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Research Article

Improved specific synthesis of [1′-^{15}N]- and [3′-^{15}N]L-histidine

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Summary

Specifically, ^{15}N-enriched L-histidines have been prepared. The labelling methodology involves introduction of labels in its precursor 1-benzyl-5-hydroxy methyl imidazole, which is converted into L-histidine via the Schöllkopf method. The procedure allows the preparation of the intermediates and finally histidine with high ^{15}N enrichment (99%) at each position, in 29% overall yield starting with $^{15}NH_4Cl$ and 56% with $KSC^{15}N$, respectively.

Key Words: ^{15}N-labelling; ^{13}C-labelling; 1-benzyl-5-hydroxymethylimidazole; L-histidine

Introduction

L-histidine residues play an important role in life processes. In many enzymes, the τ- and π-nitrogen atoms of the heterocyclic imidazole side chain play a key-role in both protonation and tautomerization reactions. In photosynthesis,[1–11] the energy and material generating process in the biosphere, the Mg^{2+} ions of the (bacterio)chlorophylls are axially co-ordinated to the 1′-N atom of L-histidine residues both in

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the antenna complexes and photosynthetic reaction centres and special properties of the imidazole ring system in the conversion of the light energy into chemical energy are expected.[1–10] NMR spectroscopy is the method of choice to determine the univocal protonation, tautomeric and co-ordination states. The incorporation of highly enriched ^{15}N-labelled L-histidines into the protein is achieved by growing micro-organisms on a well-defined substrate that is supplemented with the specifically labelled amino acid.

Earlier, we reported the synthesis of ^{15}N- and ^{13}C-labelled L-histidines in which 1-benzyl-5-hydroxymethyl-imidazole has been coupled via its chloride derivative to the chiral bislactim ether of cyclo-(D-valylglycine) to produce the enantiomerically pure labelled L-histidines.[11] However, attempted growth of photosynthetic micro-organisms on media that contained this [1′-^{15}N]L-histidine lead to the death of the living cells, which meant that it contained analytically undetectable traces of toxic substances.

In the present paper, we describe an improved synthesis of 1-benzyl-5-hydroxymethyl-imidazole and the ^{15}N-histidines prepared via this new approach which do not exhibit harmful effects on the growth of photosynthetic microorganisms.

Results and discussion

For the synthesis of [1-^{15}N]- and [3-^{15}N]1-phenyl-5-hydroxymethylimidazole, **8a** and **8b** respectively, we optimized the new route depicted in the scheme, which is partially based on a literature method.[12] The [1-^{15}N]Im- and [3-^{15}N]Im-atoms are introduced via [15N]-labelled benzylamine (which is prepared from $^{15}NH_4Cl$ and benzoylchloride) and KSC^{15}N, respectively. However, first the scheme was optimized using synthons with natural isotopic abundance.

In the first step, methyl bromoacetate (**2**) is treated with benzylamine to give N-benzylglycine methyl ester (**3**). Via treatment with formic acid and acetic acid anhydride, **3** is N-formylated to N-benzyl-N-formylglycine methyl ester (**4**).

Compound **4** is subsequently carbon formylated with methyl formate in the presence of sodium methoxide, which gives the sodium salt **5**. Treatment of **5** with potassium thiocyanate in the presence of aqueous HCl gives the imidazole derivative **6**. The compound is converted by treatment with nitric acid in the presence of sodium nitrite, followed by

addition of sodium carbonate to give methyl 1-benzyl-5-imidazole-carboxylate (**7**).

$LiAlH_4$ reduction the ester function gives the required 1-benzyl-5-hydroxymethylimidazole (**8**). The latter compound has been converted into the corresponding chloromethyl derivative and finally to the isotopomers of L-histidine by the method described before.[11] The isotopic enrichments at the chosen positions, determined by mass spectrometry, were the same (99%) as that of the starting materials. The conversions could be executed without scrambling and isotope dilution. The synthetic scheme has also been optimized to label the ^{13}C-atoms of histidine by using reagents (that can be commercially obtained in ^{13}C-enriched form) in stoichiometric quantities. This means that **8** and L-histidine can now be obtained in isotopically enriched form (^{13}C, ^{15}N) on each position and combinations of positions.

