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# Synthesis of L-histidine specifically labelled with stable isotopes

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**Abstract.** (2′-$^{13}$C)-, (1′-$^{15}$N)- and (3′-$^{15}$N)-L-Histidine were prepared according to a synthetic scheme that allows the $^{13}$C or $^{15}$N labelling of all carbon and nitrogen positions or any combination of positions. A 1,5-disubstituted imidazole ring was constructed via condensation of tosylmethyl isocyanide with 3-phenylpropenal and subsequent cycloaddition of benzylamine. The imidazole intermediate was converted into 1-benzyl-5-(chloromethyl)-imidazolium chloride which was coupled to a glycine moiety via an enantioselective coupling with the bislactim ether of cyclo-D-valylglycine. Deprotection of the coupling product afforded L-histidine in high optical purity. Syntheses for the isotopically labelled synthons were developed starting from simple, commercially available, highly enriched compounds. The labelled L-histidines were characterized by mass spectrometry and $^{1}$H-, $^{13}$C- and $^{15}$N-NMR spectroscopy.

## Introduction

Histidine (**1**) forms a unique amino acid residue in proteins and peptides because of the special properties of the imidazole moiety in the side chain (Figure 1)[1]. Under physiological conditions both the protonated imidazolium form and the neutral imidazole forms are present in about equal amounts. In protein systems the protonated histidine residue is the strongest acid present whereas the neutral histidine is the strongest base. Histidine residues therefore account to a large extent for the buffer capacity of protein systems. The imidazole side chain is often found in the active sites of enzymes, where switching between the protonation states can induce the making or breaking of bonds as, for example, in the active sites of serine proteases. Furthermore, histidine residues play a role in hydrogen-bond interaction with prosthetic groups, and coordinate metal ions in metallo-proteins.

In the photosynthetic reaction centre (RC) of purple bacteria, such as *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides*[2–6], histidine residues play a central role in the protein–cofactor interactions with several of the chromophores. Four histidine residues, which are conserved in all bacterial RCs, act as axial ligands of the $Mg^{2+}$ ions in the centre of the four bacteriochlorophylls present in the RC[7–9]. In addition, histidine residues are involved in the coordination of the non-haem $Fe^{2+}$ and in hydrogen bonding with two quinones $Q_A$ and $Q_B$[10–12]. Both nitrogen atoms in the imidazole side-chain (N1′ and N3′) can in principle be involved in these protein–cofactor interactions, because of the tautomeric nature of the imidazole ring. From the known X-ray diffraction structures of the RCs of *Rps. viridis* and *Rb. sphaeroides*[4,5,6,11] it is not clear how the histidine residues actually interact with the cofactors, since these structures are based on rigid, crystalline RCs and the resolution is of the same order of magnitude as the interaction distances (2–3 Å). Specific $^{15}$N labelling of the individual imidazole nitrogen atoms and $^{13}$C labelling of selected positions in the histidine residues afford a tool for the identification of the interactions in spectroscopic studies. Investigation of specifically $^{15}$N- or $^{13}$C-His-enriched RCs with spectroscopic techniques such as magic-angle-spinning NMR (MAS NMR), Fourier-Transform Infrared (FT-IR), Resonance-Raman and Electron-Spin-Resonance (ESR) spectroscopy, in both the dark-adapted state and the photochemical intermediate states, will provide more insight into the role of the histidine residues in the photosynthetic process.

For these investigations specifically stable-isotope-labelled L-histidines are required. Specifically enriched L-histidines are not commercially available and until now no suitable synthesis has been described in the literature[13–19]. All syntheses concerning isotopically labelled histidine have

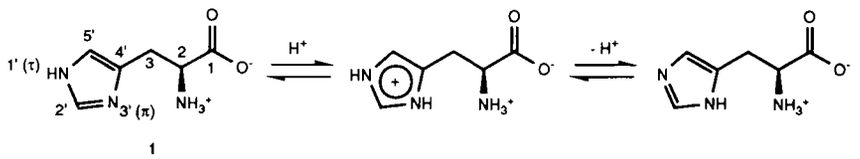


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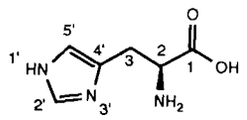
*Figure 1. Structure and numbering of* L-*histidine* (**1**) *in its neutral tautomeric and protonated forms (π: 'pros', τ: 'tele').*



**1a** 2'-$^{13}$C
**1b** 1'-$^{15}$N
**1c** 3'-$^{15}$N

*Figure 2. Synthesized isotopomers of* L-*histidine* (**1**).

dealt with the labelling of only a few atomic positions and are not suitable for the labelling of every single position and combination of positions. Moreover, most syntheses result in D,L-histidine and require optical resolution to obtain optically pure L-histidine. We therefore decided to develop a new synthesis that permits the labelling of every single carbon and nitrogen position in any combination of positions, and affords L-histidine in high optical purity.

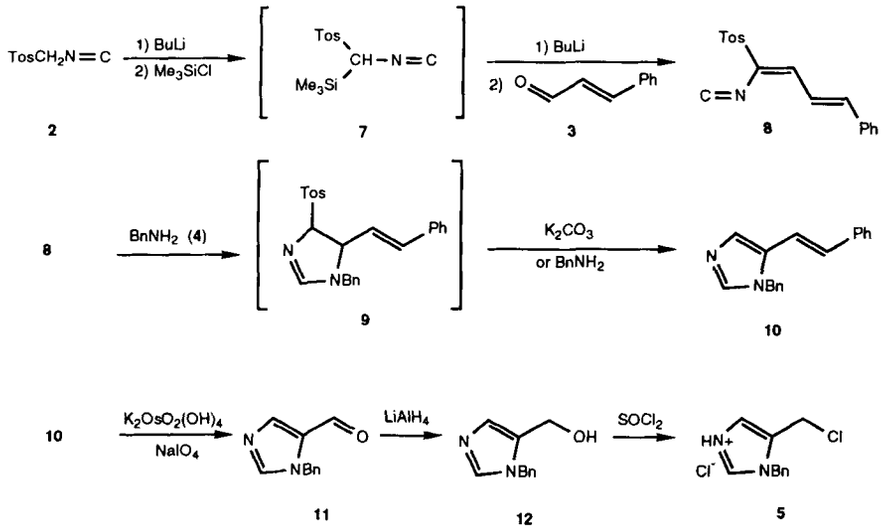
For this synthesis we needed a method for the construction of an imidazole ring with a functionalized 4(or 5)-carbon that allows the specific labelling of every carbon and nitrogen position and a method for the extension of the C4(5) functionality to an L-$\alpha$-amino acid moiety. There is a great variety in the methods available for the synthesis of imidazole rings, which differ mainly in the substitution pattern of the ring[20,21]. A general approach for the synthesis of azole rings has recently been elaborated by *Van Leusen* and coworkers and is founded on the versatile reagent tosylmethyl isocyanide (**2**, TosMIC) as central synthon[22,23]. Based on this method a 1,5-disubstituted imidazole ring can be constructed from TosMIC, an aldehyde and a primary amine[24–26]. For our purpose, the 5-substituent must be a convenient functionality for modification into a 2-amino-3-ylpropanoic acid substituent and the 1-substituent must be an easily removable imidazole-protecting group. We developed a synthesis of a 1,5-disubstituted imidazole compound that fits our requirements starting from TosMIC (**2**), (*E*)-3-phenyl-2-propenal (**3**, cinnamaldehyde) and benzylamine (**4**). For the enantioselective conversion of this intermediate 1-benzyl-5-(chloromethyl)-imidazolium chloride (**5**) into L-histidine we used the bislactim ether method of *Schöllkopf*[27]. This method has recently proved to be useful by our group in the synthesis of isotopically labelled L-lysine[28,29]. We coupled the imidazole intermediate to the optically active bislactim ether of cyclo-D-valylglycine (**6**), which afforded L-histidine after deprotection. We optimized these steps using commercially available non-enriched compounds and developed synthetic schemes for the preparation of the required synthons, TosMIC, 3-phenylpropenal and benzylamine in isotopically enriched form. Following these methods, we prepared (2'-$^{13}$C)- (**1a**), (1'-$^{15}$N)- (**1b**) and (3'-$^{15}$N)-L-histidine (**1c**) (Figure 2).

## Synthesis

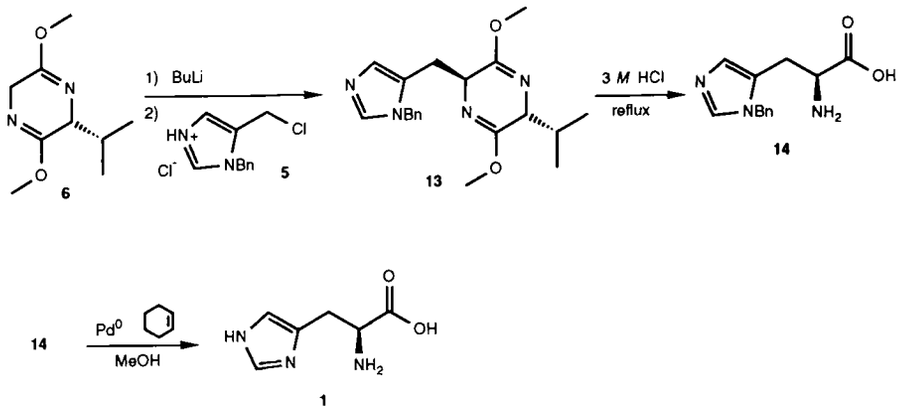
### *1,5-Disubstituted imidazole*

The construction of a 1,5-disubstituted imidazole ring is based on the dual reactivity of the TosMIC molecule, which combines an activated methylene group with a reactive isocyanide group. The key steps in this synthesis are the condensation of TosMIC (**2**) with (*E*)-3-phenyl-2-propenal (cinnamaldehyde, **3**) to form (*E*,*Z*)-1-isocyano-1-tosyl-4-phenyl-1,3-butadiene (**8**), and the subsequent cycloaddition of benzylamine (**4**) (Scheme 1). The condensation is carried out under Peterson-like conditions[26] to prevent side-reactions via an oxazolidine intermediate[25]. TosMIC is treated with butyllithium and chlorotrimethylsilane at low temperature (−80°C) to form the intermediate **7**. A second equivalent of butyllithium is added and the lithiated **7** is allowed to react with the aldehyde **3**. The formed 1-isocyano-1-tosylalkene **8** is isolated and used without purification in the next step. In this step benzylamine adds to **8** forming the intermediate imidazolidine **9**. A second equivalent of base is needed to initiate the elimination of *p*-toluenesulfinic acid (TosH) affording the imidazole ring. For this purpose an excess of potassium carbonate is used. The benzylamine–potassium-carbonate is added to a solution of **8** in methanol and stirred at room temperature for 4–5 days until all **8** has disappeared. If the benzylamine is not isotopically enriched, a second equivalent of benzylamine can be used instead of potassium carbonate. In that case the reaction time is shortened to about three days. Purification of the product using silica-gel column chromatography affords 1-benzyl-5-(2-phenylethenyl)imidazole (**10**) in 71% overall yield based on TosMIC, 3-phenylpropenal and one equivalent of benzylamine.

