Synthesis And Optical Resolution of the Neurotoxin 2-Amino-3-([^{15}N]-methylamino)propanoic acid (BMAA)

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SUMMARY

The synthesis of 2-amino-3-([^{15}N]-methylamino)propanoic acid (synonyms, BMAA, β-N-methylamino-alanine) from α-acetamidoacrylic acid and [^{15}N]-methylamine is described. Enantioselective hydrolysis of the acetamide group, mediated by the enzyme Acylase 1 (EC 3.5.1.14), yielded (R)-BMAA and the (S)-α-acetamido derivative. Acid hydrolysis of the latter compound yielded (S)-BMAA.

Key words: BMAA, neurotoxin, cycades

In a series of fascinating epidemiological studies of the abnormally high incidence of Amyotrophic Lateral Sclerosis (ALS), Parkinsonism, and Alzheimer-type dementia on Guam, Irian Jaya and the Kii peninsula of Japan, Kurland (1) and others (2) linked these diseases to the use of Cycads circinalis as a traditional food and tropical medicine in these areas. Vega and Bell described the isolation of (-)-2-amino-3-(methylamino)propanoic acid (BMAA), 1, from the seeds of Cycads circinalis (3) and demonstrated that it was toxic

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to chicks and rats (4). Recently Spencer et al. (5) reported that monkeys (Macaca mulatta) fed BMAA developed signs of motor-neuron and behavioral disfunction. A critical examination of the effect produced by BMAA requires the (S)-enantiomer (the D-enantiomer in the earlier nomenclature) and the ready availability of large quantities of (R)-BMAA for feeding experiments. Metabolic studies also demand optically pure material containing a label with high specific activity. Vega et al. (6) have described a synthesis of 1 (BMAA) from methylamine and α-acetamidoacrylic acid, 2, this synthesis would be inefficient and expensive for the preparation of a stable isotope analogue, because it requires the use of a large excess of methylamine to drive the reaction to completion. We have therefore developed a protocol for a synthesis that optimizes the conversion of labeled methylamine ([^{15}N], [^{13}C] or [^{2}H]) into BMAA, via a suitably labeled form of methylamine. It is thus now possible to prepare affordably a range of stable, isotopically labeled forms of BMAA.

In early studies, where a 1:1 ratio of methylamine to 2 was employed, the adduct (RS)-2-acetamido-3-(methylamino)-propanoic acid, 3, was obtained in very low yield. Since we were unable to obtain satisfactory yields of the desired material in the absence of an excess of methylamine, unreacted methylamine was recovered by vacuum distillation. The recovered amine was reacted with less than a stoichiometric quantity of 2 to form an additional quantity of 3. Recovered unreacted methylamine from this second cycle was reused for a third cycle. A fourth cycle did not yield isolable 3. The total yield of 3 was 31%, based on [^{15}N]-methylamine hydrochloride.

CONCLUSION

The above procedure in which excess methylamine is employed and then recovered yields ^{15}N labeled material in an acceptable yield; this procedure can also be employed for the synthesis of ^{13}C and D labeled derivatives.

EXPERIMENTAL

Synthesis

An aqueous solution of [^{15}N]-methylamine was prepared by treating a frozen solution [^{15}N]-methylamine hydrochloride (2 g) in water (8 ml) with solid NaOH (1.76 g), allowing the solution to warm slowly to room temperature and distilling the aqueous methylamine solution in vacuo. The distillate was treated with 2 (0.75 g) at 40°C for 22 hrs. Unreacted aqueous [^{15}N]-methylamine was then removed by vacuum distillation and the residue crystallized from ethanol to yield 0.64 g of 3. The recovered methylamine was reacted with a second portion of 2 (0.75 g) at 40°C as described above to yield 0.51 g of 3 after crystallization. Unreacted [^{15}N]-methylamine was again recovered from the second cycle by distillation and used for a third cycle yielding an additional 0.26 g. of 3. The total yield of 3 was 1.40 gms (31%, calculated on [^{15}N]- methylamine hydrochloride used).

