τ-Regioselective addition of (-)-N_α-*tert*-butoxycarbonyl-L-histidine methyl ester to diethyl fumarate

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Dedicated to Professor Enrique Meléndez on the occasion of his 70th birthday

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Abstract

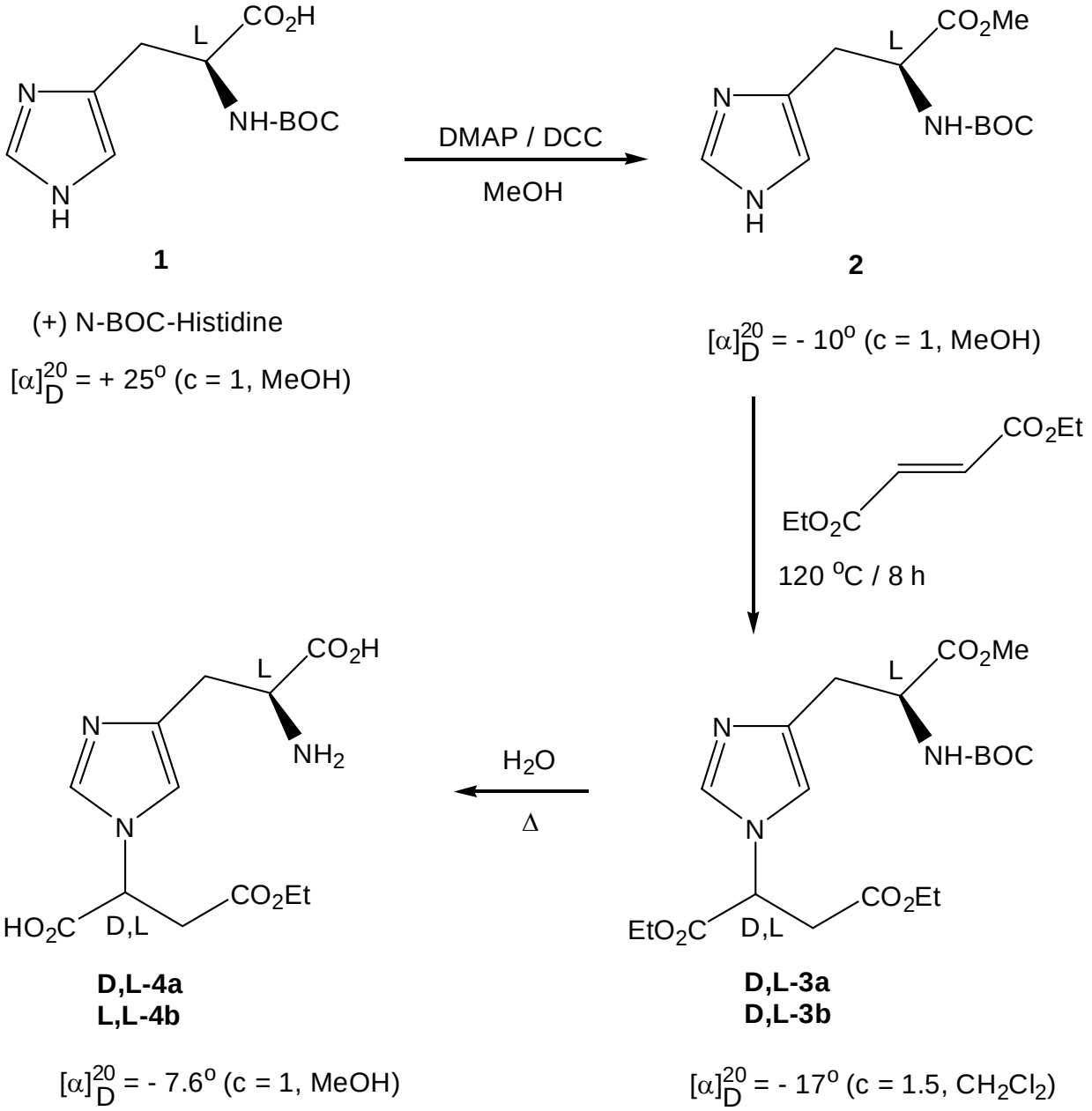
Addition of (-)-N_α-*tert*-butoxycarbonyl-L-histidine methyl ester to diethyl fumarate regioselectively yielded diethyl 2-[4-(2-methoxycarbonyl-2-*tert*-butoxycarbonylaminoethyl) imidazol-1-yl] succinate as a 1:1 mixture of diastereomers. These compounds were identified by NMR using $Eu(fod)_3$ as a stereospecific shift reagent, but were impossible to separate and characterise independently. Neutral hydrolysis of the mixture yielded the corresponding deprotected diastereomeric N_τ-(2-ethoxycarbonyl-1-carboxy)ethyl-L-histidine..