Via the present scheme, 6.7 g of [3′-^{15}N]L-histidine has been prepared in a 29% overall yield based on $^{15}NH_4Cl$. Similarly 6.2 g of [1′-^{15}N]L-histidine has been obtained in a 56% overall yield based on $KSC^{15}N$.

All experiments to incorporate [^{15}N]-L-histidine, made via the present scheme, in photosynthetic organisms did not show any harmful effect on the organisms, meaning that the biological quality of isotopically enriched histidine made via the present scheme is excellent.

Experimental

General details

All chemicals were purchased from Acros Organics or Aldrich, except for [^{15}N]-ammonium chloride (99% ^{15}N) and the [^{15}N]-potassium thiocyanate (99% ^{15}N), which were obtained from Cambridge Isotope Laboratories. [^{15}N]benzylamine (99% ^{15}N) was prepared from [^{15}N]ammonium chloride as has been described previously.[11] In all experiments distilled anhydrous solvents were used. Tetrahydrofuran (THF) was freshly distilled from sodium, prior to use. Ether refers to diethylether and all solvent mixtures are given in volume ratios (v/v). All solvents were removed by evaporation *in vacuo*.

All reactions were optimized using non-isotopically enriched starting materials and the procedures are all described for unlabelled compounds. The procedures that were used for the preparation of the isotopically enriched compounds were the same as those described for

the corresponding unlabelled compounds. Only those spectroscopic characteristics or the enriched compounds are given that are different from the unlabelled compounds.

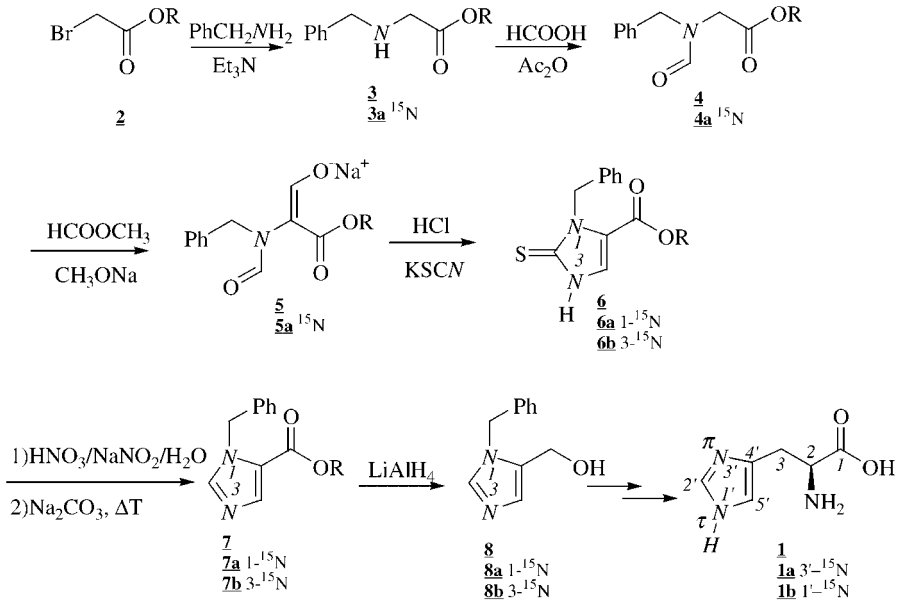
The specificity of the isotope labelling has been determined by NMR spectroscopy. All ^{1}H- and ^{13}C-NMR spectra of ^{15}N compounds were recorded using a Jeol NM FX-200 or a Bruker WM-300. ^{1}H, ^{13}C and ^{15}N spectra of the labelled ^{15}N products were recorded on a Bruker WM-300 or on a Bruker WM-600 with tetramethylsilane (TMS: δ 0.00 ppm) as internal standard for spectra recorded in $CDCl_3$, and 3-(trimethylsilyl)tetradeuteropropionic acid sodium salt (TPS: δ 0.00 ppm) for spectra recorded in D_2O. For the ^{15}N-NMR spectra we used a saturated solution of ammonium nitrate as an external standard ($^{15}NH_4NO_3$ δ 22.3 ppm relative to NH_3 (l): 0.00 ppm).

