Equilibrium Control in Bromomethylation: An Expedient Route to 2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic Acid (AMPA)‡

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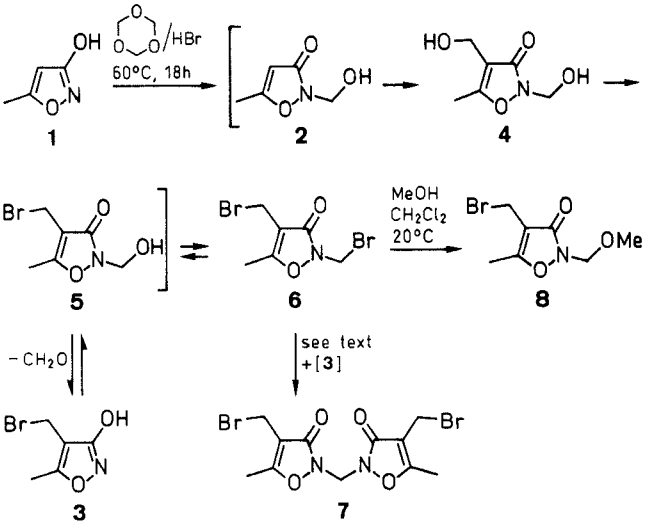
The excitatory amino acid 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) has been prepared in gram quantities in 42% total yield by a three-step procedure from 3-hydroxy-5-methylisoxazole. It is shown how bromomethylation may be optimized through control of the involved equilibria and how *N*-protecting methoxymethyl groups can be removed.

2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA, **10**) is a bioisoster of glutamic acid which acts as an excitatory neurotransmitter in the central nervous system.[1,2] AMPA is used extensively as a standard reference for the characterization of neuroreceptors of the non-*N*-methyl-D-aspartic acid (non-NMDA) type. The subgroup of these receptors which binds AMPA specifically are named AMPA-receptors.[3] Therefore, the synthesis of AMPA has received much attention.

Previous methods for the synthesis of AMPA are all quite inefficient.[1,4] They produce low yields and they are tedious multistep processes. Finally, the procedures can only be run on a small scale.

We now report on a simple three-step procedure which can be performed from an inexpensive starting material on at least a 30 g scale providing a total yield of 42%. The synthesis comprises bromomethylation at C-4 of commercially available 3-hydroxy-5-methylisoxazole (**1**, tachigaren) followed by displacement of the bromine with diethyl acetamidomalonate.