Keywords: Regioselective addition, mixture of diastereomers, NMR shift reagent

Introduction

We have previously described that 2-imidazol-1-ylsuccinic esters are easily prepared by nucleophilic addition of imidazole to fumaric esters.[1] Their regioselective neutral hydrolysis yielded the corresponding half-esters which have been proved to be excellent extrinsic probes for the measurement of extracellular pH by in vitro ^{1}H NMR.[2] In fact, proton H-2 of imidazole ring of (±)-3-(ethoxycarbonyl)-2-imidazol-1-ylpropionic acid (IEPA) has been used to measure localized magnetic resonance spectroscopic imaging (MRSI) in mouse implanted tumours.[3,4] However, IEPA is a monocarboxylate ester and is efficiently cleared from the circulation across the kidney glomerulus by the monocarboxylate transporter. In order to increase the steady state IEPA levels, we have introduced another zwitterion in the structure that increases the polarity of

the molecule. In this communication we report the preparation of the corresponding L-histidine derivative (Scheme 1) in order to improve the properties that afford these kinds of compounds the capability to act like MRI functional contrast agents.



Scheme 1. Synthesis of (-)-N_{τ}-(2-ethoxycarbonyl-1-carboxyethyl)-L-histidine.

Results and Discussion

Thermal addition of (-)-N_{α}-*tert*-butoxycarbonyl-L-histidine methyl ester to diethyl fumarate yielded regioselectively (-)-diethyl 2-[4-(2-methoxycarbonyl-2-*tert*-butoxycarbonylaminoethyl)-imidazol-1-yl]succinate **3** as an unique regioisomer. The τ attachment of the chain was confirmed spectrometrically by COSY/NOESY analysis. Also, the coupling constants values of the imidazole protons are those described for τ substituted histidines.[5] This regioselectivity is a remarkable feature because direct ring alkylation of histidine resulted in a mixture of τ and π derivatives.[6] However, the addition reaction was not stereoselective[7] and the new stereogenic center produced a mixture of diastereomers which were not differentiated by ^{1}H or ^{13}C NMR. Thus, spectra in $CDCl_3$ of the mixture of both compounds, gave single signals for all nuclei. The diastereomers could only be resolved in the presence of a lanthanide shift reagent.[8] Figure 1b depicted the split resonances of O-CH_3 group in the presence of $Eu(fod)_3$[9] that revealed a 50 % diastereomeric mixture of the two compounds obtained with the generation of a new stereogenic center.

