

On the synthesis of *N*-maleoyl amino acids in aqueous media: cautionary tales for the unwary traveller

Michael J. Paterson* and Aleksandar Jovanovic

Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD1 9SY

E-mail: Michael_J_Paterson@yahoo.co.uk

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Abstract

The high cost or protracted syntheses associated with *N*-maleoyl amino acid derivatives coupled with a considerable demand from both the life and physical sciences has spurred the search for facile, low-cost routes to these compounds. Herein, we demonstrate that a recently published method purporting to deliver *N*-maleoyl amino acids by cyclization of maleamic acids in water, instead results in a hydrolysis product, namely the corresponding hemi-maleate salt.

Keywords: Maleimides, hemi-maleate, heterobifunctional crosslinkers, cyclization

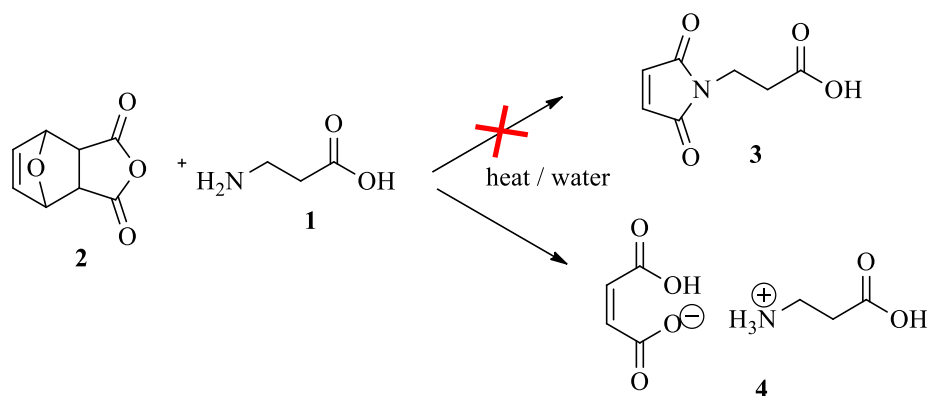
Introduction

There is a considerable demand for low-cost, high purity *N*-maleoyl amino acids (NMAAs) for use in such diverse pursuits as the preparation of polymers,¹ the elaboration of metal surfaces² and the synthesis of bioconjugates.³ The widespread use of NMAA active esters as heterobifunctional crosslinkers has resulted in the development of a number of methods⁴ for their rapid preparation; however, for applications requiring the free acids the synthetic options are less appealing.

The traditional methods used to prepare NMAAs are limited in their utility by either a lack of scalability,⁵ that they are cumbersome from a practical perspective,⁶ or that they require a protracted synthesis.⁷ It is, therefore, of particular interest when a new methodology appears in the literature offering a facile synthesis⁸ of these highly desirable compounds. Two such procedures have now been published that employ water as the reaction solvent.

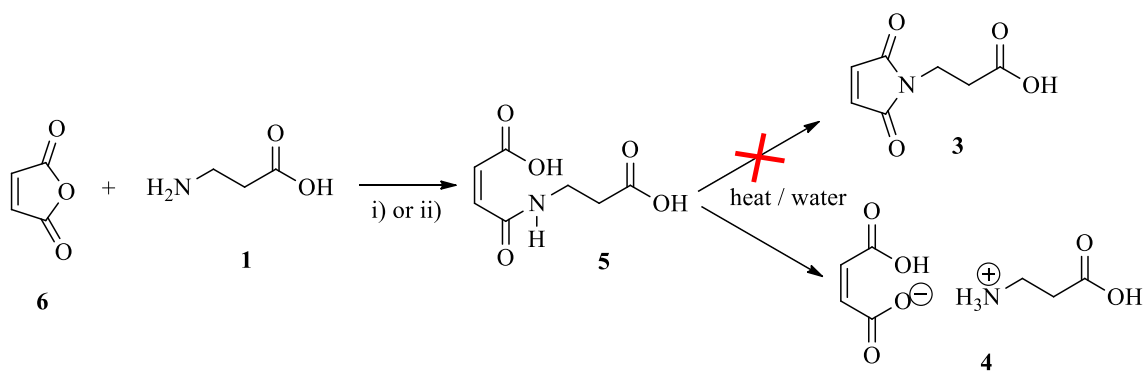
The first method to describe the preparation of NMAAs in aqueous media was that of Ondruš and coworkers⁹ (Scheme 1). This involves the reaction of the desired ω -amino acid, for example β -alanine **1**, with the commercially available *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride **2**, to furnish the corresponding NMAA, such as 3-maleimido propionic acid **3**. A detailed

analysis by Corrie and Munasinghe, however, revealed that the actual product was, in fact, a hemi-maleate **4** salt of the relevant amino acid.¹⁰



Scheme 1. Attempted preparation of maleimides using the Ondruš methodology.

