Regiospecific Synthesis of 3-Substituted L-Histidines

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3-Substituted L-histidine derivatives were prepared by a regiospecific alkylation of N,1-bis-Boc-L-histidine methyl ester with in situ-generated alkyl triflates, benzyl triflates, and benzyl mesylates. Subsequent acid hydrolysis of N-Boc and methyl ester protecting groups afforded a variety of modified L-histidines as their dihydrohalide salts in good overall yield.

Introduction

With the increasing importance of enzymes and peptide hormones as targets for pharmaceutical intervention, the modification of natural amino acids and incorporation into peptides has become a valuable process for the preparation of hormone antagonists and enzyme inhibitors of pharmacological consequence. Histidine is frequently a critical residue both at the active site of enzymes and for the recognition of peptide hormones by their receptors. Thus, recent work in our laboratories has focused on a short, efficient, and regiospecific synthesis of 3-substituted (N^{im}-substituted) histidines.[1]

Examination of the literature revealed two similar approaches for the regiospecific 3-alkylation of protected histidines. Both are limited primarily to the purpose of installing blocking groups which protect against racemization at the α-amino carbon. Aside from 3-protecting groups, the selective introduction of only 3-methyl and 3-ethyl substituents has been reported.[2] Due to the difficulty of regiospecific alkylation, even a simple molecule such as 3-benzylhistidine has been available solely by the low-yield process of fractional recrystallization of mixtures obtained by nonspecific alkylation of the parent amino acid.[3]

The first of these reported strategies employs histidine methyl ester which is protected by two acyl groups, commonly benzoyl, benzyloxycarbonyl (Cbz), or t-butyloxycarbonyl (Boc), at the N- and 1-positions.[2,4-7] Such bis-acylated histidines are conveniently and selectively prepared by treatment of histidine methyl ester with two equivalents of the appropriate acylating agent. 3-Alkylation and subsequent hydrolysis of the quaternized imidazole moiety affords 3-substituted histidines protected at amino and carboxy terminals. This method has been successful with only a limited number of alkylating agents due to the low nucleophilicity of acylated imidazoles and steric hindrance at the 3-position. Only highly reactive electrophiles such as Meerwein reagents and chloromethyl alkyl ethers are strong enough alkylating agents for practical use by this method. Attempts to use benzyl bromide and alkyl iodides in our laboratory confirmed this shortcoming, affording negligible yields of the desired products which could only be isolated by tedious chromatography of complex mixtures.

The second method utilizes 1-tritylhistidine methyl ester, protected by Boc or Cbz at the amino nitrogen.[6,8] These adducts require a slightly more elaborate synthesis but the last tritylation step is very selective for protection of the 1-position. 3-Alkylation followed by hydrolysis of the trityl group from the

imidazolium intermediate affords histidine adducts similar to those provided by the previous method. Although one might expect that in this case the higher nucleophilicity of a tritylimidazole would allow the use of a larger variety of alkylating agents, only activated alkyl halides such as phenacyl bromide or chloromethyl benzyl ether have been reported in the literature.

The ease of preparation of N,1-bis-Boc-L-histidine methyl ester (1) and its optical and chemical stability prompted us to investigate the utility of alternative alkylating agents in the first method described above. In this paper, we report that both alkyl and benzyl triflates as well as a number of benzyl mesylates are excellent electrophiles (2) for the alkylation of 1 affording 3-substituted N-Boc-L-histidine methyl esters (3) upon aqueous work-up. The large number of commercially available alcohols, ease of in situ generation of the corresponding triflates, and mild conditions required for high-yielding alkylations make this method extremely flexible and efficient. Amino- and acid-protecting groups can be removed either singly by standard methods or simultaneously by hydrolysis in 6 normal hydrochloric acid to provide a variety of synthetically useful 3-substituted histidines (4, Table).
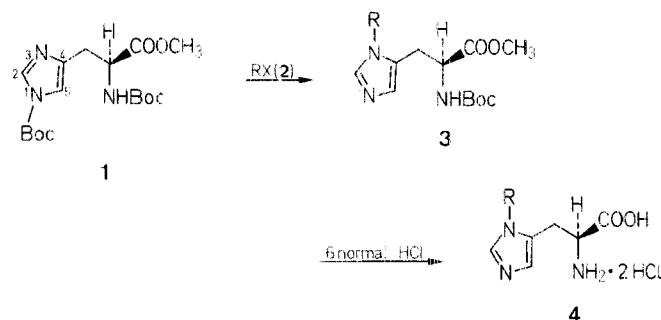

Table. 3-Substituted L-Histidine Derivatives Prepared

R–X[a]	Yield (%) of 3 (from 1)	Yield (%) of 4 (from 3)
a	$C_6H_5-CH_2-OTf$	69	95
b	$4-NO_2-C_6H_4-CH_2-OTf$	75	93
c	$4-OCH_3-C_6H_4-CH_2-OMs$	65	95
d	(R = $4-HO-C_6H_4-CH_2-$)		73[b]
e	$3-OCH_3-C_6H_4-CH_2-OTf$	57	99
f	$2-OCH_3-C_6H_4-CH_2-OMs$	42	75
g	$C_6H_5-CH_2-CH_2-OTf$	59	95
h	$c-C_6H_{11}-CH_2-OTf$	25	92
i	$c-C_6H_{11}-CH_2-CH_2-OTf$	69	86
j	$c-C_6H_{11}-OTf$	0	

[a] $Tf = SO_2-CF_3$; $Ms = SO_2-CH_3$.
[b] Obtained by ether cleavage of the methoxy derivative 3c with hydrobromic acid (see Experimental).