In order to obtain a convenient 5-substituent for side-chain manipulation, the phenylethenyl moiety is oxidatively cleaved by applying a potassium-osmate–sodium-periodate oxidation[30,31]. A solution of **10** in water/dioxane with an excess of sodium periodate and a catalytic amount




*Scheme 1. Synthetic scheme for the preparation of 1-benzyl-5-(chloromethyl)-1H-imidazolium chloride* (**5**) *from TosMIC* (**2**).



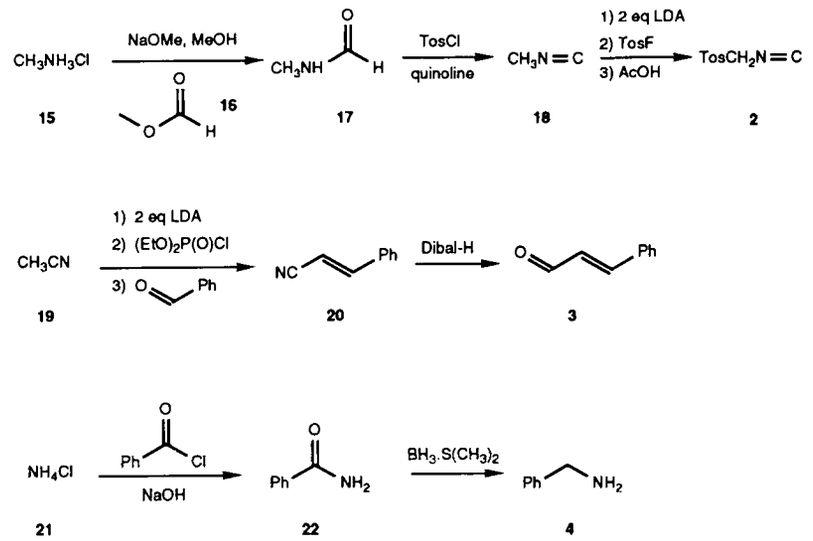

*Scheme 2. Synthesis of* L*-histidine* (**1**) *from 1-benzyl-5-(chloromethyl)-1H-imidazolium chloride* (**5**) *and bislactim ether* **6**.

of potassium osmate dihydrate is stirred for one day at 60°C. The product 1-benzylimidazole-5-carbaldehyde (**11**) is easily isolated by an extraction procedure (85% yield). The aldehyde function is converted in two steps into a chloromethyl group, which is a suitable functionality for the $S_N2$ reaction of the 1,5-disubstituted imidazole with the bislactim ether (**6**) (Scheme 2). First, the aldehyde **11** is reduced to the alcohol **12** by lithium aluminium hydride and then the alcohol is converted into the chloride **5** by treatment with thionyl chloride (both steps 99% yield). Because of the formation of hydrochloric acid in this last step the product is obtained as the 1-benzyl-5-(chloromethyl)imidazolium chloride.

### *L-Histidine*

For the enantioselective conversion of 1-benzyl-5-(chloromethyl)imidazolium chloride (**5**) into L-histidine we used the commercially available bislactim ether **6** as chiral template[27,32] (Scheme 2). This bislactim ether is an *O*-methylated cyclic dipeptide of glycine and D-valine. Deprotonation of the dihydropyrazine ring of **6** takes place selectively at the unsubstituted methylene moiety. In a nucleophilic substitution reaction of the lithiated anion of the dihydropyrazine ring, the isopropyl group on one side of the ring forces the attack to take place on the other side. In this way the configuration of the valine residue determines the chirality in the newly formed amino-acid residue. For the coupling of the (chloromethyl)imidazolium chloride **5**, two equivalents of the bislactim ether anion were needed. One equivalent of anion neutralizes the imidazolium chloride and the other equivalent is available for the $S_N2$ reaction with the chloromethyl group. The bislactim ether is treated with butyllithium in THF at low temperature (−80°C) to which the solid imidazolium chloride **5** was added. The poorly soluble hydrochloride **5** dissolves during the reaction. After **5** has completely dissolved, the mixture is allowed to warm to room temperature and after work-up the product (2*S*,5*R*)-2-[1′-benzylimidazol-5′-yl]methyl]-2,5-dihydro-3,6-dimethoxy-5-isopropylpyrazine (**13**) is purified by silica-column chromatography (84% yield). The excess of the bislactim ether **6** is entirely recovered without any racemization. Deprotection of **13** to liberate L-histidine requires the hydrolysis of the dihydropyrazine ring and the debenzylation of the imidazole ring. The hydrolysis is performed by overnight heating in 3M hydrochloric acid. The product mixture of L-3-benzylhistidine **14** and D-valine is subsequently treated with cyclohexene and palladium-black as hydrogenation catalyst in refluxing methanol, to deprotect the imidazole group of **14**[33]. Finally, the unprotected L-histidine (**1**) is separated from D-valine and purified by ion-exchange chromatography affording L-histidine in 70% yield based on the intermediate **5**.

The optical purity of histidine was determined by HPLC. The histidine is first derivatized into its diastereoisomers by *o*-phthalaldehyde and *N*-acetyl-L-cysteine[34] (OPA-NAC) and then analyzed by reversed-phase HPLC. From




*Scheme 3. Synthetic schemes for the preparation of isotopically enriched TosMIC* (**2**), *(E)-3-phenylpropenal* (**3**) *and benzylamine* (**4**).

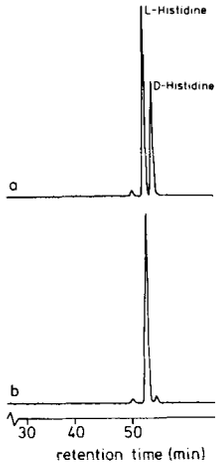



*Figure 3. Optical-purity determination of histidine* (**1**) *by HPLC after derivatization with OPA-NAC; a. 70:30 mixture of* L *and* D*-histidine; b. synthesized* L*-histidine (optical purity 96%). Conditions: C18 RP (Spherisorb ODS-2 5 μm) column (250 × 4 mm); buffer A: 30 mM sodium acetate pH 4, buffer B: 50% 30 mM sodium acetate pH 7.6 / 50% acetonitrile; linear elution gradient of 100% A to 80% A / 20% B in 60 min; flow rate 0.4 ml / min.*

the chromatograms depicted in Figure 3, the optical purity was determined to be 96%.

*Isotopically labelled synthons for* L*-histidine*

For the preparation of L-histidine, isotopically labelled at any possible carbon or nitrogen position, the availability of the building blocks in specifically enriched form is required. Since these compounds are not commercially available we developed syntheses for TosMIC (**2**), 3-phenylpropenal (**3**) and benzylamine (**4**) starting from simple, commercially available, highly enriched starting materials.

*Isotopically labelled TosMIC* (**2**)

The positions C5′, N1′ and C2′ in L-histidine originate from the methyl isocyanide moiety of TosMIC (**2**). Labelling of TosMIC is accomplished starting from the commercially available ($^{13}$C)- or ($^{15}$N)-methylammonium chloride (**15**) and methyl ($^{13}$C)-formate (**16**, Scheme 3). These starting materials are first converted into *N*-methylformamide (**17**), followed by dehydration to methyl isocyanide (**18**) and subsequent tosylylation to TosMIC. In the first step, methylammonium chloride is added to a methanolic solution of sodium methoxide and methyl formate. The methoxide neutralizes the methylammonium chloride and the free methylamine reacts with methyl formate to form *N*-methylformamide. Sodium methoxide is used in light excess to maintain slightly basic conditions, which catalyze the amidation reaction. To isolate the rather volatile *N*-methylformamide, the precipitated sodium chloride is removed by filtration and the methanol is carefully evaporated (98% yield). *N*-Methylformamide is dehydrated to form methyl isocyanide by treatment with tosyl chloride in quinoline[35]. The formed methyl isocyanide is distilled directly from the reaction mixture under vacuum (75°C, 10 mmHg) resulting in a 72% yield. To prevent polymerization upon storage and to minimize the handling of this highly toxic and smelly isocyanide, the product is immediately used in the next step. Sulfonylation of methyl isocyanide is effected by the reaction of lithiated methyl isocyanide with tosyl fluoride. For this reaction two equivalents of lithium diisopropylamide (LDA) are used at low temperature (−80°C). The first equivalent deprotonates methyl isocyanide which subsequently reacts with tosyl fluoride and the second equivalent traps the formed TosMIC into its anion. This prevents protonation of unreacted isocyanide anions by the formed TosMIC, which is more acidic than methyl isocyanide. Consequently, the use of two equivalents of LDA allows the complete reaction of the methyl isocyanide. The anion of TosMIC is subsequently quenched with acetic acid and TosMIC is isolated by silica chromatography (68% yield). The overall yield based on the labelled starting material is 49%.

*Isotopically labelled (E)-3-phenyl-2-propenal* (**3**)

For the labelling of the positions C4′ and C3 in L-histidine, 3-phenylpropenal (**3**) labelled at the C1 and C2 carbon must be synthesized. This can be achieved starting from specifically enriched, commercially available acetonitrile (**19**, Scheme 3). Acetonitrile is coupled to benzaldehyde by means of a Horner–Wadsworth–Emmons reaction. Following this procedure, acetonitrile is treated with two equivalents of lithium diisopropylamide (LDA) and one equivalent of diethyl chlorophosphate in THF at low temperature (−80°C). The first equivalent of LDA deprotonates acetonitrile, which then reacts with diethyl chlorophosphate to form diethyl (cyanomethyl)phosphonate[36]. This phosphonate is deprotonated by the second equivalent of LDA and reacts with the added benzaldehyde to give (E)-3-phenyl-2-propenenitrile (**20**, 65% yield). This nitrile is converted into the aldehyde **3** by a selective reduction with diisobutylaluminium hydride (DibalH) at low temperature (−20°C) in THF. In this way 3-phenylpropenal is obtained in 78% yield based on **20** and 51% based on acetonitrile.

