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Straightforward Synthesis of (*R*,*S*)-β-Methyleneaspartic Acid, an Inhibitor of Glutamate-Aspartate Transaminase

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Summary. A *Baylis-Hillman* adduct of methyl acrylate and ethyl glyoxalate was converted into the trichloroacetimidate that in the presence of *DABCO* rearranged to the corresponding trichloroacetamide. Eventually, hydrolysis under acidic conditions, led to the hydrochloride of racemic β-methyleneaspartic acid.

Keywords. Amino acid; Enzymes; Rearrangement; *Baylis-Hillman*; Dehydroamino acids.

Introduction

The synthesis of conformationally constrained analogues of natural amino acids is of large interest, since their insertion into peptides can cause significant changes in their conformation, which in turn may affect the ability of the peptide to fit to a receptor and they are a valuable tool for probing activity changes in a peptide [1, 2]. In addition, these compounds can act as enzymatic inhibitors, and interesting informations about the structure at the active site can be obtained by considering the conformational restrictions of the guest molecule.

Among these compounds, (*R*,*S*)-β-methyleneaspartic acid (**1**) [3] was recognized as selective inhibitor of glutamate-aspartate transaminase (Scheme 1), having higher activity than vinylglycine that irreversibly inactivates this enzyme [4].

In fact, this transaminase gained increasing interest due to its high potential for industrial production of amino acids [5], although the use is hampered by the low equilibrium constant of the catalyzed reaction [6]. Thus, a better knowledge of the structure at the active site can lead to changes in the protein sequence directed at increasing the reaction rate.

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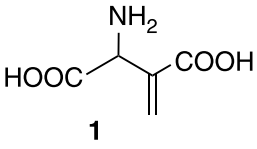

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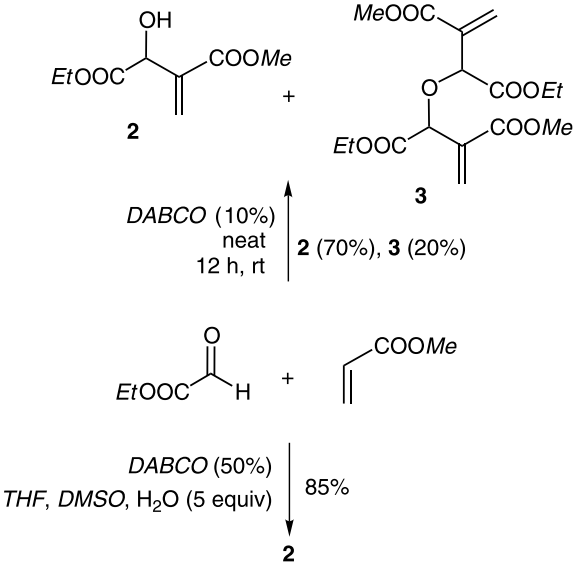
Scheme 1