Results and Discussion

The preparation of bis-Boc-protected histidine derivative (1) was accomplished according to Lit.[4] whereby commercial L-histidine methyl ester dihydrochloride is converted to its free base by treatment with triethylamine in methanol and the resulting solution is treated with di-t-butyl dicarbonate at room temperature. The reaction is essentially complete in 4 hours but we have found that the product is more easily crystallized if longer reaction times are used. It is possible that the longer reaction time serves to destroy the slight excess of di-t-butyl dicarbonate which interferes with crystallization of 1. In contrast to published reports,[4,5,8] we find 1 to be quite stable both chemically and optically. Supplies of the pure solid were stored in tightly closed bottles at room temperature for up to 9 months with no obvious deterioration as evidenced by melting point, microanalysis, optical rotation, TLC, and NMR. In addition, we have found 1 to be stable to flash chromatography on silica gel and portions of unreacted 1 can often be recovered from alkylation reactions in sufficient purity to permit recycling.

Alkyl triflates, benzyl triflates, and benzyl mesylates were generated *in situ* using the method developed[9] for the preparation of benzyl triflate as a model. In general, dichloromethane is the solvent of choice. The typical procedure involves the addition of a solution of equimolecular amounts of the desired alcohol and diisopropylethylamine in dichloromethane to a − 75 °C solution of one equivalent of triflic or methanesulfonic anhydride under a dry atmosphere. The triflate reagents are formed rapidly at this temperature. Mesylates, especially 2-methoxybenzyl mesylate, require brief warming to 0 °C to ensure complete formation. With benzyl alcohols, the choice of triflate or mesylate is based on the nature of aromatic substituents. Those with electron-withdrawing groups in any position, a 3-methoxy group, or no substituents were converted to their respective triflate reagent. In the cases of 2- and 4-methoxybenzyl alcohols, the mesylates were the reagents of choice since the respective triflates could not be detected even at − 75 °C, presumably due to electronic and resonance stabilization of the benzylic cation and the resulting formation of dibenzyl ethers subsequent to triflate formation. The use of diisopropylethylamine was found to be far superior to the use of triethylamine and triflic and methanesulfonic anhydrides were the clear reagents of choice over the corresponding acid chlorides, especially with the benzylic alkylating agents.

The alkylated intermediates 3 were frequently noncrystalline and were thus isolated as gums which were dried to constant weight at 25 °C/0.5 torr. These materials were homogeneous according to TLC and contained no NMR-detectable impurities. The final products 4 were all completely characterized, although they are very hygroscopic and frequently difficult to obtain without water of hydration. With the exception of 4a, the compounds listed in the Table are new chemical entities.

All of the alkylation reactions were achieved at room temperature or lower with the exception of the alkylation leading to 3f. The precise temperature of alkylation with the benzyl triflates listed was not determined, but since benzyl triflate itself is known to be stable only below − 20 °C,[9] the reactions presumably occur between − 75° and − 20 °C. With alkyl triflates, the alkylation reaction normally proceeds smoothly at room temperature. Even 2-phenylethyl triflate, a reagent which would be expected to be prone to decomposition by elimination, gives good yields of product 3 g. The exceptions are with cyclohexyl and cyclohexylmethyl triflates which illustrate the practical limits of steric hindrance that can be accomodated in this reaction. The low yield of 3 h obtained with cyclohexylmethyl triflate could not be improved by heating at 40 °C which suggests that elimination to

methylenecyclohexane competes with alkylation at this temperature. The absence of any detectable amounts of the alkylation product (3j) in the alkylation with cyclohexyl triflate is not surprising, given the propensity of secondary triflates toward elimination reactions.

Hydrolysis of the alkylated intermediates 3 in boiling 6 normal hydrochloric acid gives nearly quantitative yields of the corresponding histidine dihydrochlorides 4. If deprotection of the aromatic methoxy group is desired, this can be accomplished simultaneously by heating with aqueous hydrogen bromide, as exemplified by the conversion of 3c to 4d. Both deprotection conditions, although somewhat harsh, remove both the Boc and methyl ester groups simultaneously without detriment to the rest of the molecule in all cases examined. The use of standard, milder methods for removing the Boc group followed by hydrolysis of the ester would be a clear alternative if other sensitive groups were present. The alkylation of 1 with triflates and mesylates and subsequent deprotection is thus a flexible and efficient route to a variety of 3-substituted (N^{im}-substituted) histidines.