*Isotopically labelled benzylamine* (**4**)

The 3′-nitrogen in L-histidine is derived from the nitrogen of benzylamine. For the specific labelling of this position ($^{15}$N)-benzylamine is prepared in two steps from the relatively inexpensive ($^{15}$N)-ammonium chloride (**21**, Scheme 3). First, the ammonium chloride is converted into benzamide (**22**) by treatment with benzoyl chloride in 2M NaOH (85% yield)[37]. Secondly, reduction of the amide using borane–dimethyl-sulfide complex as reducing agent gives benzylamine[38]. This reduction affords benzylamine in a 92% yield from benzamide and 78% based on ammonium chloride.

## Characterization

*Mass spectrometry*

Mass spectrometry is used to determine the exact molecular mass and the isotope enrichment of the labelled histidines (**1a–c**). Because histidine has a relatively low volatility for mass spectrometric analysis it is first converted into the more volatile *n*-butyl ester[39]. The exact molecular masses are determined using double-focus chemical-ionization mass spectrometry (CI-MS, $CH_4$), since the molecular ion has a relative low abundance in electron-impact (EI) mass spectra. The isotope compositions of the synthesized isotopomers of L-histidine are determined from the measured molecular masses. The molecular ion peak [$(M + H)^+$] in the spectrum of (2′-$^{13}$C)-L-histidine (**1a**) is observed at $m/z$ 213.1445, which corresponds to $^{12}C_9{}^{13}C^1H_{18}{}^{14}N_3{}^{16}O_2$ (calculated mass: 213.1433). In the spectrum of (1′-$^{15}$N)- (**1b**) and (3′-$^{15}$N)-L-histidine (**1c**), this peak appears at $m/z$ 213.1404 and 213.1366, respectively, which is in agreement with $^{12}C_{10}{}^1H_{18}{}^{14}N_2{}^{15}N^{16}O_2$ (calculated mass: 213.1369).

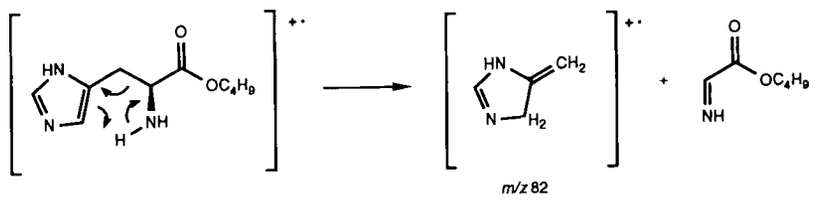



*Figure 4. Rearrangement of L-histidine n-butyl ester during mass spectral fragmentation.*

The single-focus EI (70 eV) mass spectrum of natural abundance L-histidine *n*-butyl ester displays prominent peaks at *m/z* 212 $[(M+H)^+]$ and 110 $[(M+H)^+ - (CO_2C_4H_9)^\cdot]$ and the base peak at 82 $[(M+H)^+ - HN=CHCO_2C_4H_9]$. In the spectra of the three isotopomers of L-histidine (**1a–c**) these peaks are observed at *m/z* 213, 111 and 83, respectively, which is in accordance with the presence of the isotope labels in the imidazole ring. The fragment at *m/z* 82 and 83 is formed by a McLafferty rearrangement involving a proton migration from the amine nitrogen to the imidazole ring (Figure 4)[39]. From these base peaks, and the related M − 1, M + 1 and M + 2 peaks, the isotope enrichments are calculated and corrected for the natural abundance isotope ratios. The $^{13}$C-labelled **1a** has a 99% enrichment, which is the same as the enrichment of the starting material methyl (99% $^{13}$C)-formate. The enrichment of the $^{15}$N-labelled compounds **1b** and **1c** is 98%, which is also the same enrichment as their respective starting materials (98% $^{15}$N)-methylammonium chloride and (98% $^{15}$N)-ammonium chloride. This high enrichment proves that no dilution of the labelled material occurs during the synthetic steps.

### *$^1$H-NMR Spectroscopy*

The 300-MHz $^1$H-NMR spectra of the isotopically enriched L-histidines (**1a–c**) and of natural-abundance L-histidine are presented in Figure 5. The spectra are recorded from the compounds in the protonated form in $D_2O$ at pD 1. The presence of the $^{13}$C isotope at C2′ in **1a** is easily observed by the large splitting of the H2′ signal ($^1J_{CH}$). A small additional splitting is observed in the signal of H5′ ($^3J_{CH}$). No signal of natural-abundance histidine is observed in the spectrum of **1a**, indicating a high degree of enrichment (99%). The presence of the $^{15}$N isotopes in **1b** and **1c** is confirmed by the $^{15}$N–$^1$H couplings in the signals of H2′ and H5′. Next to the geminal and vicinal $^1$H–$^1$H couplings, the spectra of histidine show long-range $^1$H–$^1$H couplings between H2′ and H5′ and between H5′ and the H3 protons. The observed homonuclear and heteronuclear coupling constants and the chemical shifts are determined from these spectra and listed in Table I.

### *$^{13}$C-NMR Spectroscopy*

In Figure 6 the $^1$H-noise-decoupled 50.1-MHz $^{13}$C-NMR spectra of L-histidine (**1**) and the isotopomers (2′-$^{13}$C)-, (1′-$^{15}$N)- and (3′-$^{15}$N)-L-histidine (**1a-c**) are shown. The spectra are recorded in $D_2O$ under conditions of full protonation (pD 1). The signal of the $^{13}$C label in **1a** is clearly observed at the expected position. The intensity of this signal is in agreement with a high degree of enrichment and no scrambling of the label is observed. The spectra of the $^{15}$N-enriched L-histidines show $^{15}$N–$^{13}$C couplings in the signals of the neighbouring carbons. The signals of C2′ and C5′ in the spectrum of the 1′-$^{15}$N-labelled compound are split, with 16.2 Hz ($^1J_{N1C2'}$) and 11.1 Hz ($^1J_{N1C5'}$), respectively. The signals of C2′ and C4′ in the spectrum of the 3′-$^{15}$N-labelled compound are split, with 16.4 Hz ($^1J_{N3'C2'}$) and 11.0 Hz ($^1J_{N3C4'}$), respectively. Since no other $^{15}$N-$^{13}$C couplings are observed, the signals of the aromatic carbons can unambiguously be assigned based on these data.

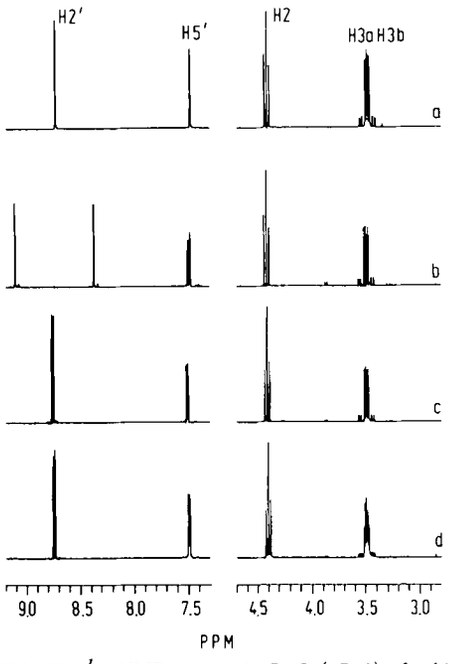



*Figure 5. 300-MHz $^1$H-NMR spectra in $D_2O$ (pD 1) of L-histidine (**1**, a) and (2′-$^{13}$C)- (**1a**, b), (1′-$^{15}$N)- (**1b**, c) and (3′-$^{15}$N)-L-histidine (**1c**, d).*

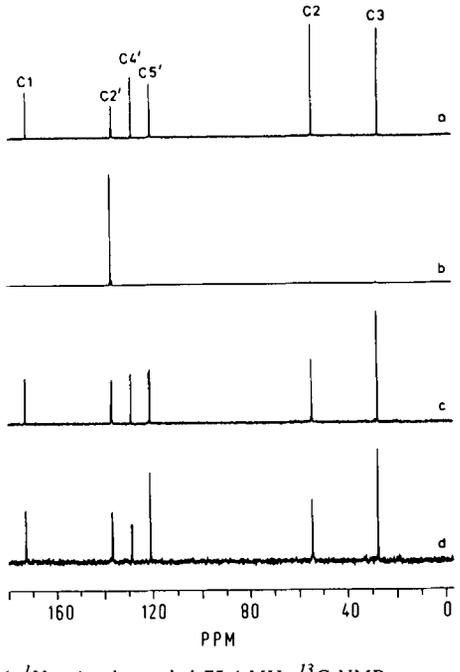



*Figure 6. $^1$H-noise-decoupled 75.4-MHz $^{13}$C-NMR spectra in $D_2O$ (pD 1) of L-histidine (**1**, a), (2′-$^{13}$C)- (**1a**, b), (1′-$^{15}$N)- (**1b**, c) and (3′-$^{15}$N)-L-histidine (**1c**, d).*

*Table I $^1H$ NMR Chemical shifts and $^1H-^1H$, $^{13}C-^1H$ and $^{15}N-^1H$ coupling constants of L-histidine (1) in $D_2O$ (pD 1).*

| δ (ppm) | | $J(^1H-^1H)$ (Hz) | | $J(^{13}C-^1H)$ (Hz) | | $J(^{15}N-^1H)$ (Hz) | |
|---|---|---|---|---|---|---|---|
| H2 | 4.42 | H3a–H3b | 15.9 | C2′–H2′ | 222 | N1′–H2′ | 5.3 |
| H3a | 3.52 | | | C2′–H5′ | 6.7 | N1′–H5′ | 4.1 |
| H3b | 3.46 | H2–H3a | 6.4 | | | | |
| H2′ | 8.74 | H2–H3b | 6.8 | | | N3′–H2′ | 5.5 |
| H5′ | 7.49 | | | | | N3′–H5′ | 4.0 |
| | | H3b–H5′ | 0.9 | | | N3′–H3 | 2.8 |
| | | H3a–H5′ | 0.8 | | | | |
| | | H2′–H5′ | 1.4 | | | | |

### *$^{15}N$ NMR Spectroscopy*

The $^1H$-noise-decoupled 30.4-MHz $^{15}N$-NMR spectra of the $^{15}N$-enriched histidines are recorded under conditions of full protonation in $D_2O$ (pD 1). The spectrum of (1′-$^{15}$N)-L-histidine (**1b**) shows a peak at −207.6 ppm relative to neat nitromethane. The spectrum of (3′-$^{15}$N)-L-histidine (**1c**) shows a peak at −205.1 ppm. These values agree with the assignment in the literature[40,41].

