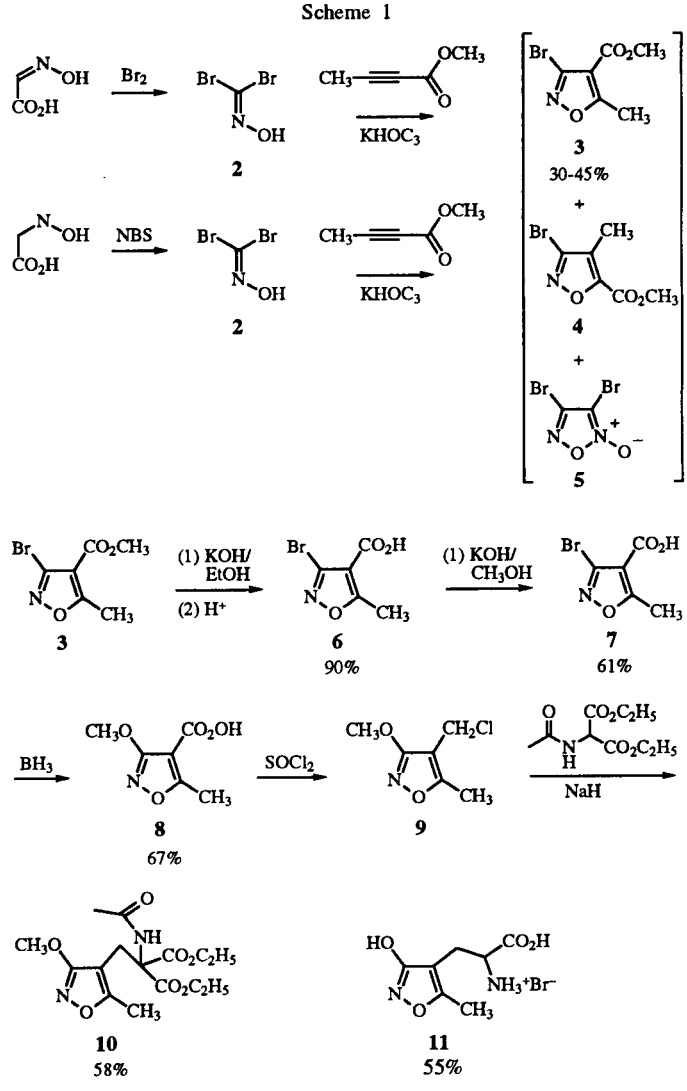
This report describes an expedient synthesis of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid hydrobromide which utilizes readily available starting materials and can provide the product on a multigram scale (Scheme 1). The synthesis used the preparation of a 3-bromoisoxazole as the key entry into the basic target structure.

The choice of methyl 3-bromo-5-methylisoxazole-4-carboxylate was based on several factors. Although both 3-chloro- and 3-bromoisoxazoles have been prepared *via* addition of the corresponding halonitrile oxide to an alkyne, the generation of the requisite dibromoformaldoxime is easier and safer than the analogous dichloroformaldoxime. Because the addition of the halonitrile oxide to an asymmetrical alkyne generates isomeric products, it was felt that a larger substituent at the 3-position would enhance the separation of isomers [6]. Finally, since the synthesis would

Scheme 1



involve a nucleophilic substitution at the 3-position by alkoxide, the more reactive bromo substituent would enhance the rate of reaction as well as improve the yield. Although this study does not compare the relative effect of the 3-bromo group directly, the results obtained in this facile synthesis of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)-propionic acid hydrobromide justify the initial premises.