All reagents were of commercial quality from freshly opened containers. Benzyl and alkyl alcohols, diisopropylethylamine, methanesulfonic anhydride, trifluoromethanesulfonic anhydride, and L-histidine methyl ester were purchased from Aldrich Chemical Co. Di-t-butyl dicarbonate was purchased from Fluka Chemical Co. Reagent quality solvents were used without further purification. Analytical TLC plates and silica gel (230–400 mesh) were purchased from EM Reagents. Melting points were taken using a MelTemp apparatus and are uncorrected. Microanalyses were obtained using a Perkin-Elmer 240 element analyser and observed rotations at the Na-D line were obtained at 25 °C using a Perkin-Elmer 141 polarimeter. Mass spectra were obtained using a VG model 7070E/HF spectrometer with either DEI or FAB ionization. IR spectra were obtained using a Nicolet IR 80 spectrometer. ^{1}H-NMR spectra were obtained using a Varian XL 200 MHz spectrometer.

N-1-Bis[t-butoxycarbonyl]-L-histidine Methyl Ester (1):

Triethylamine (60 ml, 430 mmol) is added to a stirred suspension of L-histidine methyl ester hydrochloride (52.0 g, 215 mmol) in methanol (500 ml) and stirring is continued at room temperature for 30 min (until dissolution has occurred). A solution of di-t-butyl dicarbonate (93.75 g, 430 mmol) in methanol (250 ml) is then added dropwise over 30 min and stirring is continued at room temperature for 48 h. The solvent is evaporated at reduced pressure and the residue is partitioned between dichloromethane (900 ml) and water (500 ml). The organic layer is washed with 10 % citric acid (2 × 500 ml), dried over magnesium sulfate, and concentrated at reduced pressure to a yellow oil which is dissolved in light petroleum ether (250 ml). This solution is evaporated again. The resulting oil is taken up in light petroleum ether (200 ml) and agitated with a glass rod until a crystalline solid forms. The resulting suspension is chilled overnight and filtered to give 1 as a colorless solid which is dried at 20 °C/20 torr; yield: 73.59 g (93%); m.p. 85–88 °C; $[\alpha]_D^{25}$: +19.9° (c = 1.16, CHCl₃) (Lit.[4], m.p. 90 °C; $[\alpha]_D^{25}$: +25.6° (c = 1.0, CCl₄)).

$C_{17}H_{27}N_3O_6$ calc.   C 55.27   H 7.37   N 11.38
(369.4)   found   54.98   7.47   11.17

MS (DEI): m/e = 370 (M + 1).

IR (KBr): ν = 1705 (O—CO—N); 1743 (COOCH₃) cm^{-1}.

^{1}H-NMR (CDCl₃): δ = 7.97 (s, 1 H, 2^{im}-H); 7.13 (s, 1 H, 5^{im}-H); 5.74 (d, 1 H, NH); 4.56 (m, 1 H, CH); 3.71 (s, 3 H, OCH₃); 3.03 (d, 2 H, CH₂); 1.59, 1.42 ppm (2 s, 9 H each, 2 t-C₄H₉).

3-Benzyl-L-histidine Dihydrochloride (4a):

3-Benzyl-N-t-butoxycarbonyl-L-histidine Methyl Ester (3a): To a stirred solution of triflic anhydride (2.61 ml, 15.5 mmol) in dichloromethane (30 ml) under nitrogen at − 75 °C, a solution of benzyl alcohol (1.6 ml, 15.5 mmol) and diisopropylethylamine (2.71 ml, 15.5 mmol) in dichloromethane (20 ml) is added dropwise over 10 min. Stirring at − 75 °C is continued for 20 min and a solution of N,1-bis[t-butoxycarbonyl]-L-histidine methyl ester (1; 5.0 g, 14.08 mmol) in dichloromethane (35 ml) is then added dropwise. The reaction mixture is allowed to gradually warm to room temperature over a period of 16–18 h. The mixture is

then poured into 0.25 molar phosphate buffer (pH 7; 150 ml) and this mixture is stirred vigorously for 30 min. The organic layer is diluted with dichloromethane (50 ml), washed with phosphate buffer (100 ml), dried with magnesium sulfate, and evaporated to a gum. Flash-chromatograph on silica gel (chloroform/methanol 98:2) and drying at 25°C/0.5 torr for 24 h gives product **3a** as a colorless gum; yield: 3.48 g (69%).

^{1}H-NMR (CDCl$_3$): δ = 7.41 (s, 1 H, 2^{im}-H); 7.30 (m, 3 H$_{arom}$); 7.04 (m, 2 H$_{arom}$); 6.83 (s, 1 H, 5^{im}-H); 5.07 (m, 3 H, CH$_2$—Ar + NH); 4.57 (q, 1 H, CH); 3.70 (s, 3 H, OCH$_3$); 2.95 (m, 2 H, CH$_2$); 1.40 ppm (s, 9 H, t-C$_4$H$_9$).

3-Benzyl-L-histidine Dihydrochloride (4a): A solution of the above gum in methanol (3 ml) is treated with 6 normal hydrochloric acid (74 ml) and the mixture is refluxed for 90 min. The solvent is then evaporated and the slightly colored gum is dissolved in water (75 ml). This solution is treated with activated charcoal, filtered, and concentrated. The remaining colorless gum is dissolved in ethanol (200 ml), reduced to ~10 ml and added dropwise to vigorously stirred ethyl acetate (500 ml). The resultant precipitate is collected and dried at 80°C/20 torr for 24 h to give product **4a** as a hygroscopic solid; yield: 2.25 g (95%); m.p. 199–210°C (dec); [α]$_D^{25}$: +6.5° (c = 2.0, water).