## Discussion

An enantioselective synthesis has been developed for L-histidine specifically labelled with stable isotopes. The synthetic scheme for the imidazole side-chain starts from the simple compounds ammonium chloride, methyl formate, methylammonium chloride and acetonitrile, which are commercially available in all isotopically enriched forms. The amino acid moiety of L-histidine originates from the glycine residue in the bislactim ether of D-valinylglycine (**6**), which we used in our synthesis. This bislactim ether can be synthesized from D-valine and glycine in a 72% overall yield based on glycine[29,42]. Since glycine is also commercially available in all isotopically enriched forms, it is now possible to enrich any carbon and nitrogen position specifically or any combination of positions, using this synthesis.

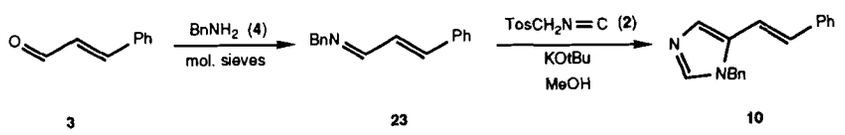
The synthesis has been performed on a gram scale and L-histidine obtained in good overall yields and high optical purity. This synthesis is demonstrated by the preparation of (2′-$^{13}$C)- (**1a**), (1′-$^{15}$N)- (**1b**) and (3′-$^{15}$N)-L-histidine (**1c**) via the labelled intermediates (1-$^{13}$C)-TosMIC (**2a**), ($^{15}$N)-TosMIC (**2b**) and ($^{15}$N)-benzylamine (**4c**), respectively. Based on spectroscopic characterization it is ascertained that these compounds are specifically enriched at the predesigned positions and that no scrambling of the labelled material has taken place during the synthetic steps. The $^{13}C$ incorporation in **1a** is 99% and the $^{15}N$ incorporation in the $^{15}N$-enriched compounds **1b** and **1c** is determined as 98%. These values are identical to the specifications of the commercial starting compounds and thus establish that no dilution of the labelled material has taken place during the synthesis.

The synthesis of the 1,5-disubstituted imidazole intermediate **10** is based on the azole formation reaction with TosMIC (**2**) as central synthon (Scheme 1). For the synthesis of imidazoles from TosMIC two approaches are available. The first, which we applied in our synthesis, is the condensation of TosMIC with an aldehyde via a Petersen olefination procedure and the subsequent reaction of the formed 1-isocyano-1-tosylalkene with a primary amine affording the 1,5-disubstituted imidazole[26] (as in Scheme 1). An alternative approach yielding the same 1,5-disubstituted imidazole, follows a 1,3-dipolar cycloaddition of TosMIC to the polarized double bond of an aldimine[25]. First, an aldehyde is condensed with a primary amine forming an aldimine, which is then reacted with the anion of TosMIC to give the 1,5-difunctionalized imidazole (as in Scheme 4). We explored both approaches for the synthesis of an imidazole intermediate that satisfied the requirements for the synthesis of isotopically enriched L-histidine.

Both methods require an aldehyde and a primary amine as starting compounds. The aldehyde determines the nature of the 5-substituent and the amine residue forms the 1-substituent. For our purpose it was important to find an aldehyde that leads to a 5-substituent that is convenient for side-chain manipulation and an amine which will end up as a removable protecting group on the 1-nitrogen. In addition, in the first approach the residue of the aldehyde must have a stabilizing effect on the 1-isocyano-1-tosylalkene intermediate and in the second approach the aldehyde and the amine must form a stable aldimine. Starting from these requirements we chose 3-phenylpropenal (**3**) and benzylamine (**4**) as building blocks, resulting in 1-benzyl-5-(2-phenylethenyl)imidazole (**10**) as the 1,5-disubstituted imidazole intermediate. The benzyl group is a well-known and easy to remove imidazole-protecting group and the phenylethenyl group can be oxidized to a carbaldehyde substituent which is a versatile group for further manipulations. Furthermore, both the 1-isocyano-1-tosylalkene intermediate and the aldimine are sufficiently stable under the reaction conditions.

The first approach, as described above (Scheme 1), results in an efficient preparation of **10** in 70% overall yield based on TosMIC on a scale up to 20 mmol (5 g). The second approach, via the aldimine intermediate (Scheme 4), results in comparable yields on a small scale (up to 5 mmol)[43]. This alternative method, however, differs considerably in reproducibility and yield after upscaling. The yield drops notably to 20 to 40% when the synthesis is carried out on a larger scale. This decline is probably due to the low stability of the TosMIC anion under the reaction conditions and the longer reaction times necessary on a larger scale. The first method, via the 1-isocyano-1-tosylalkene intermediates, does not suffer from the low stability of TosMIC anions because these are generated and converted in fast reactions at low temperature (−80°C).

The first step in the extension of the 5-substituent of the 1,5-disubstituted imidazole intermediate **10** into a L-α-amino-acid moiety, is the oxidative cleavage of the phenylethenyl substituent. For this purpose we applied a potassium-osmate–sodium-periodate oxidation. The osmate oxidizes the ethenyl double bond to form a vicinal diol which is oxidatively cleaved by the action of the periodate. This method selectively cleaves the ethenyl double bond without interference with the aromatic imidazole and phenyl rings. In our examination of this step, we first employed osmium tetraoxide ($OsO_4$) as a reagent for the hydroxylation of the double bond. We then substi-




3 23 10

*Scheme 4. Synthesis of 1-benzyl-(2-phenylethenyl)imidazole (**10**) from TosMIC (**2**) via the aldimine **23**.*

tuted the osmium tetraoxide by potassium osmate dihydrate [$K_2OsO_2(OH)_4$], which was reported to be equally effective as an hydroxylating reagent[31]. Both reagents afforded the same, high yield of 1-benzylimidazole-5-carbaldehyde (**11**) and could be applied in catalytic amounts. Both reagents are highly toxic, but the osmate is not volatile, in contrast to osmium tetraoxide. For this reason the osmate is much easier and safer to handle and therefore preferred.

For the labelling of the imidazole ring, we developed a method for the specific $^{13}C$ and $^{15}N$ enrichment of TosMIC. Next to imidazoles, TosMIC is a versatile reagent for the preparation of other heteroaromatic compounds, such as oxazoles and pyrroles[23]. Isotopically labelled TosMIC is, therefore, a valuable building block for the synthesis of these heteroaromatic compounds in a specifically enriched form. For the synthesis of TosMIC two pathways are described in the literature. The first method starts with a Mannich condensation of formaldehyde, formamide and *p*-toluenesulfonite resulting in *N*-tosylmethylformamide, which provides TosMIC after dehydration[44]. This method is not practical for isotopic labelling because of the large excess of formaldehyde and formamide that is used in the first step. The second method is based on the dehydration of *N*-methylformamide into methyl isocyanide[35] and subsequent sulfonylation[45]. This method has no obvious limitations and we therefore optimized and extended this method for the purpose of isotopic labelling.

This preparation of TosMIC starts with *N*-methylformamide which is not commercially available in an isotopically enriched form. We therefore had to extend the method by preparing of *N*-methylformamide from commercially available enriched synthons. A literature procedure describing the pyrolysis of methylammonium chloride with sodium formate gave very poor yields in our hands[46]. We reached a much better yield (98%) with the amidation of methyl formate with methylamine as described above (Scheme 3). The *N*-methylformamide (**17**) obtained was subsequently converted into methyl isocyanide (**18**) according to a large-scale procedure described in the literature[35]. The last step in the synthesis of TosMIC, the sulfonylation of methyl isocyanide, also had to be adapted. The synthesis described in the literature requires two equivalents of lithiated methyl isocyanide relative to tosyl fluoride, because the more acidic TosMIC protonates unreacted lithiated methyl isocyanide. In our situation, this would result in the loss of half of the labelled material. We therefore changed the procedure by application of the non-nucleophilic base LDA instead of butyllithium. The use of two equivalents of LDA allows the use of one equivalent of methyl isocyanide, because the formed TosMIC is now trapped into its anion by the second equivalent of LDA. This anion of TosMIC is required in the next step of the synthetic sequence, the preparation of the 1-isocyano-1-tosylalkene (**8**) (Scheme 1). The direct application of the anion obtained in this synthesis of TosMIC in the next step is, however, not practicable, because of a cumulation of impurities. We therefore quenched the anion of TosMIC with acetic acid and isolated the freshly synthesized TosMIC. The development and optimization of these steps has resulted in a convenient three-step synthesis of specifically enriched TosMIC. This preparation may prove to be very useful, given the broad scope of this reagent[22,23].

In addition to L-histidine, the presented synthetic schemes are also applicable to the synthesis of other isotopically labelled compounds. The bislactim-ether method, which we used for the enantioselective introduction of the $\alpha$-amino acid moiety into L-histidine, can also be used for the synthesis of D-histidine. Replacement of the 2*R*-bislactim ether (**6**) by the 2*S* variant allows the preparation of the optical antipode in the same high optical purity. Furthermore, the described synthesis offers the possibility of the preparation of selectively 3'-protected histidines. In the present synthesis, the L-histidine obtained (**14**) is specifically protected with a benzyl group at the 3'-imidazole nitrogen. This selective protection cannot be accomplished by straightforward protection of histidine, because this normally results in a mixture of 1'- and 3'-protected histidine.

The 1,5-difunctionalized imidazole intermediate is, as far as we know, the first imidazole compound that can be specifically $^{13}C$- and $^{15}N$-enriched in all possible (combinations of) positions. This intermediate can be used as a basis for the preparation of other imidazole-containing (bio)molecules, such as the biosynthetically related histamine (1*H*-imidazole-4-ethanamine) and urocanic acid [3-(1*H*-imidazol-4-yl)-2-propenoic acid][47]. In addition to imidazole compounds, the availability of specifically enriched TosMIC offers new possibilities for the labelling of a whole range of other compounds.

In conclusion, we have developed an enantioselective total synthesis of L- and D-histidine from simple starting compounds that are commercially available in highly isotopically enriched form. Following this synthesis, we have prepared three specifically enriched L-histidines. These compounds are prepared without any scrambling or dilution of the labelled material and are thus obtained with a very high isotope enrichment (> 98%). Using these synthetic schemes, optically pure histidines specifically $^{13}C$- or $^{15}N$-enriched in any combination of positions can be made available on a gram scale. Incorporation of these specifically enriched histidines into proteins, such as the photosynthetic RC, followed by spectroscopic studies of these labelled proteins, will provide information on the role of the histidine residues in these protein systems.