Mass spectrometry is used to determine the exact molecular mass and the isotope enrichment of the synthesized histidines. The exact molecular mass is determined with double focus mass spectroscopy (Finnigan MAT 900 mass spectrometer, equipped with a direct insertion probe (EI-MS, 70 eV), using a soft ionization (ESI) technique, which results in a protonated molecule. For [1′-^{15}N]- and [3′-^{15}N]L-histidines the experimental m/z values are 157.07421 and 157.07426, respectively. Both values correspond to the calculated one (m/z 157.07434) of the protonated histidine molecule $^{12}C_6\,^{1}H_9\,^{14}N_2\,^{15}N^{16}O_2$).

Prior to the determination of the isotope enrichment the labelled histidines are derivatized to form the N,O-ethoxycarbonyl ethyl ester (N,O-ECEE) derivatives and the enrichment is calculated from the intensities of the peaks measured by GC/MS. The measurements were performed on a Finnigan MAT ITD 700 (EI, 70 eV) coupled to a Chrompack 438A gas chromatograph equipped with a 25 m fused silica column (CP-Sil-5CB; 0.25 mm i.d.). The amino acids were derivatized by reaction with ethyl chloroformate (ECF).[13] The sample was dissolved in 200 µl of a mixture of water/ethanol/pyridine (60/32/8) and 10 µl of ECF was added. The tube was vortexed for 5 s.; gas evolution occurred (CO_2 release). A solution of 1% ECF in chloroform (200 µl) was added and the derivatives were extracted into the organic phase by vortexing for 15 s.; 1 µl of this organic layer was injected (Scheme 1).

Preparation of the compounds

*N-Benzylglycine methylester (**3**)*. Methyl bromoacetate (**2**) (7.66 g, 50 mmol, 4.74 ml) in 50 ml THF was treated with 13.9 ml (10.1 g,



Scheme 1. Synthesis of 1-benzyl-5-hydroxymethylimidazole (8) and the [1-^{15}N]- and [3-^{15}N]-isotopomers (8a and 8b), the precursor for the synthesis of [1′-^{15}N]- and [3′-^{15}N]histidine (1a and 1b). Labelled positions are indicated by italics.It should be noticed that in the literature two different systems of numbering the atoms of the imidazole ring of histidine had been used for a long time (biochemists generally numbering as 1, the nitrogen atom adjacent to the side chain, and organic chemists designed it as 3). For this reason the by IUPAC recommended notation *pros* ('near', abbreviated by π) and *tele* ('far' abbreviated τ) has also been included[14]

100 mmol) Et_3N. To this solution 5.58 g (52 mmol, 5.69 ml) of benzylamine was added. After stirring (1 night, at room temperature) the precipitated Et_3NHBr was removed by filtration and the filtrate was concentrated under vacuum to yield pure N-benzylglycine methylester (**3**) (8.26 g, 46 mmol, 92% yield).

^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 2.03 (bs, NH), 3.37 (s, 2H, CH_2), 3.67 (s, 3H, CH_3), 3.76 (s, 2 H, $PhCH_2$), 7.29 (m, 5 H, CH_{Ph}).

^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): 49.9 (CH_2), 51.4 (CH_3), 53.1 (CH_2), 126.8 (CH_{Ph}), 127.8 (CH_{Ph}), 127.9 (CH_{Ph}), 139.2 ($C_{Ph,q}$), 172.5 (CO).

*[^{15}N]Benzylglycine methylester (**3a**).* 20.4 g (114 mmol) of [^{15}N]benzylglycine methylester (**3a**) was prepared as described for **3** from 18.7 g (122 mmol) [^{15}N]benzylamine (94% yield).

^{1}H NMR (300 MHz, $CDCl_3$): the signal at 2.00 ppm is broadened and the signal at 3.36 ppm is split into a doublet, $^{2}J_{NH}$ 1.0 Hz.

^{13}C NMR (75.4 MHz, $CDCl_3$): the signals at 49.9 and 53.1 ppm appear as doublets, $^{1}J_{NC}$ 4.4 Hz and $^{1}J_{NC}$ 4.4 Hz, respectively.

^{15}N NMR (30.4 MHz, $CDCl_3$): 32.4 (bs) ppm.