C$_{13}$H$_{15}$N$_3$O$_2$ · 2 HCl · 1/2H$_2$O   calc.   C 49.07   H 5.38   N 13.21
(327.2)   found   48.83   5.41   13.05

MS (FAB) (245.3): m/e = 246 (M + 1).

IR (KBr): ν = 3410 (OH); 2890 (NH); 1745 (CO) cm^{-1}

^{1}H-NMR (D$_2$O): δ = 8.78 (s, 1 H, 2^{im}-H); 7.49 (m, 4 H, 3 H$_{arom}$ + 5^{im}-H); 7.35 (m, 2 H$_{arom}$); 5.48 (s, 2 H, CH$_2$—Ar); 3.88 (t, 1 H, CH); 3.30 ppm (m, 2 H, CH$_2$).

3-Benzyl-L-histidine: An alternative purification procedure which provides **4a** as the free amino acid is as follows: A solution of the crude dihydrochloride **4a** (3 g) in water (5 ml) is loaded onto an ion-exchange bed (Dowex 50 × 2–200, H$^+$ form; 30 ml). The column is eluted with water until the eluent is neutral to pH paper. Elution with 15% aqueous ammonia (300 ml) and evaporation of the basic eluent affords the free amino acid as a slightly colored solid. Recrystallization from water gives colorless needles; yield: 1.75 g; m.p. 288–231°C (dec); [α]$_D^{25}$: +6.04° (c = 1.49, 1 normal hydrochloric acid).

C$_{13}$H$_{15}$N$_3$O$_2$ calc.   C 63.33   H 6.16   N 17.13
(245.3)   found   63.32   6.42   17.11

MS (FAB): m/e = 246 (M + 1).

IR (KBr): ν = 3000 (NH$_3^+$); 1631 (COO$^-$) cm^{-1}.

^{1}H-NMR (DMSO-d$_6$): δ = 7.65 (s, 1 H, 2^{im}-H); 7.28 (m, 3 H$_{arom}$); 7.10 (m, 2 H$_{arom}$); 6.82 (s, 1 H, 5^{im}-H); 5.18 (AB, 2 H, CH$_2$—Ar); 4.0–3.0 (br., 3 H, NH$_3^+$); 3.30 (q, 1 H, CH); 2.85 ppm (m, 2 H, CH$_2$).

3-(4-Nitrobenzyl)-L-histidine Dihydrochloride (4b):

N-(t-Butoxycarbonyl)-3-(4-nitrobenzyl)-L-histidine Methyl Ester (3b): Prepared as **3a** but using 4-nitrobenzyl alcohol in place of benzyl alcohol; yield of **3b**: 75%.

^{1}H-NMR (CDCl$_3$): δ = 7.45 (s, 1 H 2^{im}-H); 8.15, 7.16 (AA'BB',4H$_{arom}$); 6.83 (s, 1 H, 5^{im}-H); 5.20 (AB, 2 H, CH—Ar); 5.09 (m, 1 H, NH); 4.36 (q, 1 H, CH); 3.66 (s, 3 H, OCH$_3$); 2.89 (m, 2 H, CH$_2$); 1.40 ppm (s, 9 H, t-C$_4$H$_9$).

3-(4-Nitrobenzyl)-L-histidine Dihydrochloride (4b): Obtained from **3b** by hydrolysis with 6 normal hydrochloric acid as described for **4a**; yield of **4b**: 93%; m.p. 145–155°C (dec); [α]$_D^{25}$: +8.8° (c = 2.2, methanol).

C$_{13}$H$_{14}$N$_4$O$_4$ · 2 HCl   calc.   C 42.99   H 4.44   N 15.42
(363.2)   found   42.91   4.37   15.62

MS (FAB) for C$_{13}$H$_{14}$N$_4$O$_4$ (290.3): m/e = 291 (M + 1).

IR (KBr): ν = 3410 (OH); 2900 (NH); 1748 (CO); 1522, 1351 (NO$_2$) cm^{-1}.

^{1}H-NMR (D$_2$O): δ = 8.93 (s, 1 H, 2^{im}-H); 7.61 (s, 1 H, 5^{im}-H); 8.31, 7.53 (AA'BB', 4 H$_{arom}$); 5.67 (s, 2 H, CH$_2$—Ar); 4.15 (t, 1 H, CH); 3.30 ppm (m, 2 H, CH$_2$).