## Experimental

### *General*

All chemicals were purchased from Janssen Chimica or Aldrich, except for cyclo-D-valylglycine [(2*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine] which was obtained from Merck. Methyl (99% $^{13}C$)-formate, (98% $^{15}N$)-methylammonium chloride were obtained from Cambridge Isotope Laboratories and (98% $^{15}N$)-ammonium chloride was obtained from ICON Services Inc.

In all experiments, distilled anhydrous solvents were used. Tetrahydrofuran (THF) was freshly distilled prior to use from lithium aluminium hydride. Quinoline was distilled from zinc dust under reduced pressure. Ether refers to diethyl ether and petroleum ether refers to low-boiling petroleum ether 40–60°C. All solvent mixtures are given in volume ratios (v/v). All solvents were removed by evaporation *in vacuo*.

AG 50W X8 ($H^+$ form, 200–400 mesh) ion-exchange resin was obtained from BioRad. Silica (Silica gel 60, 0.040–0.063 mm, 230–400 mesh ASTM) was obtained from Merck. The reactions were monitored by TLC (Merck $F_{254}$ silica-gel-60 sheets, 0.2 mm). Spots were visualized with an UV lamp, iodine vapour, ninhydrin (0.2% in ethanol) for amino acid detection, 3M hydrochloric acid followed by ninhydrin for bislactim-ether detection.

$^1H$-NMR spectra were recorded using a Jeol NM FX-200, a Bruker WM-300 or a Bruker MSL-400, with tetramethylsilane (TMS: $\delta$ 0.00 ppm) as internal standard for spectra recorded in $CDCl_3$, $CD_3OD$ and $CD_3S(O)CD_3$, and 3-(trimethylsilyl)-pentadeuteropropionic acid (TSP: $\delta$ 0.00 ppm) for spectra recorded in $D_2O$. $^{13}C$-NMR spectra were recorded using a Jeol NM FX-200 at 50.1 MHz, a Bruker WM-300 at 75.4 MHz or a Bruker MSL-400 at 100.3 MHz with chloroform ($CDCl_3$: $\delta$ 77.0 ppm), methanol ($CD_3OD$: $\delta$ 49.0 ppm) or tetramethylsilane (TMS: $\delta$ 0.00 ppm) as internal standard. $^{15}N$-NMR spectra were recorded using a Bruker WM-300 at 30.4 MHz with a saturated aqueous solution of ammonium nitrate ($^{15}NH_4NO_3$: $\delta$ −359.6 ppm) as external standard. The $^{15}N$-NMR chemical shifts are given relative to neat nitromethane ($\delta$ 0.0 ppm). All chemical shifts are given in ppm.

The optical purities of the histidines were determined by HPLC and are given as molar fractions (%). The apparatus consisted of a Spherisorb ODS-2 (5 $\mu$m) column (250×4 mm), a Pharmacia LKB gradient delivery system, a Rheodyne injection valve fitted with a 5-$\mu$l loop, and a SpectroVision FD-300 fluorescence detector. Prior to chromatography the histidines were derivatized using *o*-phthalaldehyde (OPA, Janssen) and *N*-acetyl-L-cysteine (NAC, Aldrich)[36]. The excitation and emission wavelengths were 360 and 405 nm, respectively. Derivatization was carried out using a solution of 2 mg of histidine in $H_2O$ (I), a 0.2M Borax buffer (pH 10.4) (II), a solution of 10 mg of OPA in 5 ml of methanol (III) and a solution of 10 mg of NAC in 5 ml of methanol (IV). 10 $\mu$l of I, 20 $\mu$l of II, 10 $\mu$l of III and 10 $\mu$l of IV were added together. The mixture was shaken for 2 min and injected. The diastereomers were separated employing a linear elution gradient of 100% buffer A2 (30mM sodium acetate pH 4) to 80% buffer A2 and 20% buffer B2 (50% 30mM sodium acetate pH 7.6/50% acetonitrile) in 60 min (0.4 ml/min). The retention time was 50 min.

Mass spectra were recorded on a Finnigan MAT-90 mass spectrometer (CI-MS, $CH_4$) or on a Finnigan MAT ITD 700 mass spectrometer (EI, 70 eV) coupled to a Hewlett Packard 438A gas chromatograph equipped with a Hewlett Packard 25-m SE 30 capillary column (GC-MS). Prior to EI-MS and CI-MS the amino acids were derivatized to their *n*-butyl esters. The sample was treated with 3M HCl in *n*-BuOH for 15 min at 100°C under an anhydrous nitrogen atmosphere in a sealed Kimax tube. The solvent was evaporated and the sample was injected as a solution in dichloromethane.

The procedures used for the preparation of the isotopically enriched compounds were the same as those described for the corresponding unlabelled compounds. Only spectroscopic characteristics of the enriched compounds that differ from those of the unlabelled compounds are given.

N-*Methylformamide* (**17**)

Sodium metal (1.38 g, 60 mmol) was dissolved in 100 ml of anhydrous methanol under gentle reflux. To this solution of sodium methoxide, first 3.7 ml (60 mmol) of methyl formate (**16**) was added and then 4.00 g (59 mmol) of methylammonium chloride (**15**) under cooling with an ice bath. The solution was stirred overnight at room temperature. The suspension was filtered over Celite and the filtrate was carefully concentrated *in vacuo* (30°C, 20 mmHg). The residue was taken up in dichloromethane and the precipitated salts were removed by filtration. The filtrate was dried on $MgSO_4$, filtered again and the solvent was carefully evaporated *in vacuo*. This resulted in 3.49 g of **17** (liquid, 99% yield). $^1$H NMR (200 MHz, $CDCl_3$): d 2.82 (d, 3H, $^3J_{HH}$ 5 Hz, $CH_3$), 7.5 (bs, 1H, NH), 8.14 (s, 1H, CHO) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CDCl_3$): d 23.9 ($CH_3$), 161.9 (CO) ppm.

(*1-$^{13}$C*)-**17** (**17a**). Compound **17a** (1.93 g) was prepared from 2.00 g of methyl ($^{13}$C)-formate and a small excess of methylammonium chloride (1.05 eq) in 98% yield. $^1$H NMR: signal at 2.82 was split into a double doublet, $^3J_{CH}$ 3.1 Hz, and the signal at 8.14 ppm was split into a doublet, $^1J_{CH}$ 91.2 Hz. $^{13}$C NMR: strong signal at 161.9 ppm.

(*$^{15}$N*)-**17** (**17b**). Compound **17b** (3.50 g) was prepared from 4.00 g of ($^{15}$N)-methylammonium chloride (99% yield). $^1$H NMR: signals at 8.14 and 7.5 ppm were split into doublets, $^2J_{NH}$ 15.4 Hz and $^1J_{NH}$ 93.3 Hz, respectively. $^{13}$C NMR: signals at 23.9 and 161.9 ppm were split into doublets, $^1J_{C3N}$ 9.8 Hz and $^1J_{C1N}$ 13.2 Hz, respectively.

*Methyl isocyanide*[35] (**18**)

A 50-ml three-necked flask was equipped with a 25-ml pressure-equalizing dropping funnel, a special liquid-nitrogen-cooled vacuum trap and a stopper. The dropping funnel was connected to a nitrogen inlet. The outlet was successively connected to an empty wash bottle, a bottle filled with 6M HCl, a second empty bottle, a three-way valve and a water aspirator (vacuum line). Under a dry nitrogen atmosphere, 14.3 g (75 mmol) of oven-dried tosyl chloride was added to the flask, followed by 20 ml of quinoline. *N*-Methylformamide (**17**, 2.95 g, 50 mmol) was dissolved in 10 ml of quinoline and loaded into the dropping funnel. The flask was heated to 75°C, the nitrogen inlet was closed and the three-way valve was switched to the vacuum line. The solution of **17** was slowly added to the heated and vigorously stirred mixture over a period of $\frac{1}{2}$ h, resulting in violent bubbling. After bubbling had ceased the product had been collected in the trap. The temperature was slightly increased to 85–90°C and the mixture was left under vacuum for another $\frac{1}{2}$ h. The nitrogen inlet was carefully opened until atmospheric pressure was reached and then the three-way valve was switched away from the vacuum line. The trap was allowed to warm under a gentle stream of nitrogen. As soon as the methyl isocyanide had melted, the trap containing the smelly product was removed, capped with a stopper and refrozen until it was needed for the next step. In this way 1.58 g of **18** was obtained (liquid, 77% yield). $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 3.14 (t, 3H, $^1J14_{NH}$ 2.3 Hz, $CH_3$) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CDCl_3$): d 26.5 [t, $^1J(^{14}NC)$ 14.7 Hz, $CH_3$], 156.0 [t, $^1J(^{14}NC)$ 11.7 Hz, CN] ppm.

(*1-$^{13}$C*)-**18** (**18a**). Compound **18a** (1.15 g) was prepared from 1.93 g of **17a** (83% yield). $^1$H NMR: signal at 3.14 ppm was split into a double triplet, $^3J_{CH}$ 2.6 Hz. $^{13}$C NMR: strong triplet at 156.0 ppm.

(*$^{15}$N*)-**18** (**18b**). Compound **18b** (1.64 g) was prepared from 3.50 g of **18b** (67% yield). $^1$H NMR: signal at 3.14 ppm was split into a doublet, $^2J(^{15}NH)$ 3.1 Hz.

p-*Toluenesulfonylmethyl isocyanide (TosMIC)* (**2**)

Under a dry-nitrogen atmosphere, 4.0 ml (28 mmol) of diisopropylamine was dissolved in 100 ml of anhydrous THF and cooled to −40°C with an ethanol/liquid-nitrogen bath. A 1.6M solution of butyllithium in hexanes (16.3 ml, 26 mmol) was added via a syringe. The mixture was stirred for 15 min and cooled to −80°C. Methyl isocyanide (**18**, 0.54 g, 13 mmol) was dissolved in THF (10 ml) and slowly added to the solution of lithium diisopropylamide (LDA). The mixture was stirred for 15 min and a solution of 2.26 g (13 mmol) of oven-dried tosyl fluoride in THF (20 ml) was added, so that the temperature did not exceed −65°C. The mixture was kept cold for 1 h and allowed to warm to −50°C. Glacial acetic acid (0.74 ml, 13 mmol) was slowly added. When the temperature had risen to 0°C, 100 ml of saturated aqueous $NH_4Cl$ was added. The organic phase was separated and washed with saturated aqueous NaCl. The aqueous $NH_4Cl$ layer was extracted with dichloromethane (2×150 ml). The combined organic layers were dried on $MgSO_4$, filtered and concentrated. Both organic fractions were combined and the product was purified by silica column chromatography (eluent: dichloromethane). This resulted in 1.72 g of **2** (solid, 68% yield). $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 2.50 (s, 3H, $CH_3$), 4.57 (s, 2H, $CH_2$), 7.45 (d, 2H, $^3J_{HH}$ 8 Hz, $CH_{Ph}$), 7.90 (d, 2H, $^3J_{HH}$ 8 Hz, $CH_{Ph}$) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CDCl_3$): $\delta$ 21.0 ($CH_3$), 60.5 ($CH_2$), 128.7, 129.6 and 131.7 ($C_{Ph}$), 146.1 (CN) ppm.