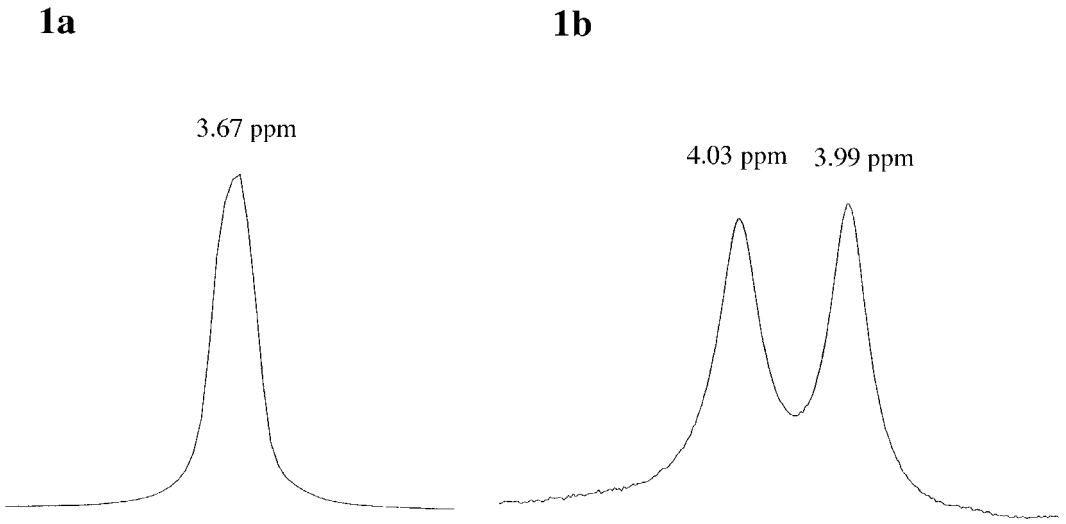


Figure 1. ^{1}H NMR spectra in $CDCl_3$ of diasteromers **3**. 1a. Resonance of proton O-CH_3 group. 1b. Resonance of proton O- CH_3 group in the presence of $Eu(fod)_3$ 0.5 L/S molar ratio.

In the same way, studies performed by GC (using non-chiral and chiral columns) and HPLC (employing a ChiralPack OD column), showed only a single peak after the injection of the sample resulting from the addition of conveniently protected histidine to diethyl fumarate. The mixture of diastereomers could not be separated by column chromatography because they appear as a single spot in TLC chromatographs in all solvent mixtures, and always the sample put in on the column, was eluted as the same non enriched mixture in one diastereomer.

In the last step of the synthesis, the neutral hydrolysis[1] of the succinate derivative yielded the half succinic ester and unexpectedly, the unprotected amino and acid groups of the histidine. This is a remarkable and very convenient result because this is an unusual and not previously reported deprotection method for these funtional groups. Cleavage of *tert*-butoxycarbonyl groups (BOC) are normally carried out in stronger conditions, with CF_3COOH, either neat or in combination with CH_2Cl_2[10] Milder methods have been described with the assistance of microwaves[11] or solid-phase[12] methodologies. Additionally selective hydrolysis of methyl esters in the presence of ethyl esters can be conveniently achieved by treatment with LiI in pyridine[13] or with NaCN in HMPA[14] The results obtained by us can be interpreted as a cascade hydrolytic process. Thus, it starts with the hydrolysis of the imidazolylacetate type ester in the histidyl succinate derivative, through a B_{AC_3} mechanism.[1] The presence of the succinic hemiester would promote a more acidic hydrolytic reaction of the methyl ester and BOC group.

Experimental Section

General Procedures. Melting points were obtained on a microscope hot stage and are uncorrected. Elemental analyses were performed with a Perkin-Elmer 240 apparatus. Optical rotations were measured in a 1 dm cell of 1 mL capacity using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded with a Bruker AM-360 (360.13 MHz for ^{1}H, 90.55 MHz for ^{13}C) and a Bruker DRX-400 (400.13 MHz for ^{1}H, 100.61 MHz for ^{13}C). ^{1}H and ^{13}C chemical shifts (δ) in $CDCl_3$ are given from internal tetramethylsilane.. ^{1}H δ in D_2O are given from internal 3-(trimethylsilyl)tetradeuteropropionic acid sodium salt, and ^{13}C in D_2O are given from external DMSO-d_6, with an accuracy of ± 0.01 ppm for ^{1}H and ± 0.1 ppm for ^{13}C. The residual water signal in ^{1}H NMR spectra in D_2O solutions was suppressed using a 1 s (low power, 0.5 watts) presaturating pulse applied with the decoupler. ^{1}H-^{1}H coupling constants (J) are accurate to ± 0.2 Hz for ^{1}H NMR spectra. AB and X parts refer to the ABX system formed by H-2 and H-3 protons of 2-substituted succinic acid derivatives. TLC chromatography was performed on DC-Alufolien/Kieselgel 60 F_{254} (Merck, 0.2 mm) and column chromatography through silica gel Merk 60 (70-230 mesh). The pK'_a of the H-2 proton in compound **4** was determined by ^{1}H NMR spectroscopy. pH Titration (22 ^{o}C) was performed using 25 mM solutions of the compound in D_2O adjusting the pD with the addition of NaOD or DCl. Products were purchased from commercial sources and were used without additional purification.