3-(4-Methoxybenzyl)-L-histidine Dihydrochloride (4c):

N-(t-Butoxycarbonyl)-3-(4-methoxybenzyl)-L-histidine Methyl Ester (3c):
A solution of methanesulfonic anhydride (10.7 g, 61.6 mmol) in dichloromethane (60 ml) is chilled to −50°C under nitrogen and treated dropwise with a solution of 4-methoxybenzyl alcohol (8.5 g, 61.6 mmol) and diisopropylethylamine (10.7 ml, 61.6 mmol) in dichloromethane

(60 ml). Upon completion of the addition, the cold bath is removed and the mixture is allowed to warm to 0°C over 30 min. The solution is then recooled to −50°C and a solution of N,1-bis[t-butoxycarbonyl]-L-histidine methyl ester (1; 20 g, 56.3 mmol) in dichloromethane (60 ml) is added dropwise. The ice bath is removed and stirring is continued at room temperature for 22 h. The mixture is then poured into 0.25 molar phosphate buffer (pH 7; 500 ml) and stirred vigorously for 30 min. The organic layer is diluted with dichloromethane (100 ml), washed with phosphate buffer (400 ml), dried over magnesium sulfate, and evaporated to a gum which is purified by flash chromatography on silica gel (chloroform/methanol 98:2) to afford product **3c** as a colorless gum; yield: 14.5 g (65%).

^{1}H-NMR (CDCl$_3$): δ = 7.42 (s, 1 H, 2^{im}-H); 6.95, 6.78 (AA'BB', 4H$_{arom}$); 6.80 (s, 1 H, 5^{im}-H); 5.12 (d, 1 H, NH); 5.00 (AB, 2 H, CH$_2$—Ar); 4.49 (m, 1 H, CH); 3.80, 3.73 (2 s, 3 H each, 2 OCH$_3$); 2.96 (m, 2 H, CH$_2$); .40 ppm (s, 9 H, t-C$_4$H$_9$).

3-(4-Methoxybenzyl)-L-histidine Dihydrochloride (4c): The hydrolysis of compound **3c** (10 g, 25.4 mmol) and purification of product **4c** are performed as described for **4a**; yield of **4c** as a hygroscopic solid: 8.5 g (95%); m.p. 125–128°C (dec); [α]$_D^{25}$: +6.5° (c = 1.63, H$_2$O).

C$_{14}$H$_{17}$N$_3$O$_3$ · 2 HCl · 1/3H$_2$O   calc.   C 47.47   H 5.50   N 11.86
(354.2)   found   47.47   5.50   12.14

MS (FAB): m/e = 276 (M + 1), 245 (M—CH$_3$O + 1), 232 (M − CO$_2$ + 1), 121 (CH$_2$—C$_6$H$_4$—OCH$_3$).

IR (KBr): ν = 3410 (OH); 2920 (NH); 1742 (CO) cm^{-1}.

^{1}H-NMR (D$_2$O): δ = 8.61 (s, 1 H, 2^{im}-H); 7.40 (s, 1 H, 5^{im}-H); 7.22, 6.95 (AA'BB', 4 H$_{arom}$); 5.29 (s, 2 H, CH$_2$—Ar); 3.79 (t, 1 H, CH); 3.73 (s, 3 H, OCH$_3$); 3.22 ppm (m, 2 H, CH$_2$).

3-(4-Hydroxybenzyl)-L-histidine Dihydrobromide (4d):
To a solution of compound **3c** (4.5 g, 11.4 mmol) in methanol (5 ml) is added aqueous 47% hydrobromic acid (100 ml) and the mixture is heated at reflux temperature for 1 h, then evaporated. The remaining solid is dissolved in water (100 ml) and this solution is treated with activated charcoal, filtered, and evaporated to a solid. This crude product is triturated with anhydrous ethanol. The resultant solid is isolated and dried at 80°C/20 torr overnight to give product **4d** as an off-white solid; yield: 3.55 g (73%); m.p. 257–260°C (dec); [α]$_D^{25}$: +4.7° (c = 2.1, H$_2$O).

C$_{13}$H$_{15}$N$_3$O$_3$ · 2 HBr   calc.   C 36.90   H 4.05   N 9.93
(423.1)   found   36.72   4.08   9.97

MS (FAB): m/e = 262 (M + 1), 169 (M − C$_6$H$_5$O).

IR (KBr): ν = 3430 (OH); 2840–3190 (NH); 1750 (CO) cm^{-1}.

^{1}H-NMR (D$_2$O): δ = 7.38 (s, 1 H, 2^{im}-H); 6.18 (s, 1 H, 5^{im}-H); 5.93, 5.61 (AA'BB', 4 H$_{arom}$); 4.04 (s, 2 H, CH$_2$—Ar); 2.61 (t, 1 H, CH); 2.02 ppm (m, 2 H, CH$_2$).

3-(3-Methoxybenzyl)-L-histidine Dihydrochloride (4e):

N-(t-Butoxycarbonyl)-3-(3-methoxybenzyl)-L-histidine Methyl Ester (3e): Prepared as **3a** but using 3-methoxybenzyl alcohol in place of benzyl alcohol. After chromatography, product **3e** is obtained as a gum which slowly crystallizes on standing. Trituration with light petroleum ether affords product **3e** as an off-white solid; yield: 57%; m.p. 108–110°C; [α]$_D^{25}$: −6.3° (c = 2.05, methanol).

C$_{20}$H$_{27}$N$_3$O$_5$ calc.   C 61.68   H 6.99   N 10.79
(389.4)   found   61.49   6.81   10.60

MS (FAB): m/e = 390 (M + 1), 334 (M − C$_4$H$_8$ + 1), 121 (CH$_2$—C$_6$H$_4$—OCH$_3$).