In a recent paper, Song and coworkers¹¹ describe the preparation of NMAAs by heat-induced cyclization of maleamic acids. This method is akin to that of Rich and coworkers,⁶ but in stark contrast to carrying out the reaction in toluene with azeotropic removal of water, as Rich did, Song and coworkers employed water as a solvent. This method is extremely attractive as it avoids all the problems associated with the traditional methods of preparing these compounds and even removes the need for harsh organic solvents, as used in a contemporary methodology.⁸



Scheme 2. Preparation and attempted cyclization using the Song methodology i) Method A acetic acid 8 h ii) Method B water 4 h.

Unfortunately, analysis the products from the attempted aqueous cyclization of maleamic acids reveals that they are hydrolyzed.

Results and Discussion

The cyclization of *N*-(3-carboxy-acryloyl)- β -alanine **5**, which was prepared, as described by Song and coworkers (Method A), by reacting maleic anhydride **6** with β -alanine **1** (Scheme 2) in acetic acid, resulted in a product with an NMR spectrum identical to that described by the authors; however, this material has different physical and chemical properties to that of an authentic sample of 3-maleimido propionic acid **3** (Bachem GmbH). In order to establish if contamination by residual acid was impeding the cyclization in our hands, a quantity of the maleamic acid **5** was prepared in water, using a known methodology (Method B),¹² and then subjected to the same cyclization conditions. When the proton and carbon-13 NMR spectra of the products from both attempted cyclizations were compared (Figures 1 and 2) with the spectrum of an authentic sample of 3-maleimido propionic acid **3** as well as one of β -alanine hemi-maleate **4** it was clear that the products of the attempted cyclizations were, as with the Ondruš methodology, unfortunately, the corresponding hemi-maleate **4**.

The NMR shifts and coupling constants (all taken in DMSO- d_6) for the hemi-maleate and the putatively cyclized materials were essentially identical and contrasted sharply with that of the authentic maleimide **3**. As noted by Corrie,¹⁰ the olefinic protons can be particularly diagnostic when comparing maleates to maleimides; in this instance, having a shift of 6.06 ppm in the hemi-maleate **4** and one of 7.02 ppm in the authentic maleimide **3**. The two methylene triplets have shifts of 3.01 and 2.58 ppm with a coupling constant of 6.8 Hz in the hemi-maleate and 3.63 and 2.51 ppm with a constant of 7.3 Hz in the maleimide species (Figure 1).

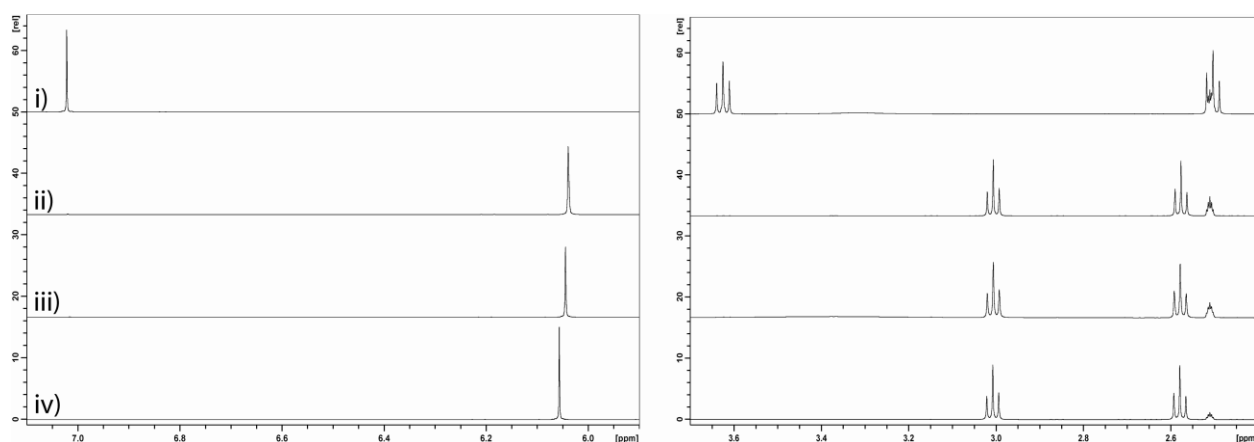


Figure 1. Selected regions of the proton spectra of i) Authentic 3-maleimido propionic acid **3** ii) Attempted cyclization of *N*-(3-carboxy-acryloyl)- β -alanine prepared using Method A iii) Attempted cyclization of *N*-(3-carboxy-acryloyl)- β -alanine prepared using Method B iv) β -alanine hemi-maleate **4**.