(*1-$^{13}$C*)-**2** (**2a**). Compound **2a** (2.71 g) was prepared from 1.15 g of **18a** (51% yield). $^1$H NMR: signal at 4.57 ppm was split into a doublet, $^3J_{CH}$ 2.7 Hz. $^{13}$C NMR: strong signal at 146.1 ppm, signal at 60.5 ppm was split with 45 Hz ($^2J_{CC}$).

(*$^{15}$N*)-**2** (**2b**). Compound **2b** (4.67 g) was prepared from 1.64 g of **18b** (61% yield). $^1$H NMR: signal at 4.57 ppm was split into a doublet, $^2J(^{15}NH)$ 2.4 Hz. $^{13}$C NMR: signal at 21.0 and 60.5 ppm were split into doublets, $^1J(^{15}NC)$ 10 and 12 Hz, respectively.

(E)-*3-Phenyl-2-propenenitrile* (**20**)

Under a dry-nitrogen atmosphere, 7.0 ml (50 mmol) of diisopropylamine was dissolved in 100 ml of anhydrous THF and cooled to −40°C with an ethanol/liquid-nitrogen bath. A 1.6M solution of butyllithium in hexanes (31 ml, 50 mmol) was added via a syringe and the mixture was stirred for 15 min. This solution of lithium diisopropylamide (LDA) was cooled to −80°C and 1.27 ml (24.3 mmol) of acetonitrile (**19**) was added as a solution in THF (10 ml). After stirring for 20 min at −80°C, 3.50 ml (24.3 mmol) of diethyl chlorophosphate in THF (10 ml) was added. The solution was allowed to warm to 0°C in 1–1$\frac{1}{2}$ h and 2.47 ml (24.3 mmol) of benzaldehyde was added in THF (10 ml). After stirring for 3 h at 0°C, the benzaldehyde had disappeared according to TLC (eluent: dichloromethane/petroleum ether, 1/1). Saturated aqueous $NH_4Cl$ (100 ml) was added and the organic layer was separated. The aqueous layer was extracted with dichloromethane (2×100 ml). The combined organic layers were dried on $MgSO_4$, filtered and concentrated *in vacuo*. This crude product was purified using silica column chromatography (eluent: dichloromethane/petroleum-ether, 1/1), yielding 2.04 g of **20** (oily, 65% yield). $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 5.85 (d, 1H, $^3J_{HH,trans}$ 16.9 Hz, CHCN), 7.36 (d, 1H, $^3J_{HH,trans}$ 16 Hz, PhCH), 7.4 (m, 5H, $CH_{Ph}$) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CDCl_3$): $\delta$ 95.8 (C2), 117.6 (C1), 126.8 (C6), 128.4 (C5), 130.5 (C7), 133.0 (C4), 149.6 (C3) ppm.

*(E)-3-Phenyl-2-propenal* (**3**)

Under a dry-nitrogen atmosphere, 2.04 g (16 mmol) of **20** was dissolved in 100 ml of anhydrous THF and was cooled to −70°C with an ethanol/liquid-nitrogen bath. A 1.0M solution of diisobutylaluminium hydride (DibalH, 32 ml, 32 mmol) was added via a syringe. The solution was allowed to slowly warm to −20°C. When the reaction was completed according to TLC (eluent: ether/petroleum-ether, 1/4), a homogeneous mixture of 20 g of silica and 16 g of water was added carefully to the cooled (0°C) mixture. The resulting suspension was stirred for 1 h at 0°C. $MgSO_4$ was added and the stirring was continued for 15 min. The mixture was filtered and the solids were thoroughly washed with dichloromethane. The solvents were evaporated *in vacuo*, which resulted in 1.62 g of **3** (oily, 78% yield). $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 6.71 (dd, 1H, $^3J_{HH}$ 7.7 Hz, $^3J_{HH,trans}$ 15.9 Hz, CHCO), 7.4 (m, 3H, $CH_{Ph}$ + PhCH), 7.5 (m, 3H, $CH_{Ph}$), 9.96 (d, 1H, $^3J_{HH}$ 7.7 Hz, CHO) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CDCl_3$): $\delta$ 127.7 (C2), 127.9 (C6), 128.4 (C5), 130.5 (C7), 133.3 (C4), 152.0 (C3), 193.0 (C1) ppm.

*Benzamide*[37] (**22**)

In 200 ml of 2M aqueous NaOH, 0.44 g (8.2 mmol) of ammonium chloride was dissolved under cooling with an ice/water bath. To this cooled solution, 1.74 g (12.4 mmol) of benzoyl chloride was slowly added, while the pH was kept constant with a solution of 2M aqueous NaOH. After stirring for 6 h (TLC eluent: 2-propanol/dichloromethane, 1/4), the precipitate formed was isolated by filtration and washed with cold water. The water layer was extracted three times with 50 ml of dichloromethane. The organic layers were combined, dried on $MgSO_4$ and concentrated *in vacuo*. This resulted in a total yield of 0.84 g of **22** (85% yield). $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 4.9 (bs, $NH_2$), 7.43 (m, 3H, $CH_{Ph}$), 7.87 (m, 2H, $CH_{Ph}$) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CDCl_3$): $\delta$ 128.5 (C3), 129.4 (C4), 132.8 (C5), 134.7 (C2), 172.3 (C1) ppm.

*($^{15}N$)-22* (**22c**). Compound **22c** (15.2 g) was prepared from 8.00 g of $^{15}NH_4Cl$ (85% yield). All NMR data were as described for **22**.

*Benzylamine*[38] (**4**)

A 250-ml flask was equipped with a Vigreux distillation column and a dropping funnel. Under a dry-nitrogen atmosphere, 5.00 g (41.3 mmol) of benzamide (**22**) was dissolved in 100 ml of anhydrous THF. This mixture was boiled under reflux and 9.0 ml (95 mmol) of borane–dimethyl sulfide complex was added via a syringe. The dimethyl sulfide formed was removed by distillation and some additional THF was added to keep a constant volume. The refluxing was continued until all **22** had disappeared according to TLC (eluent: dichloromethane/2-propanol, 97/3) (8 h). The mixture was cooled with an ice bath (0°C) and then, 40 ml of 3M HCl was carefully added dropwise. After the vigorous bubbling had ceased, some more THF was added, the excess dimethyl sulfide was removed by distillation and the mixture was heated under reflux for another 15 min. The mixture was again cooled to 0°C and the pH was adjusted to > 11 using 6M NaOH. The organic layer was separated and washed with saturated aqueous NaCl and the aqueous layer was extracted with dichloromethane. The combined organic fractions were dried on $K_2CO_3$, filtered and concentrated, resulting in 3.98 g of **4** (oily, 90% yield). $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ 1.4 (bs, $NH_2$), 3.82 (s, 2H, $PhCH_2$), 7.28 (m, 5H, $CH_{Ph}$) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CDCl_3$): $\delta$ 45.5 ($PhCH_2$), 125.7 (C5), 126.1 (C3), 127.5 (C4), 142.6 (C2) ppm.

*($^{15}N$)-4* (**4c**). Compound **4c** (11.4 g) was prepared from 14.7 g of **22c** (87% yield). All NMR data were as described for **4**.

*1-Benzyl-5-(2-phenylethenyl)-1H-imidazole* (**10**)

*Method I.* According to the procedure of *Van Leusen* and *Wildeman*[26], 3.75 g (19.2 mmol) of TosMIC (**2**) was dissolved in 150 ml of anhydrous THF under a dry-nitrogen atmosphere. This mixture was cooled to −80°C with an ethanol/liquid-nitrogen bath and 12.0 ml (19.2 mmol) of a 1.6M solution of butyllithium in hexanes was added via a syringe. After stirring for 5 min, 2.43 ml (19.2 mmol) of chlorotrimethylsilane was slowly added as a solution in THF (15 ml) via the dropping funnel. After stirring for 10 min, another 12.0 ml (19.2 mmol) of the butyllithium solution was added via a syringe. The mixture was stirred for another 10 min and a solution of 2.42 ml (19.2 mmol) of **3** in 15 ml of THF was added via the dropping funnel. The stirring was continued for 30 min and the solution was allowed to warm to −30°C. Then, saturated aqueous $NH_4Cl$ was added and the solution was warmed to room temperature. The organic layer was separated, washed with saturated aqueous NaCl and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried on $MgSO_4$, filtered and concentrated to ~100 ml. To this solution of the intermediate **8**, 200 ml of anhydrous methanol was added and the solution was again concentrated to ~150 ml. To this concentrate, 4.20 ml (38.5 mmol) of **4** was added and the mixture was stirred at room temperature until all **8** had disappeared according to TLC (eluent: dichloromethane/2-propanol, 97/3) (3 days). Then, 75 ml of water and 150 ml of dichloromethane were added to the reaction mixture and the organic layer was separated. The aqueous layer was extracted with dichloromethane and the combined organic layers were dried on $MgSO_4$, filtered and concentrated. The crude product was purified by silica column chromatography (eluent: dichloromethane/methanol, 99/1 to 95/5), resulting in 3.67 g of **10** (solid, 73% yield). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 5.14 (s, 2H, $PhCH_2$), 6.68 (d, 1H, $^3J_{HH,trans}$ 16 Hz, =CH), 6.90 (d, 1H, $^3J_{HH,trans}$ 16 Hz, =CH), 7.09 (m, 2H, $CH_{Ph}$), 7.3 (m, 8H, $CH_{Ph}$), 7.36 (s, 1H, H4), 7.48 (s, 1H, H2) ppm. $^{13}$C NMR (100 MHz, $^1$H-noise-decoupled-APT, $CDCl_3$): $\delta$ 48.5 ($PhCH_2$), 113.8 (C4), 126.0, 126.4, 127.5, 127.6, 127.9, 128.5, 128.9 and 129.2 ($C_{Ph}$ and =CH), 130.9 (C5), 135.9 ($C_{Ph,q}$), 136.6 ($C_{Ph,q}$), 138.4 (C2) ppm.