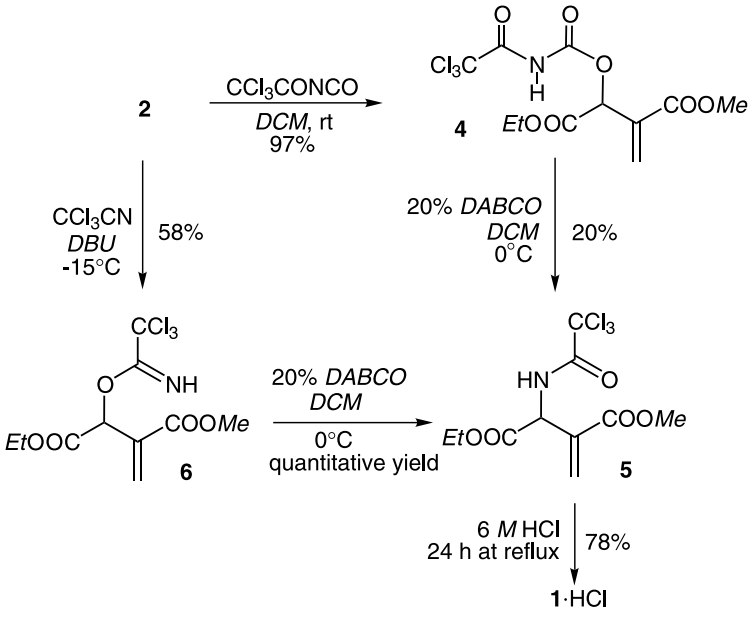
Results and Discussions

Within a programme dealing with the preparation of non-proteinogenic amino acids with conformational restrictions starting from *Baylis-Hillman* adducts [7], we directed our attention towards the adduct **2** and devised to introduce a nitrogen-containing functionality in place of the hydroxy group. Although **2** can be obtained starting from methyl acrylate and ethyl glyoxalate [8], a side reaction was invariably observed leading to substantial amounts of diastereomeric compounds **3**. Thus, in order to avoid the formation of these products, the *Baylis-Hillman* reaction was carried out in the presence of water (5 equiv) and we were pleased to obtain **2** in higher yield (Scheme 2) [9].

Then, according to a procedure earlier developed in our laboratory [7], we first treated **2** with trichloroacetyl isocyanate to give the corresponding trichloroacetyl carbamate **4** in quantitative yield (Scheme 3). However, the subsequent reaction with *DABCO* in *DCM* failed to give the expected trichloroacetylamino derivative **5**, that was obtained in very low yield, whereas trichloroacetamide was formed as the major product. Thus, as an alternative route, we considered that the amide **5** could be obtained starting from the trichloroacetimidate **6**. However, the procedures reported in literature did not allow to obtain **6** in satisfactory yield [10]. Accordingly, a modified approach to give **6** was devised using trichloroacetonitrile as both solvent and reactant [11]. The subsequent treatment of **6** with a small amount of



Scheme 2

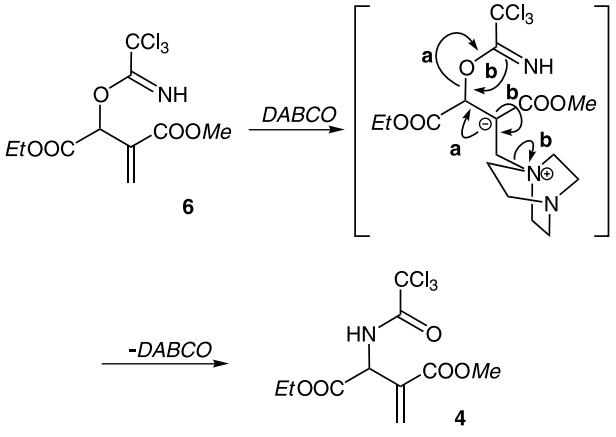


Scheme 3

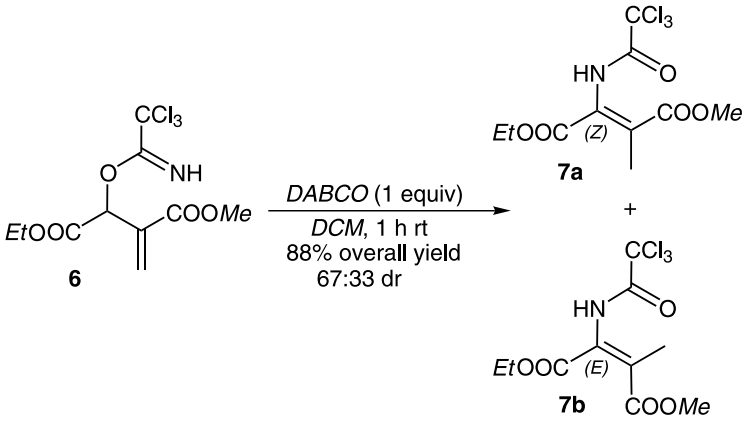
DABCO in *DCM* allowed to obtain in nearly quantitative yield the trichloroacetylamino derivative **5** that was eventually converted into the hydrochloride of (*R*,*S*)-β-methyleneaspartic acid (**1**) by reaction in refluxing 6 *M* HCl (Scheme 3) [3c].