IR (KBr): ν = 3195 (NH); 1708 (O—CO—N); 1753 (COOCH$_3$) cm^{-1}.

^{1}H-NMR (CDCl$_3$): δ = 7.45 (s, 1 H, 2^{im}-H); 7.23 (t, 1 H, 5-H$_{arom}$); 6.80 (m, 2 H, 5^{im}-H + 4-H$_{arom}$); 6.63 (d, 1 H, 6-H$_{arom}$); 6.55 (s, 1 H, 2-H$_{arom}$); 5.13 (d, 1 H, NH); 5.04 (AB, 2 H, CH$_2$—Ar); 4.47 (q, 1 H, CH); 3.74, 3.70 (2 s, 3 H each, 2 OCH$_3$); 2.94 (m, 2 H, CH$_2$); 1.39 ppm (s, 9 H, t-C$_4$H$_9$.

3-(3-Methoxybenzyl)-L-histidine Dihydrochloride (4e): The hydrolysis of compound **3e** and the work-up are performed as described for **4a**. Product **4e** is crystallized from ethanol/ethyl acetate; yield: 98.7%; m.p. 220–225°C (dec); [α]$_D^{25}$: +5.2° (c = 2.04, methanol).

C$_{14}$H$_{17}$N$_3$O$_3$ · 2 HCl   calc.   C 45.91   H 5.77   N 11.47
(348.2)   found   46.21   5.60   11.04

MS (DEI): m/e = 275(m), 202 (M—C$_2$H$_5$NO$_2$), 121 (CH$_2$—C$_6$H$_4$—OCH$_3$).

IR (KBr): ν = 3410 (OH); 2900 (NH); 1745 (CO) cm^{-1}.

^{1}H-NMR (D$_2$O): δ = 8.80 (s, 1 H, 2^{im}-H); 7.53 (s, 1 H, 5^{im}-H); 7.44 (t, 1 H, 5-H$_{arom}$); 7.07 (d of d, 1 H, 4-H$_{arom}$); 6.95 (m, 2 H, o-H$_{arom}$); 5.47 (s, 2 H, CH$_2$—Ar); 3.84 (m, 4 H, CH + OCH$_3$); 3.34 ppm (m, 2 H. CH$_2$).

3-(2-Methoxybenzyl)-L-histidine Dihydrochloride (4f):

N-(t-Butoxycarbonyl)-3-(2-methoxybenzyl)-L-histidine Methyl Ester (3f): A solution of methanesulfonic anhydride (8.0 g, 46.2 mmol) in dichloromethane (45 ml) is chilled to $-50°$ under nitrogen and treated dropwise with a solution of 2-methoxybenzyl alcohol (6.15 ml, 46.2 mmol) and diisopropylethylamine (8.0 ml, 46.3 mmol) in dichloromethane (45 ml). The cold bath is removed and the reaction mixture is warmed to 0°C over 30 min. A solution of N,1-bis[t-butoxycarbonyl]-L-histidine methyl ester (1; 15 g, 42.2 mmol) in dichloromethane (45 ml) is then added in a slow stream. The ice bath is removed and stirring is continued for 18 h at room temperature. TLC analysis (chloroform/methanol, 9:1) shows the formation of only a trace of product 3f (R$_f$: 0.35) and a large amount of 1 (R$_f$: 0.7). The reaction mixture is heated at reflux temperature for 24 h, during which time TLC analysis shows the formation of 3f at the expense of 1. The reaction mixture is then poured into 0.25 molar phosphate buffer (pH 7; 350 ml), and this mixture is vigorously stirred for 30 min. The organic phase is washed this phosphate buffer (350 ml), dried with magnesium sulfate, and evaporated. The residue is purified by flash chromatography on silica gel (chloroform/methanol, 98:2) to give product 3f as a colorless gum; yield: 7.0 g (42%). Deletion of the 18 h period at room temperature gives a comparable yield.

^{1}H-NMR (CDCl$_3$): δ = 7.42 (s, 1 H, 2^{im}-H); 7.27 (m, 1 H$_{arom}$); 6.82 (m, 4 H, 5^{im}-H + 3 H$_{arom}$); 5.12 (m, 1 H, NH); 5.02 (s, 2 H, CH$_2$—Ar); 4.52 (m, 1 H, CH); 3.80, 3.70 (2 s. 3 H each, 2 OCH$_3$); 3.02 (m. 2 H. CH$_2$); 1.36 ppm (s, 9 H, t-C$_4$H$_9$).

3-(2-Methoxybenzyl-L-histidine Dihydrochloride (4f): The hydrolysis of compound 3f and the work up are performed as described for 4a. Product 4f slowly crystallizes upon concentration. Anhydrous isopropyl alcohol is added to the wet crystalline mass, and the resultant slurry is chilled for several hours. The solid is filtered and dried at 70°C/20 torr for 24 h to give analytically pure 4f; yield: 4.7 g (75%). m.p. 226–228°C (dec); [α]$_D^{25}$: +5.7° (c = 1.15, H$_2$O).