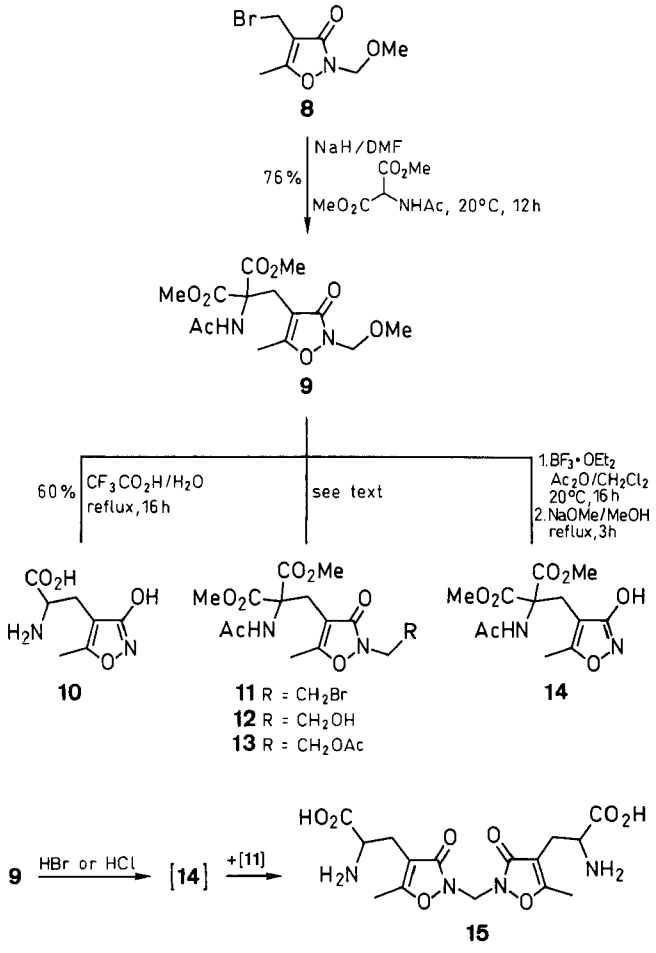
The bromomethylation of 3-hydroxy-5-methylisoxazole (**1**) proved to be intricate. The use of aqueous hydrogen bromide and trioxane under standard conditions[5] gave complicated mixtures. Therefore, a more systematic study of the course of the reaction was carried out using ^{1}H and ^{13}C NMR spectroscopy. The spectra indicated that the starting material **1** first undergoes hydroxymethylation at the 2-position to give **2**, the reaction being complete in ca. 10 minutes when 48% HBr and trioxane were used. Further reaction of the 2-hydroxymethyl-5-methylisoxazolin-3-one (**2**) required prolonged heating at 60°C. Under these conditions 2,4-di(bromomethyl)-5-methylisoxazolin-3-one (**6**) could be isolated, but it was invariantly contaminated with the dimer **7**. When the reaction was performed in aqueous deuterium bromide the NMR spectra surprisingly revealed that 4-bromomethyl-2-hydroxymethyl-5-methylisoxazolin-3-one (**5**) is solely present in the reaction mixture. Therefore, the isolated 2,4-di(bromomethyl)-5-methylisoxazolin-3-one (**6**) must be formed during the workup procedure which essentially is an extraction procedure with dichloromethane. Apparently, the extraction removes the bromomethyl compound **6** from its equilibrium with the hydroxymethyl compound **5**. If 48% aqueous hydrogen bromide is used, the equilibrium mixture contains both **5** and **6**. The 4-bromomethyl-2-hydroxymethyl compound **5**, in its turn, is in equilibrium with formaldehyde and the 4-bromomethyl compound **3**. The latter compound is *N*-alkylated by the 2,4-di(bromomethyl) compound **6** producing the dimer **7**. If, however, 62% aqueous hydrogen bromide is employed, the equilibrium between **5** and **6** is pushed completely in favor of the 2,4-di(bromomethyl) compound **6** which can then be extracted with dichloromethane in virtually quantitative yield.



The interrelations between the observed equilibria may have implications for the outcome of other hydroxyalkylations and halomethylations. In general, these reactions are often hampered by low yields and the formation of byproducts. Optimization of these reactions may be successful if proper attention is paid to the equilibria during the planning of reaction conditions and workup.

The isolated 2,4-di(bromomethyl) compound **6** is extremely sensitive to hydrolysis of the 2-bromomethyl group. This leads to the 2-hydroxymethyl-4-bromomethyl compound **5** which eliminates formaldehyde and gives **3**. A substantial part of the latter reacts with unchanged **6** and produces the dimer **7** which is difficult to separate from **3**. Therefore, aqueous hydrolysis of the 2,4-di(bromomethyl) compound **6** is unsuitable for the removal of its *N*-substituent. As a consequence, the 2-bromomethyl group of **6** was converted selectively to a more stable functionality which could then be removed under mild conditions. This is feasible since the 2-bromomethyl group in **6** is more reactive towards nucleophiles than the 4-bromomethyl group. Thus, treatment of **6** with methanol in dichloromethane at room temperature for ca. 1 hour gave the 4-bromomethyl-2-methoxymethyl compound **8** in quantitative yield. Compound **8** is more stable than **6** and is therefore an appropriate intermediate in further conversions. On the other hand, the 2-methoxymethyl group can then be cleaved off cleanly under mild conditions. The 4-bromomethyl-2-methoxymethyl compound **8** could be obtained in 93% yield from the starting material **1** by a one-pot procedure.