*Method II.* A dry 100-ml three-necked flask, equipped with a reflux condenser and a dropping funnel, was one-third filled with molecular sieves (3A) and 40 ml of anhydrous methanol. The methanol was heated to reflux and 0.55 ml (5.0 mmol) of **4** and 0.65 ml (5.0 mmol) of **3** were added. After refluxing for $\frac{1}{2}$ h the formation of the aldimine was complete and 0.98 g (5.0 mmol) of **2** was added[25]. A solution of 0.57 g (5.0 mmol) of potassium *tert*-butoxide in 5 ml of methanol was slowly dripped into the mixture. When all the aldimine had disappeared according to TLC (eluent: acetone/methanol, 9/1) (4 h) the mixture was allowed to cool, the molecular sieves were removed by filtration and were washed with dichloromethane. The filtrate was washed with saturated aqueous NaCl, and the organic layer was separated. The aqueous layer was extracted with dichloromethane (3×100 ml) and the combined organic layers were dried on $MgSO_4$. After filtration, the solvents were evaporated *in vacuo* and the product was purified by silica column chromatography (eluent: dichloromethane/ethanol, 99/1). This resulted in 0.98 g of **10** (solid, 75%). The NMR parameters were as described above.

*(2-$^{13}C$)-10* (**10a**). Compound **10a** (1.45 g) was prepared from 2.71 g of **2a** (40% yield) according to Method I. $^1$H NMR: the signals at 7.48 and 5.14 ppm were split into doublets, $^1J_{C2H2}$ 207 Hz, $^3J_{C2HBz}$ 4.1 Hz. $^{13}$C NMR: strong signal at 138.4 ppm, signals at 130.9 and 136.6 ppm were split into doublets, $^2J_{C2C5}$ 11.7 Hz, $^3J_{C2CPh}$ 5.9 Hz.

*(3-$^{15}N$)-10* (**10b**). Compound **10b** (4.34 g) was prepared from 4.67 g of **2b** (70% yield) in two batches according to Method I. $^1$H NMR: the signals at 7.36 and 7.48 ppm were split into doublets, $^2J_{N3H4}$ 7 Hz, $^2J_{N3H2}$ 11.3 Hz. $^{13}$C NMR data were as described for **10**.

*(1-$^{15}N$)-10* (**10c**). Compound **10c** (8.13 g) was prepared from 13.56 of **2** and 7.47 g of **4c** (45% yield) in five batches according to Method I, using one equivalent of **4c** and an excess of potassium carbonate. $^1$H NMR: the signals at 7.36 and 7.48 ppm were split into doublets, $^3J_{N1H4}$ 2.1 Hz, $^2J_{N1H2}$ 8.2 Hz. $^{13}$C NMR: signals at 48.5, 130.9 and 138.4 ppm were split into doublets, $^1J_{N1CPh}$ 10.3 Hz, $^1J_{N1C5}$ 14.7 Hz, $^1J_{N1C2}$ 11.7 Hz.

*1-Benzyl-1H-imidazole-5-carbaldehyde* (**11**)

**10** (2.32 g, 8.9 mmol) was dissolved in a mixture of 80 ml of dioxane and 50 ml of water. Potassium osmate dihydrate (0.16 g, 0.45 mmol) and 3.8 g (17.8 mmol) of sodium periodate were added and the mixture was stirred at 60°C. After 24 h, all **10** had disappeared according to TLC (eluent: toluene/*n*-butanol, 4/1). The mixture was cooled to room temperature and acidified with 30 ml 3M HCl. The aqueous mixture was extracted with ether (3×100 ml) and the pH was subsequently adjusted to > 10 with 6M NaOH. The aqueous layer was extracted with dichloromethane (3×100 ml) to isolate the product. The combined dichloromethane fractions were dried on $MgSO_4$, filtered and the product was obtained by evaporation of the solvent *in vacuo*. This gave 1.41 g of **11** (solid) in 85% yield. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 5.48 (s, 2H, $PhCH_2$), 7.20 (m, 2H, $CH_{Ph}$), 7.29 (m, 3H, $CH_{Ph}$), 7.71 (s, 1H, H4), 7.80 (s, 1H, H2), 9.71 (s, 1H, CHO) ppm. $^{13}$C NMR (75 MHz, $^1$H-noise-decoupled-APT,

$CDCl_3$): δ 49.9 ($PhCH_2$), 128 (C4), 127.3, 128.0 and 128.6 ($C_{Ph}$), 130.8 (C5), 135.4 ($C_{Ph,q}$), 143.5 (C2), 178.8 (CHO) ppm.

*(2-$^{13}$C)*-**11** (**11a**). Compound **11a** (0.92 g) was prepared from 1.45 g of **10a** (89% yield). $^1$H NMR: the signals at 7.80, 7.71 and 5.48 ppm were split into doublets, $^1J_{C2H2}$ 208 Hz, $^3J_{C2H4}$ 11.8 Hz, $^3J_{C2HBz}$ 4.6 Hz. $^{13}$C NMR: strong signal at 143.5 ppm, signal at 130.8 was split into a doublet, $^2J_{C2C5}$ 11.7 Hz.

*(3-$^{15}$N)*-**11** (**11b**). Compound **11b** (2.41 g) was prepared from 4.34 g of **10b** (77% yield). $^1$H NMR: the signals at 7.71 and 7.80 ppm were split into doublets, $^2J_{N3H4}$ 4.1 Hz, $^2J_{N3H2}$ 1.0 Hz. $^{13}$C NMR data were as described for **11**.

*(1-$^{15}$N)*-**11** (**11c**). Compound **11c** (4.00 g) was prepared from 8.13 g of **10c** (70% yield). $^1$H NMR: the signals at 7.71, 7.80 and 9.71 ppm were split into doublets, $^3J_{N1H4}$ 2.1 Hz, $^2J_{N1H2}$ 8.2 Hz, $^3J_{N1H(CO)}$ 3.6 Hz. $^{13}$C NMR: signals at 49.9, 130.8 and 143.5 ppm were split into doublets, $^1J_{N1CPh}$ 7.3 Hz, $^1J_{N1C5}$ 13.2 Hz, $^1J_{N1C2}$ 9 Hz.

*1-Benzyl-5-(hydroxymethyl)-1*H*-imidazole* (**12**)

In a 100-ml three-necked flask equipped with a reflux condensor, 0.24 g (6.4 mmol) of lithium aluminium hydride was suspended in 20 ml of THF under a dry nitrogen atmosphere. To the stirred suspension, a solution of 2.17 g (11.7 mmol) of **11** in 10 ml of THF was added and the mixture was stirred for 15 min. The mixture was carefully diluted with 10 ml of water and subsequently with 10 ml of 6M HCl. The pH was adjusted to 9 using 6M NaOH and the aqueous mixture extracted with dichloromethane (8×75 ml). The organic layers were combined and dried on $MgSO_4$. After filtration, the solvents were evaporated *in vacuo*, resulting in 2.18 g of **12** (solid, 99% yield). $^1$H NMR (200 MHz, $CDCl_3$): δ 1.6 (s, 1H, OH), 4.25 (s, 2H, $PhCH_2$), 5.24 (s, 2H, $CH_2O$), 7.02 (s, 1H, H4), 7.25 (m, 5H, $CH_{Ph}$), 7.51 (s, 1H, H2) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CD_3S(O)CD_3$): δ 47.5 ($CH_2O$), 52.8 ($PhCH_2$), 127.4 (C4), 127.0, 127.8, 128.6 and 131.6 ($C_{Ph}$), 137.6 (C5), 138.4 (C2) ppm.

*(2-$^{13}$C)*-**12** (**12a**). Compound **12a** (0.84 g) was prepared from 0.92 g of **11a** (90% yield). $^1$H NMR: the signals at 7.51, 7.02 and 5.24 ppm were split into doublets, $^1J_{C2H2}$ 207 Hz, $^3J_{C2H4}$ 1.0 Hz, $^3J_{C2HBz}$ 5.2 Hz. $^{13}$C NMR: strong signal at 138.4 ppm.

*(3-$^{15}$N)*-**12** (**12b**). Compound **12b** (2.33 g) was prepared from 2.41 g of **11b** (96% yield). $^1$H NMR: the signals at 7.02 and 7.51 ppm were split into doublets, $^2J_{N3H4}$ 9.2 Hz, $^2J_{N3H2}$ 13.8 Hz. $^{13}$C NMR data were as described for **11**.

*(1-$^{15}$N)*-**12** (**12c**). Compound **12c** (3.61 g) was prepared from 3.75 g of **11c** (95% yield). $^1$H NMR: the signals at 4.25, 7.02 and 7.51 ppm were split into doublets, $^3J_{N1H6}$ 5.3 Hz, $^3J_{N1H4}$ 3.1 Hz, $^2J_{N1H2}$ 8.2 Hz. $^{13}$C NMR: signals at 47.5 and 138.4 ppm were split into doublets, $^1J_{N1CPh}$ 10.3 Hz, $^1J_{N1C2}$ 11.7 Hz.

*1-Benzyl-5-(chloromethyl)-1*H*-imidazolium chloride* (**5**)

A 50-ml flask, equipped with a reflux condensor, was charged with 1.94 g (10.3 mmol) of **12**. Thionyl chloride (10 ml) was added and the mixture was refluxed for 20 min. The mixture was allowed to cool and the volatile compounds were evaporated *in vacuo* in the fume hood. The last traces of thionyl chloride were removed by applying vacuum (0.5 mmHg) to the product overnight. This resulted in 2.48 g of **5** (solid, 99% yield). $^1$H NMR (200 MHz, $CD_3S(O)CD_3$): δ 4.90 (s, 2H, $CH_2Cl$), 5.53 (s, 2H, $PhCH_2$), 7.39 (m, 5H, $CH_{Ph}$), 7.86 (s, 1H, H4), 9.33 (s, 1H, H2) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CD_3S(O)CD_3$): δ 33.1 ($CH_2Cl$), 49.6 ($PhCH_2$), 120.5 (C4), 128.6, 128.9, 128.9 and 130.0 ($C_{Ph}$), 134.9 (C5), 137.3 (C2) ppm.