C$_{14}$H$_{17}$N$_3$O$_3$ · 2HCl calc. C 48.29 H 5.50 N 12.07 Cl 20.36
(348.2)   found 48.00   5.55   12.09   20.50

MS (FAB): m/e = 551 (2 M + 1), 276 (M + 1).

IR (KBr): v = 3430 (OH); 2860 (NH); 1751 (CO) cm^{-1}.

^{1}H-NMR (D$_2$O): δ = 8.68 (s, 1 H, 2^{im}-H); 7.43 (m, 3 H, 5^{im}-H + 2 H$_{arom}$); 7.05 (m, 2 H$_{arom}$); 5.37 (s, 2 H, CH$_2$—Ar); 4.07 (t, 1 H. CH); 3.77 (s, 3 H, OCH$_3$); 3.40 ppm (m, 2 H, CH$_2$).

3-(2-Phenylethyl)-L-histidine Dihydrochloride (4g):

N-(t-Butoxycarbonyl)-3-(2-phenylethyl)-L-histidine Methyl Ester (3g): A solution of triflic anhydride (2.6 ml, 15.5 mmol) in dichloromethane (20 ml) is chilled to $-75°$C under nitrogen and a solution of 2-phenylethanol (1.85 ml, 15.5 mmol) and diisopropylethylamine (2.7 ml, 15.5 mmol) in dichloromethane (20 ml) is added dropwise over 15 min. The resultant solution is stirred for 15 min at $-75°$C and then a solution of N,1-bis[t-butoxycarbonyl]-L-histidine methyl ester (1; 5.72 g, 15.5 mmol) in dichloromethane (20 ml) is added dropwise. The mixture is allowed to warm to room temperature over 5 h and stirring is continued for 24 h. The mixture is then poured into 0.25 molar phosphate buffer (pH 7; 150 ml), stirring vigorously for 30 min. The organic phase is diluted with dichloromethane (100 ml), washed with phosphate buffer (150 ml), dried with magnesium sulfate, and evaporated. The residue is purified by flash chromatography on silica gel (chloroform/methanol 98:2) to give product 3g as a colorless gum; yield: 3.43 g (59%).

^{1}H-NMR (CDCl$_3$): δ = 7.25 (m, 4 H, 2^{im}-H + 3 H$_{arom}$); 7.03 (m, 2 H$_{arom}$); 6.73 (s, 1 H, 5^{im}-H); 5.07 (d, 1 H, NH); 4.47 (m, 1 H, CH); 4.06 (d of t, 2 H, N—CH$_2$); 3.70 (s, 3 H, OCH$_3$); 2.92 (m, 4 H, CH$_2$ + CH$_2$—Ar; 1.38 ppm (s, 9 H, t-C$_4$H$_9$).

3-(2-Phenylethyl)-L-histidine Dihydrochloride (4g): Acid hydrolysis of compound 3g and the work-up are performed as described for 4a. Product 4g is precipitated by addition of ethanolic solution to ethyl acetate; yield: 2.7 g (88%); m.p. 130–135°C (glass); [α]$_D^{25}$: +12.4° (c = 2.05, methanol).

C$_{14}$H$_{17}$N$_3$O$_2$ · 0.5 H$_2$O calc. C 49.28 H 5.90 N 12.31
(341.2)   found   49.17   5.71   12.33

MS (DEI): m/e = 260 (M + 1), 185 (M — NH$_2$CHCOOH). 105 (C$_6$H$_5$—CH$_2$—CH$_2$)

IR (KBr): v = 3430 (OH); 2850–3150 (NH); 1744 (CO) cm^{-1}.

^{1}H-NMR (D$_2$O): δ = 8.51 (s, 1 H, 2^{im}-H); 7.35 (m, 4 H. 5^{im}-H + 3 H$_{arom}$); 7.12 (m. 2 H$_{arom}$); 4.49 (t, 2 H, N—CH$_2$); 3.99 (t, 1 H. CH); 3.14 ppm (m, 4 H, CH$_2$—Ar + CH$_2$).

3-(Cyclohexylmethyl)-L-histidine Dihydrochloride (4h):

N-(t-Butoxycarbonyl)-3-(cyclohexylmethyl)-L-histidine Methyl Ester (3h): This compound is prepared according to the procedure described for 3g but using cyclohexylmethanol in place of 2-phenylethanol. Product 3h is obtained as an oil which slowly crystallizes on standing. The resultant solid is triturated with light petroleum ether and dried at 35°C/20 torr overnight to afford analytically pure 3 h; yield: 25%; m.p. 96–99°C; [α]$_D^{25}$: —6.6° (c = 2.07, methanol).

C$_{19}$H$_{31}$N$_3$O$_4$ calc. C 62.44 H 8.55 N 11.49
(365.5)   found 62.66   8.43   11.31

MS (DEI): m/e = 365 (M$^+$), 248 (M—BocNH$_2$); 177 (M—BocNH—CH—COOCH$_3$).

IR (KBr): v = 3346 (NH); 2977–2855 (CH); 1744 (COOCH$_3$); 1684 (NH—COO) cm^{-1}.