The dibromoformaldoxime **2** was prepared by the addition of bromine in methylene chloride to a cold aqueous solution of glyoxylic acid aldoxime **1** [7]. The dibromoformaldoxime was isolated in pure form for the subsequent, base-promoted addition to methyl butynoate. Alternatively, the dibromoformaldoxime **2** was generated *in situ* by the addition of *N*-bromosuccinimide to the glyoxylic acid aldoxime **1** followed by addition to the methyl butynoate in the presence of base. Separation of the organic phase and purification by flash chromatography gave the methyl 3-bromo-5-methylisoxazole-4-carboxylate **3** in a 30-45% yield. The remainder of the product consisted of the dibromofuroxan **5** [8] and the isomeric methyl 3-bromo-4-methylisoxazole-5-carboxylate **4**. Subsequent hydrolysis of the methyl ester with cold ethanolic potassium hydroxide followed by acidification provided the carboxylic acid **6** in a 90% yield. Heating the 3-bromo-5-methylisoxazole-4-carboxylic acid with methanolic potassium hydroxide at reflux for 96 hours gave a 61% yield of the 3-methoxy-5-methylisoxazole-4-carboxylic acid **7**. The remainder of the reaction mixture was a combination of product and starting material which could be recycled for a more efficient conversion. The desired 4-hydroxymethyl intermediate **8** was obtained by borane reduction in a 67% yield (after recrystallization) and converted with thionyl chloride in a 70% yield to the 3-methoxy-4-chloromethyl-5-methylisoxazole **9**, identical to the previously reported compound [3,8]. Conversion with diethyl acetamidomalonate and deprotection with hydrobromic acid using literature procedures [3,4] gave the described product **11**, identical to authentic material.

The overall yield for (±) 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid hydrobromide **11** is 3.3% for the seven step synthesis. It should be noted that the step with lowest yield is the first one which can be performed on a greater than 100 g scale with relative ease and safety. The subsequent reactions have not been optimized but one can anticipate that each step can be enhanced to some extent. This synthesis, therefore, represents an improvement upon previous reports and also possesses the ability to be applied to the preparation of other analogs.

EXPERIMENTAL

The ^{1}H-nmr Spectra were obtained on a Varian XL300 multinuclear spectrometer using TMS as an internal standard. The ir spectra were recorded with a Perkin Elmer model 1600 FTIR. Melting points were determined with a Thomas Hoover melting point apparatus and are uncorrected. Elemental analysis (C,H,N) were provided by Atlantic Microlab, Inc. (Norcross, GA). Analytical tlc was performed on plastic backed Silica gel 60 F_{254} sheets (Bakerflex) Column chromatography employed silica gel (Baker flash chromatography grade). Solvents and reagents were obtained commercially and used without further purification.

Dibromoformaldoxime (**2**).

To a solution of glyoxylic acid monohydrate (58 g) in 500 ml of water was added hydroxylamine hydrochloride (47 g). The reaction solution was stirred at ambient temperature for 24 hours, then neutralized by the portionwise addition of sodium bicarbonate and mixed with 500 ml methylene chloride. The reaction mixture was cooled to 4° with an ice bath and a solution of bromine (48 ml) in 250 ml of methylene chloride was added dropwise with stirring over 1.25 hours. The reaction was stirred for an additional 2 hours during which the orange-red color gradually disappeared. The phases were separated and the aqueous layer was extracted with methylene chloride. The organic phases were combined, evaporated to give a white solid that was washed with cold hexanes and collected by filtration. The product **2** was dried to give 24 g (mp 67-71°, lit 65-69°).

Methyl 3-Bromo-5-methylisoxazole 4-carboxylate (**3**).

Method A.

To a vigorously stirred mixture of methyl butynoate (13.7 g, 140 mmoles) and potassium bicarbonate (20 g) in 100 ml of methylene chloride was added dropwise with stirring a solution of dibromoformaldoxime **2** (20.3 g, 100 mmoles) in 100 ml of methylene chloride. The reaction mixture was stirred at ambient temperature for 48 hours, then filtered and evaporated to dryness. The residue was purified by flash chromatography on 400 g silica gel using hexane-methylene chloride (20:1 → 10:1) to give the product in a 44% isolated yield (9.7 g, 44 mmoles), mp 66-69°. Recrystallization from hexanes gave colorless needles, mp 77-78°.

Method B.