The carbon-13 spectra (Figure 2) also contain marked differences. In particular, one of the carbonyl peaks which resonates at 167.29 ppm in the hemi-maleate **4** is at 170.69 ppm in the

maleimide **3**; the olefinic carbons are at 135.94 ppm in the hemi-maleate **4** and upfield at 134.56 ppm in the maleimide **3** and the methylene carbons are at 34.82 and 31.45 ppm, in the hemi-maleate **4**, and at 33.29 and 32.39 ppm in the maleimide **3**. In addition to the NMR data, the melting points of the products from the attempted cyclizations were in good accord with that of the hemi-maleate **4** (121-124 °C for Method A, 122-123.5 °C for Method B and 123-124 °C for the hemi-maleate). The melting point of the authentic maleimide **3** is significantly lower at 110-112 °C (lit. 108-109 °C).⁸ In conclusion, contrary to the published method, maleamic acids are hydrolyzed on refluxing in water.

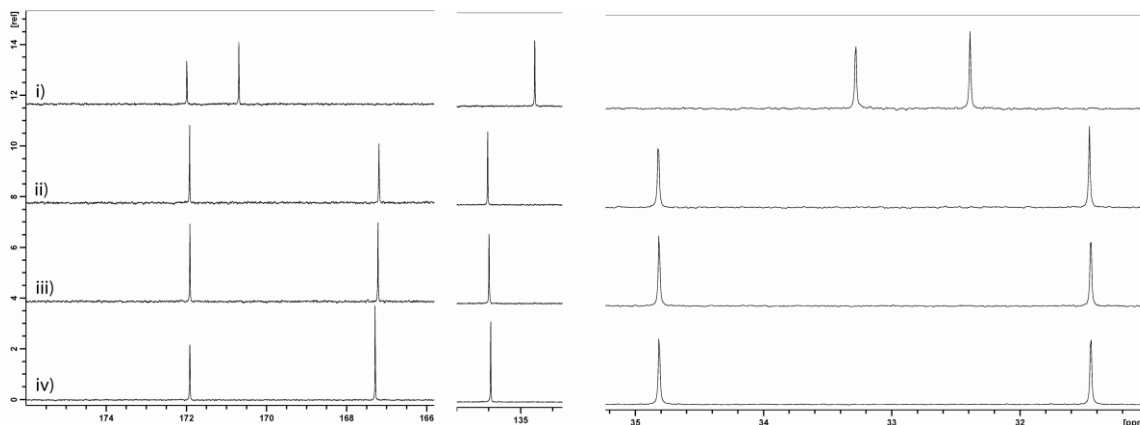


Figure 2. Selected regions of the carbon-13 Spectra of i) Authentic 3-maleimido propionic acid **3** ii) Attempted cyclization of *N*-(3-carboxy-acryloyl)- β -alanine prepared using Method A iii) Attempted cyclization of *N*-(3-carboxy-acryloyl)- β -alanine prepared using Method B iv) β -alanine hemi-maleate **4**.

Experimental Section

General. NMR spectra were recorded on a Bruker Avance II 500 MHz instrument using DMSO- d_6 as a solvent. Proton and carbon-13 spectra were recorded at 500 MHz and 126 MHz, respectively. Chemical shifts are reported in ppm and are calibrated against the residual solvent signal. Infrared spectra were recorded in the research laboratories of Dr. B. D. Alexander, University of Greenwich at Medway, UK, on a Perkin-Elmer Spectrum RX1 FT-IR instrument, and are reported in reciprocal centimetres. Melting points were determined on a Reichert hot-plate apparatus and are uncorrected. Elemental analysis was carried out by Medac Ltd, Egham, Surrey, UK.

Analysis of 3-maleimido propionic acid (**3**)

A sample of commercially available 3-maleimido propionic **3** (Bachem GmbH) was obtained and used as an authentic standard, m.p. 110-112 °C (lit.⁸ 108-109 °C); ^1H NMR (DMSO- d_6) δ

2.54-2.47 (m, 3H), 3.63 (t, *J* 7.3 Hz, 2H), 7.02 (s, 2H); ^{13}C NMR (DMSO- d_6) δ 32.39, 33.29, 39.02, 39.19, 39.36, 39.52, 39.69, 39.86, 40.02, 134.56, 170.69, 171.99; IR (KBr) 3455, 3101, 2951, 1705, 1454, 1412, 1399, 1370, 1314, 1250, 1230, 1154, 1081, 1054, 947, 834, 696.