The rearrangement leading to the trichloroacetamide **5** starting from the trichloroacetimidate **6** probably involves attack of *DABCO* to the conjugate double bond followed by elimination of the anion of trichloroacetamide *via* an S_N' reaction (Scheme 4, path a). In turn, this anion attacks at C-2 by a further S_N' reaction to give the trichloroacetamide **5**, *DABCO* being the leaving group (Scheme 4, path b).

Eventually, it is worth noting that when **6** was treated with an equimolar amount of *DABCO* for 1 h, a diastereomeric mixture of 1-ethyl 4-methyl (*E*)-3-methyl-2-trichloroacetylamino-2-butendioate (**7a**) and its (*Z*)-isomer (**7b**) was



Scheme 4



Scheme 5

obtained in 88% overall yield and 67:33 ratio. Their configurations were assigned on the basis of ^{1}H NMR data and confirmed by NOE experiments. In fact the signal for (3-CH_3) in the (*E*)-diastereomer is more deshielded (2.01 ppm) than the corresponding signal in the (*Z*)-diastereomer (1.87 ppm). Moreover, the methyl group at 2.01 ppm in the (*Z*)-diastereomer afforded a positive NOE (5.1%) over the adjacent methylene of the ethoxycarbonyl group, suggesting a *syn* relationship, whereas this effect was missing in the (*E*)-diastereomer for the methyl group at 1.87 ppm (Scheme 5).

Considerable interest in recent years has been directed toward the synthesis of α,β-dehydroamino acids [12], which are constituents of a variety of biologically important peptides. They are also valuable intermediates for the preparation of both natural and unnatural amino acids. Thus, compounds **7a** and **7b** can provide useful precursors for both the enantiomerically pure forms of 3-methyl aspartic acid by hydrogenation of the double bond [13], carried out in the presence of an appropriate chiral catalyst, and work directed towards this goal is currently underway in our laboratory.

Experimental

IR spectra were recorded in $CHCl_3$ on a Nicolet FT-IR 20-XS spectrophotometer. Unless otherwise noted, NMR spectra (200 MHz for ^{1}H, 50 MHz for ^{13}C, chemical shifts as ppm in the δ scale, coupling constants *J* in Hz) were recorded at 25°C on a Varian Gemini 200 spectrometer. Diastereomeric ratios were determined by glc analysis by using a Chrompack 9001 gas-chromatograph equipped with a capillary column Chrompack 7720 (50 m × 0.25 mm id; stationary phase CP-Sil-5 CB). EIMS and CI-MS analyses were carried out on a Hewlett-Packard spectrometer model 5890, series II. Elemental analyses (C, H, N) were conducted using a Carlo-Erba 1106 elemental analyzer, and their results were found to be in good agreement (±0.2%) with the calculated values. Column chromatography was performed using Kieselgel 60 Merck (230–400 mesh ASTM).

1-Ethyl 4-methyl 2-hydroxy-3-methylenebutanedioate (**2**, $C_8H_{12}O_5$) *and 1-Ethyl 4-methyl 2-(1-ethoxycarbonyl-2-methoxycarbonylallyloxy)-3-methylenebutanedioates* (**3**, $C_{16}H_{22}O_9$)
Method A. To a mixture containing 20.4 g ethyl glyoxalate (50% solution in toluene, 100 mmol) and 8.6 g methyl acrylate (100 mmol), 1.12 g *DABCO* (10 mmol) were added. After stirring for 12 h at rt, the oil was purified by silica gel chromatography (cyclohexane:ethyl acetate = 80:20) to give first

13.2 g (70%) **2** [8] and then a diastereomeric mixture 70:30 of 1-ethyl 4-methyl 2-(1-ethoxycarbonyl-2-methoxycarbonylallyloxy)-3-methylenebutanedioates (**3**), as colorless oils.