(-)-*N*$_\alpha$-*tert*-Butoxycarbonyl-L-histidine methyl ester (2). To a solution of DMAP (2.391 g, 19.6 mmol) in CH_2Cl_2 (20 mL) under argon, a solution of (+)-*N*$_\alpha$-*tert*-butoxycarbonyl-L-histidine (1) (5.000 g, 19.6 mmol) in CH_3OH (20 mL) was added. The reaction mixture was cooled to 0 ^{o}C followed by a slow addition of a solution of DCC (3.861 g, 19.6 mmol) in CH_2Cl_2 (10 mL).[15] The reaction was stirred for 5 min at 0 ^{o}C, and left at room temperature for two days. Afterwards, the solvent was evaporated *in vacuo* and the residue was treated with CH_2Cl_2 to

precipitate DCU that was filtered off *in vacuo*. The remaining solution was concentrated *in vacuo* and the residue was purified through a silica gel column using CH_2Cl_2/EtOH: 95/5 as eluent to obtain **2** as a white solid (4.00 g, 75 %), mp 118-120 °C (from EtOH); $[\alpha]_D^{20}$ = - 10° (c = 1, MeOH); IR (film) ν_{max}/cm^{-1} 3010, 2960, 1745 and 1710 (CO), 1510, 1380, 1070, 1040; δ_H(400.13 MHz; $CDCl_3$; Me_4Si) 1.42 (s, 9 H), 2.43 (s, 3 H), 3.09 (m, AB part, 2 H), 3.69 (s, 3 H), 4.55 (m, X part, 1 H), 5.75 (m, 1 H, NH), 6.79 (bs, 1 H), 7.53 (bs, 1 H); δ_C(100.61 MHz; $CDCl_3$; Me_4Si) 28.3 (q), 29.7 (t), 52.3 (q), 53.6 (d), 116.3 (d), 133.7 (s), 135.2 (d), 155.6 (s), 172.6 (s); m/z 196 (M+-73, 9 %), 152 (44), 136 (7), 110 (26), 82 (100), 81 (64), 57 (91).

(-)-Diethyl 2-[4-(2-methoxycarbonyl-2-*tert*-butoxycarbonylaminoethyl)-imidazol-1-yl]succinate (3). A mixture of (-)-N_α-*tert*-butoxycarbonyl-L-histidine methyl ester (**2**) (1.425 g, 5.3 mmol) and diethyl fumarate (1.823 g, 10.6 mmol) was heated at 120 °C for 8 h. After cooling to room temperature, the residue was purified through a silica gel column using CH_2Cl_2/EtOH : 98/2 as eluent to obtain 1.730 g (74 %) of a brown oil (two diastereomers).as a mixture of **3a** (50 %) and **3b** (50 %). Picrate mp 128-130 °C (from ethanol); $[\alpha]_D^{20}$ = 17.0° (c = 1.5, CH_2Cl_2). Anal. Calcd for $C_{26}H_{34}N_6O_{15}$ (picrate): C, 46.56; H, 5.12; N, 12.53. Found: C, 46.71; H, 5.18; N, 12.54.; IR (film) ν_{max}/cm^{-1} 3050, 3010, 1745br (CO), 1505, 1380, 1180, 1070, 1040; δ_H(400.13 MHz; $CDCl_3$; Me_4Si) 1.22, (t, 3 H, J = 7,1 Hz)1.25 (t, 3 H, J = 7,1 Hz), 1.43 (s, 9 H), 2.85-3.25 (m, 2 AB parts, 4 H), 3.68 (s, 3 H), 4.08 (m, 2 H), 4.17 (m, 2 H), 4.53 (X part, 1 H), 5.17 (X part, 1 H, J = 7.2 Hz), 5.82 (m, 1H), 6.74 (bs, 1 H), 7.46 (d, 1 H, J = 1.2 Hz); δ_C(100.61 MHz; $CDCl_3$; Me_4Si) 13.8 (q), 13.9 (q), 28.2 (q), 30.2 (t), 37.4 (t), 52.0 (q), 53.4 (d), 55.8 (d), 61.4 (t), 62.4 (t), 79.5 (s), 115.4 (d), 136.8 (d), 138 (s), 155.5 (s), 168.2 (s), 169.0 (s), 172.3 (s); m/z (EI): 441 (M^+, 3%), 368 (12), 324 (50), 282 (32), 253 (65), 179 (11), 136 (12), 107 (32), 81 (57), 57 (100).