*N-Benzyl-,N-formylglycine methylester (**4**)*. To a solution of 3.43 g (19.2 mmol) N-benzylglycine methylester (**3**) in 15 ml 98% formic acid, 15 ml of acetic anhydride was added in small portions. When the vigorous reaction had subsided, the mixture was heated on an oil bath (100°C) for 1 h and then evaporated under vacuum to remove the formic and acetic acids. The residual material was taken up into dichloromethane. This solution was washed four times with 1 M $NaHCO_3$ solution to remove the last traces of acid, once with water and once with a saturated solution of NaCl and subsequently dried over anhydrous $MgSO_4$. Concentration in vacuo yielded 3.67 g (17.71 mmol, 92% yield) of N-benzyl-N-formyl-glycine methylester (**4**).

^{1}H NMR (300 MHz, $CD_3S(O)CD_3$, δ in ppm): *rotamer* **A**: 3.62 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 4.50 (s, 2H, CH_2Ph), 7.21 (m, 2H, CH_{Ph}), 7.31 (m, 3H, CH_{Ph}), 8.22 (s, 1H, OCH). *Rotamer* B: 3.60 (s, 3H, CH_3), 3.90 (s, 2H, CH_2), 4.56 (s, 2H, $PhCH_2$), 7.21 (m, 2H, CH_{Ph}), 7.31 (m, 3H, CH_{Ph}), 8.42 (s, 1H, OCH).

^{13}C NMR (50.1 MHz, $CD_3S(O)CD_3$, δ in ppm): *rotamer* **A**: 42.6 (CH_2), 51.1 (CH_2Ph), 51.6 (CH_3), 127.4 (CH_{Ph}), 127.8 (CH_{Ph}), 128.4 (CH_{Ph}), 134.7 ($_{Cq,Ph}$), 162.7 (HCO), 168.3 (CO). *Rotamer* B: 45.8 (CH_2), 47.3 (CH_2Ph), 51.6 (CH_3), 128.0 (CH_{Ph}), 128.2 (CH_{Ph}), 128.4 (CH_{Ph}), 135.1 ($C_{q,Ph}$), 162.4 (HCO), 169.0 (CO).

*N-benzyl,N-formyl-[^{15}N]glycine methylester (**4a**)*. Compound **4a** (21.5 g, 165 mmol) was prepared as described for **4** from 20.4 g (184 mmol) of N-benzyl-[^{15}N]glycine methylester (**3a**) (91% yield).

^{1}H NMR (300 MHz, $CDCl_3$): *rotamer* **A**: signal at 8.42 ppm was split into a doublet, $^{2}J_{NH}$ 15.8 Hz. *Rotamer* **B**: signal at 8.22 ppm was also split into a doublet, $^{2}J_{NH}$ 15.5 Hz.

^{13}C NMR (75.4 MHz, $CDCl_3$): *Rotamer* **A**: signals at 42.6 and 51.1 ppm were split into doublets, $^{1}J_{NC}$ 13.2 Hz and $^{1}J_{NC}$ 11.7 Hz, respectively.

Rotamer **B**: signals at 45.8 and 47.3 ppm were also split into doublets, $^{1}J_{NC}$ 13.2 Hz and $^{1}J_{NC}$ 12.8 Hz respectively.

^{15}N NMR (30.4 MHz, $CDCl_3$, δ in ppm): rotamer **A**: 121.8 (s). Rotamer **B**: 122.5 (s).

*N-Benzyl,N-formyl,C-formylglycine methylester sodium salt (**5**).* In a three-necked flask provided with a stirrer and thermometer was placed 2.19 g (10.57 mmol) of N-benzyl,N-formylglycine methylester and 1.80 g (29.98 mmol) of dry methyl formate. The reaction mixture was cooled in an ice bath whilst the mixture was stirred. Then, 0.78 g (14.44 mmol) of sodium methoxide and 5 ml of dry toluene were added. The temperature of the reaction mixture was maintained below 15°C, and stirring was continued for 1 h after all the sodium methoxide had been added. The mixture was then placed in the refrigerator and allowed to stand for approximately 24 h. To isolate the sodium salt, extremely dry ether was added to the reaction mixture, and the yellowish solid was collected on a glass filter, washed with extremely dry ether and finally dried under vacuum. The result was 2.35 g (8.66 mmol, 88% yield) of the desired product.