Enantioselective Hydrolysis (Resolution)

An aqueous solution (80 ml) of (RS)-3 (1.38 g) was prepared and the pH adjusted to 7.5 with 2M LiOH. To this solution was added Acylase I (EC 3.5.1.14) (70 mg), from porcine kidney (Sigma Chemical Co.), and the mixture incubated for 30 hrs at 37°C. The pH of the solution was adjusted to 5.0 with acetic acid, charcoal was added and the solution refrigerated overnight. The solution was filtered and passed through the acid form of a Bio-Res 70/200 mesh column. Unhydrolyzed 3 was eluted with water, while the (R)-amino acid was retained on the column (6). The filtrate was concentrated to a volume of 80 ml; and the pH adjusted to 7.5 with 2M LiOH. After treatment with Acylase I (70 mg) for 30 hrs. at 37°C., the solution was again passed through the ion exchange column. The filtrate was concentrated to yield 3 (446 mg). The (R)-amino acid, (+)-1, was eluted by washing the column with 0.3M HCl. The eluant was concentrated and the residue crystallized from aqueous ethanol to afford 429 mg (64%) of the hydrochloride m.p.

181-182°C., $[\alpha]_D$ +30.0 (c 2.45, H_2O). CIMS: m/z 120 (M+H); m/z 104 ($M-CH_3$); ^1H NMR (D_2O) 2.82 (3H, s, $N-CH_3$); 3.47 (2H, m,$-CH_2-$); 4.09 (1H, m, -CH-); e.e.>99%.

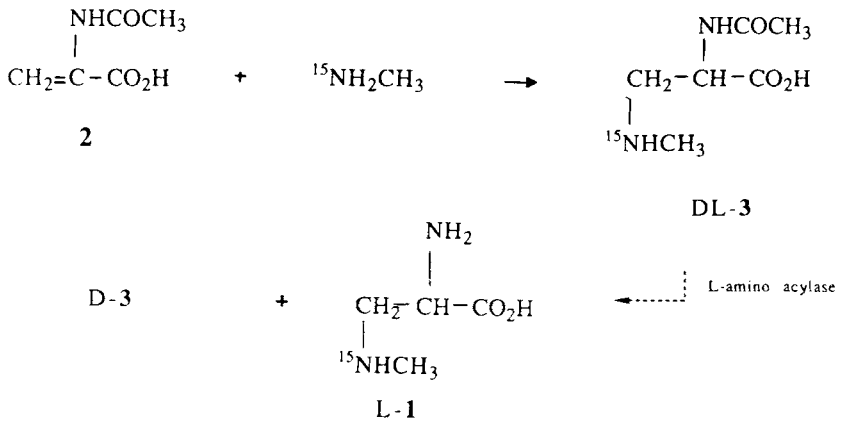
An [^{15}N]-labeled sample of (S)-BMAA was obtained by refluxing 0.446 mg of 3 (recovered from the above Acylase catalyzed hydrolysis) in 2M HCl for 3 hrs. The solution was concentrated in vacuo to yield 0.257 mg of (S)-BMAA hydrochloride (60%), $[\alpha]_D$= -24.3 (c 1.25, H_2O); e.e. 99%. Analysis of [^{15}N] incorporation:

Gas chromatography/mass spectrometry of the di-N-pentafluoropropionyl iso-butyl ester of [^{15}N]-BMAA under electron impact conditions gave a total ion chromatogram consisting of a single peak with a retention time identical to that obtained for the same derivative of unlabeled (authentic) BMAA (7). The fragmentation pattern for a mass spectral scan from this peak was consistent with the incorporation of a single [^{15}N]-atom. The base peak was m/z 191 (i.e. $CF_3CF_2CO[^{15}N](CH_3)CH_2$ cf.m/z 190 for the unlabeled material) and all other assignments are consistent with the structure as previously described (7).

Determination of optical purity (enantiomeric excess):

A sample of BMAA was methylated by refluxing it in methanol (2 ml) containing thionyl chloride (0.2 ml). The solvent was removed in vacuo, the residue dissolved in methylene chloride (2 ml) and treated with a solution (3 ml) trifluoroacetyl-L-prolyl chloride in methylene chloride (0.1 mmole/ml) and 0.2 ml triethylamine. The solution was refluxed (2 hrs.) and then concentrated in vacuo. The mixture of diastereomeric amides were separated @ 250°C by GC (Hewlett Packard 5890) on a 25 m capillary column (Ultra 1). The area under each band was determined using a HP 3392A integrator.

Synthetic Scheme for the preparation of ^{15}N-BMAA and its Enantiomers



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