2: IR ($CHCl_3$): $\bar{\nu} = 3490, 1732, 1636\,cm^{-1}$; ^1H NMR (200 MHz, $CDCl_3$): $\delta = 1.24$ (t, $J = 7.2$ Hz, 3H, $OCH_2C\underline{H}_3$), 3.51 (d, $J = 6.2$ Hz, OH), 3.76 (s, 3H, OCH_3), 4.22 (q, $J = 7.2$ Hz, 2H, $OC\underline{H}_2CH_3$), 4.83 (d, $J = 6.2$ Hz, 1H, 2-CH), 5.92 (s, 1H, vinyl), 6.34 (s, 1H, vinyl) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 14.0, 52.0, 62.2, 71.2, 128.8, 138.1, 165.6, 172.2$ ppm; EIMS (70 eV): m/z (%) = 188 (M^+, 2), 143 (22), 116 (40), 84 (100).

3: IR ($CHCl_3$): $\bar{\nu} = 1743, 1720\,cm^{-1}$; ^1H NMR (200 MHz, $CDCl_3$): $\delta = 1.22$ (t, J = 7.2 Hz, 6H, $OCH_2C\underline{H}_3$, 70%), 1.26 (t, J = 7.2 Hz, 6H, $OCH_2C\underline{H}_3$, 30%), 3.75 (s, 6H, OCH_3, 30%), 3.78 (s, 6H, OCH_3, 70%), 4.18 (q, J = 7.2 Hz, 4H, $OC\underline{H}_2CH_3$, 70%), 4.20 (q, J = 7.2 Hz, 4H, $OC\underline{H}_2CH_3$, 30%), 4.97 (br s, 2H, CH–O), 6.02 (s, 2H, vinyl, 30%), 6.12 (s, 2H, vinyl, 70%), 6.42 (s, 2H, vinyl, 30%), 6.45 (s, 2H, vinyl, 70%) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 13.6$ (70%), 13.7 (30%), 51.6, 61.1 (30%), 61.5 (70%), 70.6, 76.0 (30%), 76.5 (70%), 128.2, 135.5 (30%), 135.6 (60%), 165.0, 165.3, 168.5 (70%), 168.6 (30%), 171.8 ppm; EIMS (70 eV): m/z (%) = 358 (M^+, 8), 343 (25), 185 (16), 136 (32), 84 (100).

Method B. To a mixture containing 21.4 g ethyl glyoxalate (50% solution in toluene, 105 mmol) and 9.1 cm^3 methyl acrylate (100 mmol) in 35 cm^3 *THF* and 15 cm^3 *DMSO* containing 9.0 cm^3 H_2O (500 mmol), 5.6 g *DABCO* (50 mmol) were added. After stirring for 12 h at rt, the oil was purified by silica gel chromatography (cyclohexane:ethyl acetate = 80:20) to give 16.0 g (85%) **2** [8] as a colorless oil. EIMS (70 eV): m/z (%) = 188 (M^+, 2), 143 (22), 116 (40), 84 (100).

1-Ethyl 4-methyl 2-trichloroacetylaminocarbonyloxy-3-methylenebutanedioate
(**4**, $C_{11}H_{12}Cl_3NO_7$)

To a solution containing 1.9 g **2** (10 mmol) in 50 cm^3 dry *DCM*, 1.9 g trichloroacetylisocyanate (15 mmol) dissolved in 10 cm^3 dry *DCM* were added at 0°C and the mixture was stirred at rt for 2 h [5]. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (cyclohexane:ethyl acetate = 80:20) to give 3.7 g (97%) **4** as a viscous oil. IR (neat): $\bar{\nu} = 3355, 1741, 1724\,cm^{-1}$; ^1H NMR (200 MHz, $CDCl_3$): $\delta = 1.24$ (t, $J = 7.2$ Hz, 3H, $OCH_2C\underline{H}_3$), 3.79 (s, 3H, OCH_3), 4.22 (q, $J = 7.2$ Hz, 2H, O–$C\underline{H}_2CH_3$), 5.98 (s, 1H, 2-CH), 6.11 (s, 1H, vinyl), 6.55 (s, 1H, vinyl), 8.78 (s, 1H, NH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 13.9, 52.4, 62.3, 72.4, 91.4, 132.3, 133.7, 148.5, 157.5, 164.5, 166.7$ ppm; EIMS (70 eV): m/z (%) = 377–375 (M^+, 2), 143 (22), 116 (40), 84 (100).