Compound **8** was then treated with the anion of dimethyl acetylamidomalonate in dimethylformamide to give methyl 2-acetamido-2-methoxycarbonyl-3-(2-methoxymethyl-5-methylisoxazolin-3-on-4-yl)propanoate (**9**) in 76% yield. Deprotection of this compound to yield AMPA (**10**) involves removal of the 2-methoxymethyl group, hydrolysis of the two ester groups, decarboxylation of one of the resulting carboxyl groups, and hydrolysis of the amide group. All of these processes should be feasible under conditions of acidic hydrolysis. Hydrochloric and hydrobromic acid proved inappropriate producing AMPA (**10**) in only 5–20% yields. The major product was the dimer **15**. Most likely this compound is formed by alkylation of the *N*-deprotected compound **14** with the 2-bromomethyl compound **11**, formed when bromide ions displace methanol of the protonated 2-methoxymethyl group. In order to avoid formation of the dimer **15** an acid with a non-nucleophilic counterion was employed. Trifluoroacetic acid proved to be suitable having the additional advantage that excess is readily removed by evaporation. In this way all protecting groups could be removed in one sequence giving rise to 60% analytically pure 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA, **10**), in gram-scale preparations.



Alternative stepwise deprotection starting with selective removal of the 2-methoxymethyl group of **9** was also found feasible. Several reagents were tried. Zinc bromide gave no conversion while titanium tetrachloride led to a ca. 1:1 mixture of deprotected material **14** together with the corresponding 2-hydroxymethyl derivative **12**.

Selective demethoxymethylation was achieved by treatment of **9** with acetic anhydride in the presence of diethyl ether–boron trifluoride complex. This afforded the 2-acetoxymethyl compound **13**. The success of this reaction may be due to the formation of a cyclic intermediate involving the carbonyl oxygen atom of the isoxazolone. A related intermediate has been proposed by cleavage of *O*-methoxyethyl protecting groups.[6] Treatment of the 2-acetoxymethyl compound **13** with sodium methoxide in methanol produced the selectively deprotected methyl 2-acetamido-3-(3-hydroxy-5-methylisoxazol-4-yl)-2-methoxycarbonylpropanoate (**14**) in high yield. The latter compound can be converted to AMPA (**10**) by known procedures.[1,10]

CH_2Cl_2 was dried over NaH. DMF and MeOH were purified as described.[7,8] Unless otherwise stated $MgSO_4$ was used to dry organic extracts. Solvents were removed in vacuo by rotary evaporation. All new compounds were colorless, unless otherwise stated. The purity of all compounds were confirmed by melting points, TLC, and ^1H and ^{13}C NMR spectra, recorded at 200 and 50.32 MHz, respectively, on a Bruker AC-200 instrument. Signal positions are given in ppm relative to TMS when $CDCl_3$ is used as the solvent and relative to dioxane when water is used. ^{13}C NMR signals were assigned through their multiplicity in the coupled spectra or through DEPT spectra. Multiplicity resulting from long range C–H couplings are given in brackets.

Tachigaren (**1**) was supplied by Cheminova A/S, Denmark. The technical quality can be purified by dissolution in Et_2O, filtration through activated carbon and recrystallization from Et_2O/hexane. The mp of the pure colorless compound is 84–85°C.

All new compounds gave satisfactory microanalyses data: C ± 0.17, H ± 0.09, N ± 0.1.

4-Bromomethyl-2-methoxymethyl-5-methylisoxazolin-3-one (8):
3-Hydroxy-5-methylisoxazole (tachigaren, **1**; 9.91 g, 0.10 mol) and 1.3.5-trioxane (13.5 g, 0.15 mol) were placed in a 250 mL flask. 62% Aq HBr (100 mL) was added, the flask was closed with a glass stopper and the mixture was stirred at 60°C in an oil bath for 18 h. Extraction with CH_2Cl_2 (5 × 100 mL), addition of MeOH (250 mL), mixing well, and standing for 2 h gave a mixture which was divided into two equal parts each of which was worked up as follows: Addition of CH_2Cl_2 (250 mL), washing with water (3 × 400 mL), drying, filtration and removal of the CH_2Cl_2 produced **8** as a colorless oil; yield: 22 g (93%).

^1H NMR ($CDCl_3$): δ = 2.34 (s, 3H, CH_3), 3.40 (s, 3H, CH_3O), 4.20 (s, 2H, CH_2Br), 5.16 (s, 2H, NCH_2O).