To the cold stirred solution of glyoxylic acid aldoxime **1** (98 g, 110 mmoles) in 100 ml dimethoxyethane and 30 ml of water was added portionwise *N*-bromosuccinimide (35.6 g, 200 mmoles) over a 35 minute period. When the addition was complete, the reaction solution was allowed to warm to ambient temperature and stirred for an additional 30 minutes. The resultant solution of dibromoformaldoxime **2** was added dropwise over a 2.5 hour period to stirred mixture composed of methyl butynoate (20 g, 200 mmoles), potassium bicarbonate (40 g) and 100 ml, of dimethoxyethane. The reaction mixture was stirred at ambient temperature for 16 hours filtered and evaporated to dryness. The residue was partitioned between ethyl acetate and water and the organic phase was separated, washed sequentially with water, brine, dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. The crude product was purified by flash chromatography on silica gel using hexane-methylene chloride (10:1 → 8:1) as the eluent. The resultant solid was recrystallized from cyclohexane to give the colorless product **3** in a 30% yield (6.7 g, 30 mmoles); ^{1}H-nmr (deuteriochloroform): 2.71 (s, 3H, CH_3), 3.88 (S, 3H, O-CH_3); ir (potassium bromide): 2960 (w), 1727 (s), 1679 (m), 1984 (s), 1957 (s),1400 (s), 1250 (s).

Anal. Calcd. for $C_6H_6NO_3Br$: C, 32.76; H, 2.75; N, 6.37. Found: C, 32.87; H, 2.76; N, 6.32.

3-Bromo-5-methylisoxazole-4-carboxylic Acid (**6**).

To a mixture of the ester **3** (9.2 g, 42 mmoles) in 35 ml of ethanol was added potassium hydroxide (4.4 g, 78 mmoles) in 7 ml of water. The reaction was stirred at ambient temperature for 4 hours. The reaction was diluted with 100 ml of water and extracted twice with ether. the aqueous phase was acidified with 2 *N* hydrochloric acid to give a two phase system. The mixture was extracted three times with ether. The organic phase was washed with brine, dried over anhydrous magnesium sulfate, filtered and evaporated to dryness to give the crude product. The solid was recrystallized from hexane-ethyl acetate to give the pure product in a 90% yield (6.8 g, 36 mmoles), mp 181-183°; ^{1}H-nmr: 2.78 (s, 3H, CH_3), ir (potassium bromide): 3440 (m), 1741 (s), 1582 (s), 1463 (s), 1439 (s), 7246 (m).

Anal. Calcd. for $C_5H_4NO_3Br$: C, 29.16; H, 1.96; N, 6.80. Found: C, 29.27; H, 1.96; N, 6.74.

3-Methoxy-5-methylisoxazole-4-carboxylic Acid (**7**).

The reaction solution composed of the carboxylic acid **6** (84 g, 40 mmoles), potassium hydroxide (8 g) in 60 ml of methanol was heated at reflux for 96 hours. The reaction solution was diluted with water and acidified with 20 ml of concentrated hydrochloric acid. The mixture was extracted with ether. The organic phase was washed with brine, dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. The solid was recrystallized from ethyl acetate to give the pure product in a 61% yield (4.5 g, 24.5 mmoles), mp 200-204°. The mother liquor contained a mixture of product and unreacted starting material; ^{1}H-nmr (deuteriochloroform): 2.66 (s, 3H, CH_3), 4.10 (s, 3H, OCH_3); ir (potassium bromide): 3461 (m), 1686 (s), 1617 (m), 1527 (s), 1490 (m), 1404 (m).

Anal. Calcd. for $C_6H_7NO_4$: C, 45.87; H, 4.49; N, 8.92. Found C, 45.66; H, 4.41; N, 8.77.

3-Methoxy-4-hydroxymethyl-5-methyl-isoxazole (**8**).

To a solution of the methoxy carboxylic acid **7** (4.15 g, 25 mmoles) in 50 ml of tetrahydrofuran was added 30 ml of 1 *M* borane in tetrahydrofuran. The reaction solution was heated at reflux for 16 hours, then cooled to ambient temperature and the excess borane was decomposed by the addition of methanol. The solution was evaporated to dryness and the residue was purified by column chromatography on a silica gel using methanol in methylene chloride (2% → 5%) as the eluent. The fractions containing the product were combined, evaporated to give an oil that crystallized on standing, (2.5 g, 17.5 mmoles, 67%), mp 70-71°; ^{1}H-nmr (deuteriochloroform): 2.32 (s, 3H, CH_3), 3.97 (s, 3H, -CH_3),4.39 (d, 2H,-CH_2); ir (potassium bromide): 3256 (m), 1659 (s),1525 (s), 1479 (s), 1416 (s), 1275 (s).