*(2-$^{13}$C)*-**5** (**5a**). Compound **5a** (1.08 g) was prepared from 0.84 g of **12a** (99% yield). $^1$H NMR: the signals at 9.33, 7.86 and 5.53 ppm were split into doublets, $^1J_{C2H2}$ 221 Hz, $^3J_{C2H4}$ 5.6 Hz, $^3J_{C2HBz}$ 4.6 Hz. $^{13}$C NMR: strong signal at 137.3 ppm.

*(3-$^{15}$N)*-**5** (**5b**). Compound **5b** (3.01 g) was prepared from 2.33 g of **12b** (99% yield). $^1$H NMR: the signals at 7.86 and 9.33 ppm were split into doublets, $^2J_{N3H4}$ 4.1 Hz, $^2J_{N3H2}$ 5.6 Hz. $^{13}$C NMR: the signals at 120.5 and 137.3 ppm were split into doublets, $^1J_{N3C4}$ 8.8 Hz, $^2J_{N3C2}$ 14.7 Hz.

*(1-$^{15}$N)*-**5** (**5c**). Compound **5c** (4.62 g) was prepared from 3.61 g of **12c** (99% yield). $^1$H NMR: the signals at 4.90, 7.86 and 9.33 ppm were split into doublets, $^3J_{N1H6}$ 2.6 Hz, $^3J_{N1H4}$ 3.1 Hz, $^2J_{N1H2}$ 4.6 Hz. $^{13}$C NMR: signals at 49.6 and 137.3 ppm were split into doublets, $^1J_{N1CPh}$ 8.8 Hz, $^1J_{N1C2}$ 13.2 Hz.

*(2S,5R)-2-[(1-Benzylimidazol-5-yl)methyl]-2,5-dihydro-3,6-dimethoxy-5-isopropylpyrazine* (**13**)

Under a dry nitrogen atmosphere, 2.06 g (11.2 mmol) of **6** was dissolved in 20 ml of anhydrous THF. This solution was cooled to −70°C with an ethanol/liquid-nitrogen bath and 7.0 ml (11.2 mmol) of a 1.6M solution of butyllithium in hexanes was added via a syringe. After stirring for 15 min at −70°C, 1.36 g (5.59 mmol) of **5** was added. The resulting suspension was stirred for 6 h at −70°C and subsequently warmed to room temperature. The stirring was continued for 1 h and saturated aqueous $NH_4Cl$ was added. The organic layer was separated and washed with saturated aqueous NaCl. The aqueous layer was extracted with dichloromethane. The combined organic layers were dried on $MgSO_4$, filtered and concentrated *in vacuo*. The excess of **6** and the product were isolated by silica column chromatography (eluent: methanol/dichloro-methane, 3/97). This resulted in 0.98 g of **6** (5.3 mmol) and 1.66 g of **13** (oil, 84% yield based on **5**). $^1$H NMR (300 MHz, $CDCl_3$): δ 0.65 (d, 3H, $^3J_{HH}$ 7 Hz, $CH_3$), 1.00 (d, 3H, $^3J_{HH}$ 7 Hz, $CH_3$), 2.21 (m, 1H, $CHMe_2$), 3.00 (d, 2H, $^3J_{HH}$ 4.6 Hz, $ImCH_2$), 3.68 (s, 3H, $OCH_3$), 3.70 (s, 3H, $OCH_3$), 3.70 (m, 1H, H5), 4.18 (m, 1H, H2), 5.15 (d, 1H, $^2J_{HH}$ 16 Hz, PhCH), 5.25 (d, 1H, $^2J_{HH}$ 16 Hz, PhCH), 6.81 (s, 1H, H4′), 7.02 (d, 2H, $^3J_{HH}$ 8 Hz, $CH_{Ph}$), 7.32 (m, 3H, $CH_{Ph}$), 7.42 (s, 1H, H2′) ppm. $^{13}$C NMR (50.1 MHz, $^1$H-noise-decoupled, $CDCl_3$): δ 16.2 ($CH_3$), 18.6 ($CH_3$), 27.6 ($ImCH_2$), 31.2 ($CMe_2$), 47.9 ($PhCH_2$), 51.9 (C2), 55.3 ($OCH_3$), 60.3 (C5), 127.4 (C4′), 126.1–128.4 ($C_{Ph}$), 136.3 (C5′), 137.9 (C2′), 161.7 (C6), 164.2 (C3) ppm.

*(2′-$^{13}$C)*-**13** (**13a**). Compound **13a** (0.83 g) was prepared from 1.03 g of **5a** (55% yield). $^1$H NMR: the signals at 7.42 and 6.81 ppm were split into doublets, $^1J_{C2'H2'}$ 206 Hz, $^3J_{C2'H4'}$ 10.3Hz, the signals at 5.15 and 5.25 ppm were split into double doublets, $^3J_{C2'HBz}$ 4 Hz. $^{13}$C NMR: strong signal at 137.9 ppm.

*(3′-$^{15}$N)*-**13** (**13b**). Compound **13b** (2.93 g) was prepared from 2.96 g of **5b** (68% yield). $^1$H NMR: the signals at 6.81 and 7.42 ppm were split into doublets, $^2J_{N3'H4'}$ 9.7 Hz, $^2J_{N3'H2'}$ 10.8 Hz. $^{13}$C NMR: the signal at 137.9 ppm was split into a doublet, $^2J_{N3C2}$ 20.5 Hz.

*(1′-$^{15}$N)*-**13** (**13c**). Compound **13c** (4.16 g) was prepared from 4.62 g of **5c** (55% yield) in three batches. $^1$H NMR: the signals at 6.81 and 7.42 ppm were split into doublets, $^2J_{N3'H4'}$ 3.6 Hz, $^2J_{N3'H2'}$ 8.2 Hz. $^{13}$C NMR: signal at 47.9 ppm was split into a doublet, $^1J_{N1CPh}$ 8.8 Hz.

*L-3′-Benzylhistidine hydrochloride* (**14**)

In a 50-ml reflux setup, 0.22 g (0.63 mmol) of **13** was dissolved in 40 ml of 3M HCl. The solution was refluxed overnight (16 h). After cooling down, the mixture was concentrated *in vacuo* and the residue was lyophilized. This resulted in 0.28 g of a 1:1 mixture of **14** and D-valine hydrochloride (solids, 93%) which was directly used in the next step. $^1$H NMR **14** (300 MHz, $D_2O$): δ 3.40 (m, 2H, H3), 3.97 (m, 1H, H2), 5.48 (s, 2H, $PhCH_2$), 7.40 (m, 6H, H5′ and $H_{Ph}$), 8.79 (s, 1H, H2′) ppm. $^{13}$C NMR **14** (50.1 MHz, $^1$H-noise-decoupled, $D_2O$): δ 24.8 (C3), 51.2 ($PhCH_2$), 51.9 (C2), 120.1 (C5′), 128.3–130.0 ($C_{Ph}$), 133.4 (C4′), 136.5 (C2′), 172.2 (C1) ppm.

*L-Histidine dihydrochloride* (**1**). In a 50-ml reflux setup, 0.21 g of the 1:1 product mixture of **14** and D-valine hydrochloride from the previous reaction was dissolved in a mixture of cyclohexene/methanol (1/1). A freshly prepared $Pd^0$ pellet[48] was added and crushed with a glass rod. The mixture was refluxed until the conversion was complete (65 h) according to TLC (eluent: 2-propanol/water, 7/3). The suspension was cooled and filtered over Celite. The solvents were removed *in vacuo* and the residue was dissolved in water. The aqueous solution was brought onto a column packed with AG 50W X8 ion-exchange resin ($H^+$ form). The column was washed with water and D-valine was eluted with 0.1M HCl. The product was subsequently eluted with 2M HCl. Concentration of the eluate resulted in 0.091 g of **1** (90% yield). Optical purity (HPLC): 97%. $^1$H NMR spectrum (300 MHz, $D_2O$) is depicted in Figure 5a, the parameters are listed in Table I. The $^{13}$C NMR (75.4 MHz, $D_2O$) is depicted in Figure 6a. MS (*n*-butyl ester, EI, 70 eV): *m/z* 212 (M+H, 10%), 110 (40%), 82 (100%).

*(2'-$^{13}$C)*-**1** (**1a**). Compound **1a** (0.50 g) was prepared from 0.83 g of **13a** (94% yield). $^{1}$H NMR spectrum (300 MHz, $D_2O$) is depicted in Figure 5b, the parameters are listed in Table I. The $^{13}$C NMR (75.4 MHz, $D_2O$) is depicted in Figure 6b. MS (*n*-butyl ester, CI, $CH_4$): *m/z* 213.1445 $^{12}C_9{}^{13}C^{1}H_{18}{}^{14}N_3{}^{16}O_2$ (M+H) calcd.: 213.1433, (*n*-butyl ester, EI, 70 eV) *m/z* 213 (M+H, 5%), 111 (40%), 83 (100%).

*(1'-$^{15}$N)*-**1** (**1b**). Compound **1b** (1.66 g) was prepared from 2.93 g of **13b** (88% yield). $^{1}$H NMR spectrum (300 MHz, $D_2O$) is depicted in Figure 5c, the parameters are listed in Table I. The $^{13}$C NMR (75.4 MHz, $D_2O$) is depicted in Figure 6c. MS (*n*-butyl ester, CI, $CH_4$): *m/z* 213.1403 $^{12}C_{10}{}^{1}H_{18}{}^{14}N_2{}^{15}N^{16}O_2$ (M+H) calcd.: 213.1369, (*n*-butyl ester, EI, 70 eV) *m/z* 213 (M+H, 10%), 111 (40%), 83 (100%).

*(3'-$^{15}$N)*-**1** (**1c**). Compound **1c** (1.93 g) was prepared from 4.16 g of **13c** (72% yield). $^{1}$H NMR spectrum (300 MHz, $D_2O$) is depicted in Figure 5d, the parameters are listed in Table I. The $^{13}$C NMR (75.4 MHz, $D_2O$) is depicted in Figure 6d. MS (*n*-butyl ester, CI, $CH_4$): *m/z* 213.1366 $^{12}C_{10}{}^{1}H_{18}{}^{14}N_2{}^{15}N^{16}O_2$ (M+H) calcd.: 213.1369, (*n*-butyl ester, EI, 70 eV) *m/z* 213 (M+H, 10%), 111 (40%), 83 (100%).

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