^{13}C NMR ($CDCl_3$): δ = 11.8 (CH_3), 18.6 (CH_2Br), 56.8 (CH_3O), 75.0 (NCH_2O), 106.9 (C-4), 165.5 (triple triplet, C-3), 168.6 (triple quartet, C-5).

Methyl 2-Acetamido-2-methoxycarbonyl-3-(2-methoxymethyl-5-methylisoxazolin-3-on-4-yl)propanoate (9):
A 80% suspension of NaH in mineral oil (2.25 g, 75 mmol) was added during 20 min to a solution of dimethyl acetamidomalonate[9] (13.25 g, 70 mmol) in DMF (250 mL). After another 20 min, a solution of **8** (16.53 g, 70 mmol) in DMF (50 mL) was added with stirring during 20 min. After stirring for 12 h AcOH (5 mL) was added and the mixture was evaporated to dryness, dissolved in CH_2Cl_2 (300 mL) and washed with water (100 mL). The aqueous solution was extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic extracts were dried and filtered. Evaporation of the solvent and recrystallization from Et_2O afforded **9**; yield: 18.4 g (76%); mp 149–150°C.

^{1}H NMR ($CDCl_3$): δ = 2.03 (s, 3H, CH_3CON), 2.17 (s, 3H, 5-CH_3), 3.30 (s, 2H, 4-CH_2), 3.37 (s, 3H, CH_3O), 3.82 (s, 6H, CO_2CH_3), 5.10 (s, 2H, NCH_2O), 7.14 (s, 1H, NH).

^{13}C NMR ($CDCl_3$): δ = 11.5 (CH_3), 22.7 ($\underline{C}H_3CO$), 26.2 (4-CH_2), 53.5 ($CO_2\underline{C}H_3$), 57.0 (CH_3O), 65.2 (C), 75.0 (NCH_2O), 103.5 (C-4), 167.6 (triple triplet, C-3), 167.9 (quintet, $\underline{C}O_2CH_3$), 168.7 (triple quartet, C-5), 169.5 (double quartet, CON).

Methyl 2-Acetamido-3-(3-hydroxy-5-methylisoxazol-4-yl)-2-methoxycarbonylpropanoate (14):

$BF_3 \cdot Et_2O$ (0.5 mL, 4.0 mmol) was added to a mixture of **9** (1.03 g, 3.0 mmol), CH_2Cl_2 (15 mL) and Ac_2O (15 mL). The flask was left closed for 16 h. After removal of CH_2Cl_2 the mixture was poured into water (50 mL). Stirring for 1 h, extraction with CH_2Cl_2 (4 × 50 mL), drying, filtration, removal of the CH_2Cl_2, addition of 0.1 M NaOMe in MeOH (50 mL), heating to reflux for 3 h, addition of AcOH to pH ca. 7 and reduction of the volume to ca. 5 mL gave a residue which was poured into water (50 mL). Extraction with CH_2Cl_2 (6 × 50 mL), drying, filtration, removal of CH_2Cl_2 dissolution in boiling MeOH/EtOH (1:1, 150 mL), filtration through activated carbon which was then extracted with boiling MeOH (100 mL) and removal of the solvents afforded **14**; yield 760 mg (84%); mp 211 °C [Lit.[10] mp 220–221 °C (Corr)]. The ^{1}H NMR spectrum was identical with that reported previously.[10]

^{13}C NMR (CD_3CN with 10% (v/v) of saturated NaOD in D_2O): δ = 10.3 (5-CH_3), 21.3 ($\underline{C}H_3CON$), 28.2 (CH_2), 52.1 (CH_3O), 67.0 (C), 101.5 (C-4), 168.6 ($\underline{C}O_2CH_3$), 165.1, 170.5 and 176.6 (C-3, C-5 and $CH_3\underline{C}ON$).