(-)-N_τ-(2-Ethoxycarbonyl-1-carboxyethyl)-L-histidine (4). A solution of (-)-diethyl 2-[4-(2-methoxycarbonyl-2-*tert*-butoxycarbonylaminoethyl)imidazol-1-yl]succinate (**3a** and **3b**) (3.400 g, 7.71 mmol) in distilled water (75 mL) was heated at 100 °C for 24 h. After cooling at room temperature, the reaction mixture was concentrated in vacuo to yield 1.850 g (80 %) of **4a** and **4b** (1/1) as a white solid (two diastereomers); m.p. 195-197 °C, (from H_2O /EtOH); $[\alpha]_D^{20}$ = -7.6° (c = 1, MeOH). Anal. Calcd for $C_{12}H_{17}N_3O_6 \cdot H_2O$: C, 45.56; H, 5.73; N, 13.28. Found: C, 45.56; H, 5.72 ; N, 13.26.; IR (KBr) ν_{max}/cm^{-1} 3457, 3138, 2980, 1726 and 1619bs (CO), 1593, 1519, 1402, 1333, 1249, 1156; δ_H(400.13 MHz; D_2O; TSP) 1.15 (t, 3 H, J = 7.1 Hz), 1.16 (t, 3 H, J = 6.9 Hz), 3.32-3.14 (m, 4 AB parts, 8 H), 3.97 (m, 2 X parts, 2 H), 4.10 (m, 4 H), 5.29 (m, 2 X parts, 2 H), 7.46 (s, 2 H), 8.75 (s, 2 H); δc (100.61 MHz; D_2O; TSP) 12.2 (q, 2 C), 25.0 (t), 25.1 (t), 36.1 (t), 36.2 (t), 52.4 (d), 52.5 (d), 59.4 (d), 59.5 (d), 61.4 (t, 2 C), 119.0 (d), 119.5 (d), 127.2 (s), 127.5 (s), 134.8 (d), 135.1 (d), 171.0 (s, 2 C), 171.5 (s, 4 C); m/z 254 (M^+-45, 3%), 143 (2), 127 (53), 117 (7), 99 (100), 82 (31), 71 (20), 55 (36); pK_a' = 5.58.

Determination of pK_a'. The pK_a' of the H-2 in componund **4** was determined by ^1H NMR spectroscopy.[2] pH Titration (37 °C) was performed using 25 mM solutions of the compound in D_2O adjusting the required pD with the addition of NaOD or DCl. A different tube was used to obtain each pD value. The dependence of the H-2 chemical shift (δ) with respect to pD was obtained and computer fitted to the Henderson-Hasselbalch equation with the program Sigma Plot v 4.0.:

$$pD = pK'_a - \log [(\delta-\delta_1) / (\delta_2-\delta)]$$

where δ_1 and δ_2 refer to the inferior (anionic) and superior (protonated) chemical shift limits of the titration curve. A three parameter, non-linear regression algorithm based on least squares minimization, allowed the determination of optimal value for δ_1, δ_2 and pK_a'. Observed changes in the chemical shift of the H-2 proton are derived from the protonation/deprotonation at the imidazolic N-3 nitrogen atom.

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