^{1}H NMR (300 MHz, CD_3SOCD_3, δ in ppm): 3.36 (s, 3H, CH_3), 4,50 (s, 2H, CH_2), 7.18 (m, 3H, CH_{Ph}), 7.78 (m, 2H, CH_{Ph}), 8.48 (bs, 1H, CHO), 8.88 (bs, 1H, HCONa). ^{13}C NMR (75.5 MHz, CD_3SOCD_3, δ in ppm): 47.1 (CH_2), 49.3 (CH_3), 126.0 (C2), 128.7 (CH_{Ph}), 129.2 (CH_{Ph}), 129.5 (CH_{Ph}), 138.8 ($C_{Ph,q}$), 165.8 (NCHO), 169.3 (CO) and 173.3 (HCONa).

*N-Benzyl,N-formyl,C-formyl-[^{15}N]glycine methyl ester sodium salt (**5a**).* 4.69 g (18.2 mmol) of (**5a**) was synthesized as described above from 4.30 g (20.7 mmol) of benzyl-N-formyl-[^{15}N]glycine methylester (**4a**) (yield: 88%).

^{1}H NMR (300 MHz, CD_3SOCD_3): The signal at 8.50 ppm appeared as a doublet: $^{2}J_{NH}$ 11.8 Hz.

^{13}C NMR (75.5 MHz, CD_3SOCD_3: No splittings were observed.

^{15}N NMR (30.4 MHz, CD_3SOCD_3: 126.9 (s) ppm.

*Methyl 1-benzyl-2-mercapto-5-imidazolecarboxylate (**6**).* 2.23 g (8.67 mmol) of the dry sodium enolate salt of N-benzyl,N-formyl,C-formylglycine methyl ester (**5**) was dissolved in 10 ml of 50% methanol and 1.66 ml (19.9 mmol) of 12 M hydrochloric acid was added. After standing for approximately 20 h at room temperature, 0.84 g (8.64 mmol) of potassium thiocyanate was added and the mixture was heated on an oil bath at 80°C until most of the alcohol had evaporated. The methyl 1-benzyl-2-mercapto-5-imidazolecarboxylate (**6**) was collected by filtration, washed with water and air dried. The result was 1.79 g (7.22 mmol, 83% yield based on KSCN).

^{1}H NMR (200 MHz, $CDCl_3$, δ in ppm): 3.76 (s, 3H, CH_3), 5.70 (s, 2H, $PhCH_2$), 7.25 (m, 5H, CH_{Ph}), 7.44 (s, 1H, H4) 12.65 (bs, 1H, NH).

^{13}C NMR (50.1 MHz, $CDCl_3$, δ in ppm): 48.4 (CH_2Ph), 51.8 (OCH_3), 120.8 (C_q, C5), 123.0 (C4), 127.5 (CH_{Ph}), 128.1 (CH_{Ph}), 128.3 (CH_{Ph}), 136.2 ($C_{Ph,q}$), 158.5 (C_q, CO), 164.6 (C_q, C2).

*Methyl 1-benzyl-2-mercapto-[1-^{15}N]5-imidazolecarboxylate (**6a**).* 5.1 g (20 mmol) of (**6a**) was prepared from 4.3 g (21 mmol) of benzyl-N-formyl-[^{15}N]glycine methylester (**5a**) (98% yield) as described above for **6**.

^{1}H NMR (200 MHz, $CDCl_3$): the signal at 7.44 ppm was split into a doublet, $^{3}J_{NH}$ 2.4 Hz.

^{13}C NMR (50.1 MHz, $CDCl_3$): the signals at 48.5, 120.8 and 164.6 ppm are shown as doublets, $^{1}J_{NC}$ 10.3 Hz, $^{1}J_{NC}$ 13.2 Hz and $^{1}J_{NC}$ 14.7 Hz, respectively.

^{15}N NMR (30.4 MHz, $CDCl_3$): 173.0 (s) ppm.

*Methyl 1-benzyl-2-mercapto-[3-^{15}N]5-imidazolecarboxylate (**6b**).* Compound (**6b**) (8.52 g, 33.98 mmol) was prepared similarly from 9.56 g (36.95 mmol) of the dry sodium enolate salt of N-benzyl,N-formyl,C-formylglycine methylester (**6**) and 3.46 g (35.2 mmol) of $KSC^{15}N$ (92% yield based on the labelled starting material).

^{1}H-NMR (300 MHz, $CDCl_3$): the signal at 7.45 and 12.65 ppm are doublets, $^{3}J_{NH}$ 3.4 Hz and $^{1}J_{NH}$ 99.4 Hz, respectively.