1-Ethyl 4-methyl 2-trichloroacetylamino-3-methylenebutanedioate (**5**, $C_{10}H_{12}Cl_3NO_5$)

To a solution containing 1.9 g **4** (5.0 mmol) in 20 cm^3 *DCM* at 0°C, 65 mg *DABCO* (0.5 mmol) were added and the mixture was stirred for 15 min at 0°C. After dilution with 150 cm^3 ethyl acetate, the organic layer was washed first with 30 cm^3 1 *M* HCl and then with 100 cm^3 brine. After drying (Na_2SO_4), the solvents were removed under reduced pressure and the residue was purified by silica gel chromatography (cyclohexane:ethyl acetate = 80:20), to give 0.35 g (20%) **5** as a colorless oil, followed by 0.6 g trichloroacetamide. IR (neat): $\bar{\nu} = 3351, 1722, 1668\,cm^{-1}$; ^1H NMR (200 MHz, $CDCl_3$): $\delta = 1.26$ (t, $J = 7.1$ Hz, 3H, $OCH_2C\underline{H}_3$), 3.80 (s, 3H, OCH_3), 4.25 (q, $J = 7.1$ Hz, 2H, $OC\underline{H}_2CH_3$), 5.25 (d, $J = 7.9$ Hz, 1H, 2-CH), 6.10 (s, 1H, vinyl), 6.47 (s, 1H, vinyl), 7.81 (d, $J = 7.9$ Hz, Hz, 1H, NH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 13.9, 52.3, 56.0, 62.5, 92.0, 131.7, 134.1, 161.2, 165.3, 168.2$ ppm; EIMS (70 eV): m/z (%) = 334–332 (MH^+, 4), 318–316 (12), 260 (22), 198 (18), 158 (44), 99 (100).

1-Ethyl 4-methyl 2-trichloroacetiminoxy-3-methylenebutanedioate (**6**, $C_{10}H_{12}Cl_3NO_5$)

To a solution of 9.5 g **2** (50 mmol) in 25 cm^3 CCl_3CN (250 mmol) and 25 cm^3 dry *THF*, 375 mm^3 *DBU* (3.5 mmol) dissolved in 0.5 cm^3 dry *THF* were added in 1 min at −15°C under vigorous stirring. After 1 h the cooling bath was removed and temperature raised to rt. The mixture was directly purified by silica gel chromatography (cyclohexane:ethyl acetate = 95:5) to give 12.1 g (73%) **6** as a colorless oil.

IR (neat): $\bar{\nu} = 3339$, 1720, 1671 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): $\delta = 1.27$ (t, $J = 7.3$ Hz, 3H, $OCH_2C\underline{H}_3$), 3.82 (s, 3H, OCH_3), 4.25 (q, $J = 7.3$ Hz, 2H, $OC\underline{H}_2CH_3$), 6.12 (s, 1H, vinyl), 6.13 (s, 1H, 2-CH), 6.55 (s, 1H, vinyl), 8.53 (br s, =NH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 13.7$, 53.0, 61.5, 73.4, 90.3, 129.7, 134.1, 160.8, 164.5, 166.8 ppm; EIMS (70 eV): m/z (%) = 334–332 (MH^+, 5), 318–316 (6), 170 (32), 161 (24), 144 (100).