β -Alanine hemi-maleate (4). A solution of maleic acid (1 g, 8.6 mmol) and β -alanine **1** (0.767 g, 8.6 mmol) in water (10 mL) was stirred for 15 minutes at room temperature and then concentrated under reduced pressure. This material was then characterized without further manipulation, m.p. 123-124 °C; ^1H NMR (DMSO- d_6) δ 2.58 (t, *J* 6.8 Hz, 1H), 3.01 (t, *J* 6.8 Hz, 1H), 6.06 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 31.45, 34.82, 38.96, 39.13, 39.29, 39.46, 39.63, 39.79, 39.96, 135.94, 167.29, 171.92; IR (KBr) 3330-2820, 1695, 1625, 1506, 1398, 1344, 1232, 866, 789, 658; Anal. Calcd. for $\text{C}_7\text{H}_{11}\text{NO}_6$: C, 40.98; H, 5.40; N, 6.82. Found: C, 40.92; H, 5.24; N, 6.71.

Preparation of *N*-(3-carboxy-acryloyl)- β -alanine (5)

Method A. To a vigorously stirred solution of β -alanine **1** (4.45 g, 50 mmol) in acetic acid (50 mL) was added maleic anhydride **6** (4.9 g, 50 mmol). Stirring was continued for 8 hours at room temperature, whereafter the mixture was filtered and the precipitate washed, successively, with cold water (3 x 20 mL) and diethyl ether (20 mL) to give (8.6 g, 92%) of maleamic acid **5**, m.p. 151-155 °C (lit.⁶ 159.5-160 °C); ^1H NMR (DMSO- d_6) δ 2.48 (t, *J* 6.8 Hz, 2H), 3.37 (dt, *J* 6.7, 5.7 Hz, 2H), 6.24 (d, *J* 12.5 Hz, 1H), 6.40 (d, *J* 12.5 Hz, 1H), 9.08 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 32.99, 35.28, 39.02, 39.19, 39.35, 39.52, 39.69, 39.78, 39.86, 40.02, 131.42, 132.64, 165.36, 165.52, 172.54; IR (KBr) 3329, 3052, 1715, 1691, 1628, 1554, 1436, 1400, 1370, 1282, 1227, 1205, 1051, 991, 948, 895, 884, 867, 830, 718, 696, 603.

Method B. To a vigorously stirred solution of β -alanine **1** (4.45 g, 50 mmol) in water (10 mL) was added maleic anhydride **6** (4.9 g, 50 mmol). Stirring was continued for 4 hours at room temperature, whereafter the mixture was filtered and the precipitate washed, successively, with cold water (3 x 20 mL) and diethyl ether (20 mL) to give (8.2 g, 88%) of maleamic acid **5**, m.p. 152-154 °C (lit.⁶ 159.5-160 °C); ^1H NMR (DMSO- d_6) δ 2.48 (t, *J* 6.7 Hz, 2H), 3.36 (dt, *J* 6.7, 5.7 Hz, 2H), 6.24 (d, *J* 12.5 Hz, 1H), 6.40 (d, *J* 12.5 Hz, 1H), 9.09 (s, 1H); ^{13}C NMR (DMSO- d_6) δ 32.99, 35.28, 39.02, 39.18, 39.35, 39.52, 39.68, 39.78, 39.85, 40.02, 131.41, 132.65, 165.36, 165.52, 172.54; IR (KBr) 3332, 3052, 1712, 1691, 1628, 1551, 1435, 1398, 1370, 1281, 1225, 1205, 1051, 991, 948, 895, 884, 864, 830, 718, 696, 603.

Attempted aqueous cyclization of *N*-(3-carboxy-acryloyl)- β -alanine. A solution of *N*-(3-carboxy-acryloyl)- β -alanine **5** (1.7 g, 9 mmol) was refluxed in water (20 mL) for 30 minutes, the mixture cooled (35 °C) and then concentrated under reduced pressure to give a white solid. This material was then characterized without further manipulation, m.p. 121-124 °C (Method A), 122-123.5 °C (Method B); ^1H NMR (DMSO- d_6) (Method A) δ 2.58 (t, *J* 6.8 Hz, 2H), 3.01 (t, *J* 6.8 Hz, 2H), 6.04 (s, 2H); ^1H NMR (DMSO- d_6) (Method B) δ 2.58 (t, *J* 6.8 Hz, 2H), 3.01 (t, *J* 6.8 Hz, 2H), 6.04 (s, 2H); ^{13}C NMR (DMSO- d_6) (Method A) δ 31.45, 34.82, 38.99, 39.16, 39.33, 39.49, 39.66, 39.75, 39.83, 39.99, 135.99, 167.22, 171.92; ^{13}C NMR (DMSO- d_6) (Method B) δ 31.46, 34.82, 39.01, 39.18, 39.34, 39.51, 39.68, 39.77, 39.84, 40.01, 136.03, 167.20, 171.92; IR

(KBr) (Method A) 3346-2808, 1696, 1625, 1494, 1397, 1351, 1238, 872, 786, 655; IR (KBr) (Method B) 3314-2828, 1693, 1625, 1497, 1398, 1348, 1232, 869, 789, 655.

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