^{1}H-NMR (CDCl$_3$): δ = 7.41 (s, 1 H, 2^{im}-H); 6.77 (s, 1 H, 5^{im}-H); 5.15 (d, 1 H, NH); 4.55 (q, 1 H, CH); 3.73 (s, 3 H, OCH$_3$); 3.64 (d, 2 H, N—CH$_2$); 3.06 (d, 2 H, CH$_2$); 1.65 (m, 6 H$_{alkyl}$); 1.41 (s. 9 H, t-C$_4$H$_9$); 1.20 (m, 3 H$_{alkyl}$); 0.96 ppm (m, 2 H$_{alkyl}$).

3-(Cyclohexylmethyl)-L-histidine Dihydrochloride (4h): The hydrolysis of compound 3h (1.1 g, 3.01 mmol) and work-up are performed as described for 4a; yield: 0.90 g (92%); m.p. 160–165°C (dec); [α]$_D^{25}$: +14.5 (c = 2.07, methanol).

C$_{13}$H$_{21}$N$_3$O$_2$ · 2 HCl calc. C 48.15 H 7.14 N 12.96
(324.25)   found 48.37   7.29   12.97

MS (DEI): m/e = 251 (M$^+$), 177 (M—NH$_2$CHCOOH), 95 (C$_7$H$_{11}$).

IR (KBr): v = 3400 (OH); 3110 (NH); 2928, 2855 (CH); 1745 (CO) cm^{-1}.

^{1}H-NMR (D$_2$O): δ = 8.80 (s, 1 H, 2^{im}-H); 7.51 (s, 1 H, 5^{im}-H); 4.32 (t, 1 H. CH); 4.08 (d, 2 H, N—CH$_2$); 3.44 (m, 2 H, CH$_2$); 1.64 (m, 6 H$_{alkyl}$); 1.11 ppm (m, 5 H$_{alkyl}$).

3-(2-Cyclohexylethyl)-L-histidine Dihydrochloride (4i):

N-(t-Butoxycarbonyl)-3-(2-cyclohexylethyl)-L-histidine Methyl Ester (3i): This compound is prepared according to the procedure described for 3g but using 2-cyclohexylethanol in place of 2-phenylethanol. After chromatography as for 3g, compound 3i is obtained as gum; yield: 69%.

IR (neat): v = 3360, 3200 (NH); 2979–2854 (CH); 1750 (COOCH$_3$); 1712 (NH—COO) cm^{-1}.

^{1}H-NMR (CDCl$_3$): δ = 7.41 (s, 1 H, 2^{im}-H); 6.77 (s, 1 H, 5^{im}-H); 5.13 (d, 1 H, NH); 4.52 (q, 1 H, CH); 3.87 (t, 2 H, N—CH$_2$); 3.73 (s, 3 H, OCH$_3$); 3.06 (d, 2 H, CH$_2$); 1.65 (m, 7 H$_{alkyl}$); 1.41 (s, 9 H, t-C$_4$H$_9$); 1.20 (m, 4 H$_{alkyl}$); 0.96 ppm (m, 2 H$_{alkyl}$).

3-(2-Cyclohexylethyl)-L-histidine Dihydrochloride (4i): A solution of compound 3i (7.4 g, 19.5 mmol) in methanol (10 ml) is added dropwise to 6 normal hydrochloric acid (300 ml) and stirred for 5–10 min until homogeneous. This solution is heated at reflux temperature for 3 h, and evaporated. The residue is dissolved in water (200 ml) and this solution is treated with activated charcoal, filtered, and evaporated to a gum. The gum is sonicated with anhydrous ethanol until it begins to crystallize. The resultant suspension is evaporated at reduced pressure to give a solid which is triturated with cold ethanol and dried at 75°C/12 torr overnight to afford pure 4i as a hygroscopic solid; yield: 5.7 g (86%); m.p. 180–186°C (dec); [α]$_D^{25}$: +11.5° (c = 2.09, methanol).

C$_{14}$H$_{23}$N$_3$O$_2$ · 2HCl calc. C 49.71 H 7.43 N 12.48 Cl 20.96
(338.3)   found 49.51   7.43   12.48   20.65

MS (FAB) for C$_{14}$H$_{23}$N$_3$O$_2$ (265.35): m/e = 266 (M + 1), 191 (M — NH$_2$CHCOOH), 96 (C$_6$H$_{10}$=CH$_2$).

IR (KBr): v = 3410 (OH); 3110 (NH); 2920, 2854 (CH); 1741 (CO) cm^{-1}.

^{1}H-NMR (D$_2$O): δ = 8.79 (s, 1 H, 2^{im}-H); 7.48 (s, 1 H. 5^{im}-H); 4.68 (t, 1 H, CH); 4.27 (m, 3 H, N—CH$_2$ + CH); 3.44 (m, 2 H, CH$_2$); 1.71 (m, 7 H$_{alkyl}$); 1.16 ppm (m, 6 H$_{alkyl}$).

(1) For the locants in names of substituted histidine derivatives, Chemical Abstracts nomenclature is used in this paper. 3-Substituted is equivalent to $N(\pi)$-substituted in IUPAC nomenclature.

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