1-Ethyl 4-methyl 2-trichloroacetylamino-3-methylenebutanedioate (**5**, $C_{10}H_{12}Cl_3NO_5$)
Following method B, to a solution containing 1.7 g **6** (5.0 mmol) in 20 cm^3 *DCM* at 0°C, 61 mg *DABCO* (0.5 mmol) were added and the mixture was stirred for 2 min at 0°C. Then the mixture was diluted with 150 cm^3 ethyl acetate and the organic layer washed with 30 cm^3 3 *M* HCl and 100 cm^3 brine. After drying (Na_2SO_4) the solvents were removed under reduced pressure and the residue was purified by silica gel chromatography (cyclohexane:ethyl acetate = 80:20), to give 1.7 g (quantitative yield) **5** as a colorless oil. EIMS (70 eV): m/z (%) = 334–332 (MH^+, 4), 318–316 (12), 260 (22), 198 (18), 158 (44), 99 (100).

(R,S)-Methyleneaspartic acid hydrochloride (**1** · HCl)
To 1.0 g **5** (3.0 mmol) 20 cm^3 6 *M* HCl were added and the mixture was refluxed for 24 h. The reaction mixture was concentrated at reduced pressure leaving 0.44 g (78%) **1** · HCl [3c] as a white foam. ^1H NMR (200 MHz, D_2O, *DSS*): $\delta = 4.89$ (s, 1H, 2-CH), 6.28 (s, 1H, vinyl), 6.67 (s, 1H, vinyl) ppm; ^{13}C NMR (50 MHz, D_2O): $\delta = 54.1$, 131.9, 135.7, 167.0, 169.7 ppm; MS (CI): m/z (%) = 146 (M^+, 2), 116 (40), 84 (100).

Ethyl 4-methyl (E)-3-methyl-2-trichloroacetylamino-2-butendioate (**7a**, $C_{10}H_{12}Cl_3NO_5$)
and its (Z)-diastereomer (**7b**, $C_{10}H_{12}Cl_3NO_5$)
To a solution containing 1.7 g **6** (5.0 mmol) in 20 cm^3 *DCM* at 0°C, 0.61 g *DABCO* (5.0 mmol) were added and the mixture was stirred for 1 h at rt. Then the mixture was diluted with 150 cm^3 ethyl acetate and the organic layer washed with 30 cm^3 3 *M* HCl and 100 cm^3 brine. After drying (Na_2SO_4) the solvents were removed under reduced pressure and the residue was purified by silica gel chromatography (cyclohexane:ethyl acetate = 80:20) to give 1.5 g (88% overall yield, dr = 67:33) of **7a** and **7b** as colorless oils.

(E)-Diastereomer ***7a***: IR ($CHCl_3$): $\bar{\nu} = 3345$, 1719, 1660 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): $\delta = 1.31$ (t, $J = 7.3$ Hz, 3H, $OCH_2C\underline{H}_3$), 1.87 (s, 3H, 3-CH_3), 3.78 (s, 3H, OCH_3), 4.33 (q, $J = 7.3$ Hz, Hz, 2H, $OC\underline{H}_2CH_3$), 12.09 (br s, 1H, NH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 13.3$, 13.7, 52.6, 62.3, 91.4, 110.6, 138.1, 159.0, 162.5, 169.4 ppm; EIMS: m/z (%) = 334–332 (MH^+, 6), 274–272 (12), 186 (21), 113 (65), 85 (100).

(Z)-Diastereomer ***7b***: IR ($CHCl_3$): $\bar{\nu} = 3341$, 1722, 1665 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): $\delta = 1.29$ (t, $J = 7.3$ Hz, 3H, $OCH_2C\underline{H}_3$), 2.01 (s, 3H, 3-CH_3), 3.79 (s, 3H, OCH_3), 4.25 (q, $J = 7.3$ Hz, 2H, $OC\underline{H}_2CH_3$), 8.05 (br s, 1 H, NH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 13.8$, 16.5, 52.5, 62.4, 91.9, 126.6, 134.2, 159.4, 162.3, 168.0 ppm; EIMS: m/z (%) = 334–332 (MH^+, 6), 274–272 (12), 186 (21), 113 (65), 85 (100).

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