^{13}C NMR (75.5 MHz, $CDCl_3$): the signals at 122.9 and 164.6 ppm were split into doublets, $^{1}J_{CN}$ 10.8 Hz and $^{1}J_{CN}$ 14.6 Hz, respectively.

^{15}N-NMR (30.4 MHz, $CDCl_3$): 171.5 (d, $^{1}J_{NH}$ 99.3 Hz, NH) ppm.

*Methyl 1-benzyl-5-imidazolecarboxylate (**7**).* 3.53 g (14.22 mmol) of methyl 1-benzyl-2-mercapto-5-imidazolecarboxylate (**6**) was dissolved in 7.70 ml $NaNO_2$ solution (2.0 g $NaNO_2$ in 950 ml water) and 2.68 ml of nitric acid (35% HNO_3) was added with continuous stirring. After all the nitric acid had been added, the solution was stirred for about 10 min or until the evolution of nitrogen oxides had completely stopped. The reaction mixture was boiled with 40 ml of a solution of sodium carbonate (40%) to liberate methyl 1-benzyl-5-imidazolecarboxylate. Extraction with ether yields the maximum amount of the product. The yield was 3.01 g (13.92 mmol, 98% yield) methyl 1-benzyl-5-imidazole carboxylate (**7**).

^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 3.79 (s, 3H, CH_3), 5.50 (s, 2H, $PhCH_2$), 7.17 (m, 2 H, CH_{Ph}), 7.30 (m, 3H, CH_{Ph}), 7.62 (d, $^{4}J_{HH}$ 0.8 Hz, 1H, H4), 7.77 (d, $^{4}J_{HH}$ 0.8 Hz,1H, H2).

^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): 49.8 (CH_2), 51.3 (CH_3), 122.2 (C5), 127.1 (CH_{Ph}), 127.9 (CH_{Ph}), 128.7 (CH_{Ph}), 136.0 ($C_{Ph,q}$), 137.9 (C2), 142.1 (C4),160.4 (C_q (CO)).

*Methyl 1-benzyl,[1-^{15}N]5-imidazolecarboxylate (**7a**)*. Compound **7a** (6.4 g, 114 mmol) was prepared as described for **7** from 8.6 g (145 mmol) of methyl 1-benzyl-2-mercapto-[-^{15}N]5-imidazolecarboxylate (**6a**) (85% yield).

^{1}H NMR (200 MHz, $CDCl_3$): the signals at 7.62 and 7.76 ppm were split into double doublets, $^{2}J_{NH}$ 7.9 Hz en $^{3}J_{NH}$ 2.7 Hz.

^{13}C NMR (50.1 MHz, $CDCl_3$): the signals shown at 49.9, 122.3, 137.8, 142.1 and 160.4 ppm appear as doublets, $^{1}J_{NC}$ 8.8 Hz, $^{1}J_{NC}$ 18.4 Hz, $^{2}J_{NC}$ 5.9 Hz, $^{1}J_{NC}$ 10.2 Hz and $^{2}J_{NC}$ 2.9 Hz, respectively.

^{15}N NMR (30.4 MHz, $CDCl_3$): 118.1 (s) ppm.

*Methyl 1-benzyl-[3-^{15}N]5-imidazolecarboxylate (**7b**)*. Compound **7b** (3.63 g, 16.59 mmol) was prepared as described for **7** from 4.25 g (16.95 mmol) of methyl 1-benzyl-2-mercapto-[3-^{15}N]5-imidazolecarboxylate (**6b**) (98% yield).

^{1}H-NMR (300 MHz, $CDCl_3$): the signals at 7.62 and 7.77 ppm were split into double doublets, $^{3}J_{NH}$ 11.0 Hz and $^{3}J_{NH}$ 10.0 Hz, respectively.

^{13}C NMR (75.5 MHz, $CDCl_3$): the signals at 122.3, 137.9 and 142.1 ppm are broadened.

^{15}N-NMR (30.4 MHz, $CDCl_3$): 257.6 (m) ppm.