Anal. Calcd. for $C_6H_9NO_3$: C, 50.35; H, 6.34; N, 9.79. Found C, 50.24; H, 6.37; N, 9.78.

3-Methoxy-4-chloromethyl-5-methylisoxazole (**9**).

To a solution of 3-methoxy-4-hydroxymethyl-5-methylisoxazole **8** (3.0 g, 21 mmoles) in 40 ml of benzene was added 6 ml of thionyl chloride. The reaction was heated at reflux for 24 hours, cooled and evaporated to dryness. The residue was purified by chromatography on silica gel using hexanes-methylene chloride (2:1) as the eluent. The product was isolated in a 70% yield (2.3 g, 14.6 mmoles); ^{1}H-nmr (deuteriochloroform): 2.37 (s, 3H, CH_3), 4.00 (s, 3H, OCH_3), 4.33 (s, 2H, CH_2Cl)

Diethyl 3-Methoxy-5-methyl-(1.2-isoxazol-4-yl)methylacetamidomalonate (**10**).

To a stirred solution of diethyl acetamidomalonate (325 g, 15 mmoles) in 75 ml of ethanol was gradually added sodium hydride (99%, 0.36 g, 15 mmoles). After stirring for 10 minutes the chloromethyl isoxazole was added and the resulting mixture was heated at reflux for 24 hours, cooled to ambient temperature, filtered and evaporated to dryness. The crude material was purified by column chromatography on neutral alumina (Activity III), using methanol-methylene chloride (1:99) as the eluent. The fractions containing the product were combined, evaporated to dryness and recrystallized from hexane-ethanol to give the colorless product in a 58% yield (2.8 g, 8.8 mmoles), mp 97-99°; ^{1}H-nmr (deuteriochloroform): 1.28 (t, 6H, CH_2, CH_3), 1.99 (s, 3H,CH_3), 2.18 (s, 3H, CH_3), 3.35 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3), 4.16-4.30 (m, 4H, -CH_2 -C) ir (potassium bromide): 3252 (m), 2986 (w), 1747 (s), 1639 (m), 1574 (s), 1472 (m), 1313 (s).

Anal. Calcd. for $C_{15}H_{22}NO_2O_7$: C, 52.63; H, 6.48; N, 8.18. Found: C, 52 51; H, 6.45; N, 8.14

(+)-2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic Acid Hydrobromide (**11**).

The diethyl isoxazolylmethylacetamidomalonate **10** (3.0 g, 9.2 mmoles) was dissolved in 50 ml of 48% hydrobromic acid and the solution was heated at 130-140° for 1-5 hours. The solution was evaporated under reduced pressure. The crude red oil was dissolved in deionized water and reevaporated. This procedure was repeated three times. The residue was dissolved in 2-propanol and precipitated by the addition of ether, collected by filtration, washed with 2-propanol-ether to give the crude product (2.3 g, 86 mmoles, 93%). Recrystallization from 2-propanol-ether gave a pure colorless product in a 55% yield (1.37 g, 5.1 mmoles), mp 215-217°; ^{1}H-nmr (deuteriochloroform): 2.23 (s, 3H, CH_3), 2.93 (d, 2H, CH_2), 4.27 (t, 1H, -CH-); ir (potassium bromide): 3445 (m), 2929 (m), 1737 (s), 1664 (m), 1580 (m), 1528 (m), 1498 (m), 1210 (m).

Anal. Calcd. for $C_7H_{12}N_2O_4Br \cdot 0.5C_4H_{10}O$: C, 35.54; H, 5.30; N, 9.21. Found: C, 35.91; H, 5.28; N, 9.22.

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