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Facile Determination of the Protecting Group Location of N^{im}-Protected Histidine Derivatives by $^1H-^{15}N$ Heteronuclear Correlation NMR

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Abstract: The positioning of the imidazole protecting group of several histidine derivatives was determined by means of $^1H-^{15}N$ heteronuclear multiple-bond correlation NMR experiments. The cross-peak originated from the three-bond correlation between the histidine side-chain H^{β} and the imidazole N^{π} was used for the identification of the N^{π} signal in the ^{15}N spectrum. Therefore, based on the fact that the signal of the substituted imidazole nitrogen appears always at lower chemical shift (δ) than the unsubstituted one, the position of the blocking group could easily be inferred. The obtained data confirmed previous findings that were accomplished with other less generally applicable spectroscopic or crystallographic techniques.

The knowledge of the position of a protecting group on the imidazole ring of histidine is relevant because the two nonequivalent nitrogens (commonly referred to as π and τ)[1] engage differently in the side reactions that may affect histidine derivatives during peptide synthesis. In particular, racemization of carboxy-activated N^{α}-protected histidine is promoted by the π nitrogen,[2] and therefore its selective blockade has proven highly advisable.[3] Many imidazole protecting groups have been developed over the years,[4] but most of the resulting histidine derivatives were proposed without a definite knowledge of the location of the blocking group. In the case of the benzyloxymethyl group (Bom) developed by Jones and co-workers,[5] a chemical strategy was devised for its selective introduction on the π-position and the location of the protecting group was unequivocally established by crystallographic analysis.[6] Therefore, protecting groups introduced by the same sequence of chemical steps were assumed likewise to give the π-substituted products.[7] The actual position of Bom was further confirmed by the NMR measurement of the NOE interaction between the methylene moiety of the blocking group and the nearby imidazole protons.[8] However, this experiment is only possible if suitable proton pair connectivities between the blocking group and the imidazole ring exist. Matthews and Rapoport introduced a more general NMR approach based on the determination of the magnitude of the cross-ring coupling constants between the imidazole ring protons.[9] The problem associated with this method is that these coupling constants are rather small, ranging between 1.1 and 1.5 Hz for τ-substituted rings and 0.9−1.0 Hz for π-substituted rings, and their accurate measurement may be difficult, particularly in complex structures.

As a part of a project devoted to the synthesis of imidazole-containing biopolymers, we developed a new acyl protection for the side chain of histidine, i.e., the 2,6-dimethoxybenzoyl (2,6-Dmbz).[10] During the preparation of 9-fluorenylmethoxycarbonyl (Fmoc) histidine derivatives, we isolated two compounds that we thought were most likely two different regioisomers. Although the characterization by NMR, HPLC, and mass spectroscopy clearly proved the assumption of regioisomerism, it did not easily reveal the position of the 2,6-Dmbz, and use of NOE difference spectroscopy was precluded due to the lack of a suitable proton pair. Furthermore, because of overlapping and line-broadening of the proton signals, Rapoport's experiments did not, in our hands, provide the necessary data. However, considerations of the chemical reactivity and careful inspection of NMR data of the two regioisomers led us to a provisional assignment of the substituent location. To substantiate this assignment, we sought a more direct method and thought that this could be achieved by $^1H-^{15}N$ heteronuclear multiple-bond correlation NMR (gs-HMBC). The natural abundance of ^{15}N is low (0.37%) and the gyromagnetic ratio approximately only 10% that of the proton, thus making direct measurements of the nuclei rather difficult unless properly labeled compounds are used. However, the indirect through proton-observed experiments can provide information on the chemical structure that is otherwise not easily accessible. In the present case, the three-bond correlation between the histidine β protons and the imidazole π nitrogen was used for structural determination (Figure 1).

In *N*-alkylated imidazoles, the "pyridine-type" nitrogen is resonating at higher frequency (higher δ) compared to the substituted "pyrrole-type" nitrogen (100 ppm in the case of *N*-methylimidazole).[11,12] *N*-Acyl substituents move both the "pyrrole-type" nitrogen and the "pyridine-

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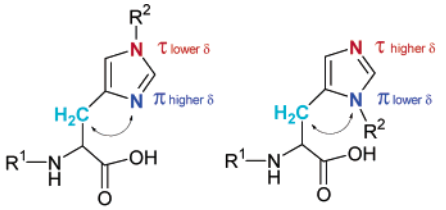

1, $R^1 = R^2$ = Boc
2, R^1 = Boc, R^2 = Tos
3, R^1 = Boc, R^2 = Dnp
4, R^1 = Fmoc, R^2 = Trt
5, R^1 = Fmoc, R^2 = 2,6-Dmbz
6, R^1 = Boc, R^2 = Bom
7, R^1 = Fmoc, R^2 = 2,6-Dmbz

FIGURE 1. Diagnostically relevant HMBC connectivity between β protons and imidazole π-nitrogen.

type" nitrogen to higher δ (50 and 10 ppm, respectively, when *N*-acetylimidazole is compared to *N*-methylimidazole).[11,13] It is therefore a reasonable assumption that in all the cases studied in this work the substituted nitrogen ("pyrrole-type") appears at lower δ than the unsubstituted one ("pyridine-type"). Hence, in the $^1H-^{15}N$ HMBC spectrum the cross-peak between the β protons of the histidine side chain and the π nitrogen would coincide with the lower δ nitrogen in a π-protected derivative and with the higher δ nitrogen in a τ-protected compound. As an example, Figure 2 shows a typical spectrum for two representative compounds, one τ-substituted and one π-substituted (compounds **1** and **6**, respectively). Apart from the diagnostically important H(β)/N(π) cross-peak, also visible are the HMBC signals of the two- or three-bond correlation between the imidazole protons and the