*1-Benzyl-5-hydroxymethylimidazole (**8**)*. In a 100 ml three-necked flask 0.13 g (3.40 mmol) lithium aluminium hydride was suspended in 15 ml of extremely dry THF under a dry argon atmosphere. To this suspension, a solution of 0.719 g (3.33 mmol) of methyl 1-benzyl-5-imidazolecarboxylate (**7**) in 15 ml extremely dry THF was dropwise added with continuous stirring, until the methyl 1-benzyl-5-imidazolecarboxylate had disappeared (3–3.5 h, TLC: toluene/*n*-BuOH 80/20) The mixture was carefully diluted with 10 ml of water and subsequently with 10 ml of 6 M HCl. The pH was adjusted to 9 using 6 M NaOH and the aqueous layer was extracted with dichloromethane. The organic layers were combined, washed with saturated aqueous NaCl and dried on anhydrous $MgSO_4$. After filtration, the solvents were evaporated in

vacuum, yielding 0.56 g (2.98 mmol, 89% yield) of 1-benzyl-5-hydroxymethylimidazole (**8**).

^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm): 3.55 (bs, 1H, OH), 4.48 (s, 2H, $PhCH_2$), 5.22 (s, 2H, CH_2), 6.88 (s, 1H, H4), 7.12 (m, 2H, CH_{Ph}), 7.30 (m, 3H, CH_{Ph}), 7.41 (s, 1H, H2).

^{13}C NMR (75.5 MHz, $CDCl_3$, δ in ppm): 48.8 (CH_2Ph), 54.0 (CH_2OH), 127.0 (CH_{Ph}), 128.1 (CH_{Ph}), 128.4 (C4), 128.9 (CH_{Ph}), 131.4 (Cq, C5), 136.2 ($C_{Ph,q}$), 138.6 (C2).

*[1-^{15}N]1-Benzyl-5-hydroxymethylimidazole (**8a**)*. Compound **8a** (5.7 g, 30 mmol) was prepared similarly from 6.6 g (31 mmol) of methyl [1-^{15}N]1-benzyl-5-imidazolecarboxylate (**7a**) (98% yield).

^{1}H NMR (600 MHz, $CDCl_3$): the signals at 4.50, 6.97 and 7.48 ppm were split into doublets, $^{3}J_{NH}$ 1.9 Hz, $^{3}J_{NH}$ 2.7 Hz and $^{2}J_{NH}$ 7.9 Hz, respectively.

^{13}C NMR (150.9 MHz, $CDCl_3$): the signals at 48.8 and 138.9 ppm appeared as doublets, $^{1}J_{NC}$ 9.7 Hz and $^{1}J_{NC}$ 11.6 Hz, respectively.

^{15}N NMR (60.8 MHz, $CDCl_3$): 150.9 (s) ppm.

*[3-^{15}N]1-Benzyl-5-hydroxymethylimidazole (**8b**)*. Compound **8b** (3.03 g, 16.09 mmol) was prepared from 3.63 g (16.59 mmol) of methyl [3-^{15}N]1-benzyl-5-imidazole-carboxylate (**7b**) (97% yield).

^{1}H-NMR (300 MHz, $CDCl_3$): the signals at 6.87 and 7.40 ppm were split into doublets, $^{3}J_{NH}$ 9.4 Hz and $^{3}J_{NH}$ 10.8 Hz, respectively.

^{13}C NMR (75.5 MHz, $CDCl_3$): the signal at 128.3 ppm appeared as a doublet, $^{1}J_{NC}$ 13.8 Hz.

^{15}N-NMR (30.4 MHz, $CDCl_3$): 251.9 (bs).

Conclusions

The synthesis of 1-benzyl-5-hydroxymethylimidazole as described is suitable for the incorporation not only of ^{15}N-labels but also for ^{13}C at all positions and combination of positions. All steps gave high yields and did not require special purification steps. Via this new strategy, 5.7 g of [1-^{15}N]1-benzyl-5-hydroxymethyl imidazole (**8a**) with an overall yield of 53% based on $^{15}NH_4Cl$ and 2.8 g of [3-^{15}N]1-benzyl-5-hydroxymethyl imidazole with an overall yield of 90% based on $KSC^{15}N$ have been synthesized. Both **8a** and **8b** could be converted into the specifically labelled [1′-^{15}N]- and [3′-^{15}N]-L-histidines via a scheme we have

described earlier.[11] The isotopically enriched L-histidine prepared via the presented method show the required properties for optimal biological studies.

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