2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic Acid (AMPA), 10):

Compound **9** (3.44 g, 10 mmol) was refluxed in 1 M aq CF_3CO_2H (80 mL, 80 mmol) for 16 h (oil bath, 120 °C). Evaporation to dryness, addition of water (50 mL) and evaporation to a viscous oil was repeated three times. After the last evaporation, water (10 mL) was added and the solution was passed through Amberlite IRA-400 (OH^--form, 80 mL). The column was washed with water (ca. 150 mL) until the eluate was neutral. Subsequent extraction with 1 M aq AcOH (600 mL) gave an acidic eluate which was evaporated to dryness. Addition of water (150 mL) and evaporation in vacuo to a volume of ca. 10 mL gave a slurry which was filtered, washed with cold water (2 × 5 mL) and dried in vacuo to give 990 mg of **10**; mp 233 °C (dec) [Lit.[4] mp 240–250 °C (dec)]. Further 135 mg was obtained by reducing the volume of the mother liquor to ca. 5 mL and cooling to 0 °C thus bringing the total yield to 1.13 g (60%). The ^{1}H NMR spectrum was identical with that reported previously.[1] ^{13}C NMR (1 M NaOD in D_2O): δ = 13.9 (5-CH_3), 29.3 (CH_2), 58.3 (CH), 106.4 (C-4), 169.6 (triple quartet, C-5), 180.2 (triplet, C-3), 183.0 (quartet with fine structure, CO_2H).

NMR Experiments

3-Hydroxy-5-methylisoxazole (**1**; 0.45 g, 4.5 mmol), trioxane (0.60 g, 6.7 mmol), 48% DBr in D_2O [from $SOBr_2$ (1.75 mL) and D_2O (4.10 mL)] were mixed and heated to 60 °C. NMR spectra of the reaction mixture showed immediate and complete conversion to the 2-hydroxymethyl compound **2**:

^{1}H NMR: δ = 6.81 (s, 1H, H-4), 5.53 (s, 2H, NCH_2O), 2.48 (s, 3H, CH_3).

^{13}C NMR (DEPT spectrum): δ = 97.8 (C-4), 70.2 (NCH_2O), 11.8 (CH_3).

The CH_2 signals were assigned by comparison with those of the 2-methoxymethyl compound **8**. After 0.5 h the reaction mixture contained **2** and **5** in the ratio 2.6:1

Compound **5**:

^{1}H NMR: δ = 5.39 (s, 2H, NCH_2O), 4.21 (s, 2H, CCH_2Br), 2.37 (s, 3H, CH_3).

^{13}C NMR (DEPT spectrum): δ = 69.1 (NCH_2O), 19.8 (CH_2Br), 13.6 (CH_3).

The CH_2Br signals were assigned by comparison with the di(bromomethyl) compound **6**. In addition, a product formed from dioxane (according to blank experiments) resonating at δ = 4.43 in the ^{1}H NMR spectra and at δ = 74.9 in the ^{13}C spectra was observed. Minor peaks were also present. The ratio between **2** and **5** decreased to be 1:1 after 2 h, and after 24 h only **5** could be observed. The mixture was then extracted with $CDCl_3$. The extract contained **6** and **7** in the ratio of 4.8:1.

Compound **6**:

^{1}H NMR ($CDCl_3$): δ = 5.63 (s, 2H, NCH_2Br), 4.14 (s, 2H,CCH_2Br), 2.36 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$): δ = 167.0 and 171.9 (C-3 and C-5), 107.6 (C-4), 40.3 (NCH_2Br), 17.8 ($C\underline{C}H_2Br$), 12.4 (CH_3).

Compound **7**:

^{1}H NMR ($CDCl_3$): δ = 5.75 (s, 2H, NCH_2N), 4.16 (s, 4H, 2 × CCH_2Br), 2.29 (s, 6H, 2 × CH_3).

^{13}C NMR ($CDCl_3$): δ = 170.6 and 167.2 (C-3 and C-5), 106.9 (C-4), 54.3 (NCH_2N), 18.3 ($C\underline{C}H_2Br$), 12.3 (CH_3).

When the experiment was performed using 62% HBr the $CHCl_3$ extract contained only the di(bromomethyl) compound **6** and an aliphatic byproduct:

^{1}H NMR: δ = 5.70 (s).

^{13}C NMR: δ = 69.0 (t).

By addition of MeOH, **6** was converted to the 2-methoxymethyl-4-bromomethyl compound **8**.

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‡ AMPA is a bioisoster of glutamic acid which acts as an excitatory neurotransmitter in the central nervous system.

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