TABLE 1. Chemical Shifts of the Nitrogen Nuclei in the *F1* Domain and Coordinates of the Cross-Peak of the Long-Range H(β)/N(π) Interaction as *F1*/*F2* Chemical Shifts[a]

histidine derivative	δ, ppm $^{15}N(\tau)$	δ, ppm $^{15}N(\pi)$	cross-peak coordinates *F1*/*F2*
1	188.6	272.2	272.2/2.85
2	207.3	273.8	273.8/2.80
3	170.1	267.6	267.6/2.91
4	188.1	261.5	261.5/2.88
5[b]	210.8	274.2	274.2/2.91
6	256.5	179.0	179.0/3.02
7	267.0	207.8	207.8/3.15[c]

[a] d_6-DMSO, 20 °C, 400.13 MHz for ^1H and 40.55 MHz for ^{15}N; δ (ppm) relative to external NH_3 (liq). [b] The experiment was performed at 60 °C to reduce the line width of imidazole signals. [c] An HMBC signal was detected between N(π) and only one of the two β protons (the lower δ one).

imidazole nitrogens. Two-bond cross-ring spin−spin interactions of *N*-substituted imidazoles in nonaqueous media are characterized by ^2J(N,H) varying in the range of 4−12 Hz,[11,13] whereas three-bond ^3J(N,H) are invariably smaller (1−4 Hz).[11] It can therefore be assumed that possible four-bond interactions would be rather weak and very difficult to detect and hence would not interfere with the diagnostic cross-peak. Indeed, none of the spectra recorded were complicated by the four-bond interactions.

The study was extended to cover some commonly used protected histidine derivatives (Figure 1), and the results are presented in the Table 1. Previously, chemical degradation of Z-His(Dnp)-OH indicated the 2,4-dinitro-

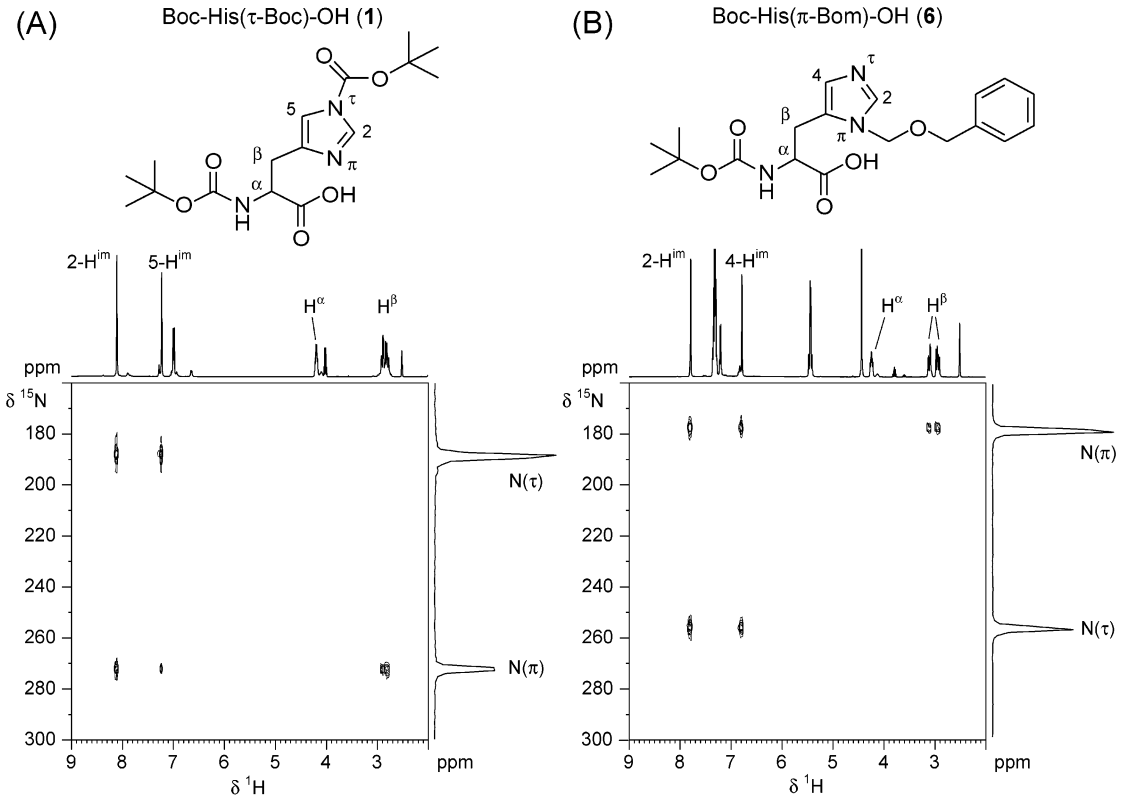

FIGURE 2. $^1H-^{15}N$ HMBC spectra of (A) **1** and (B) **6**. Only the region of the interactions of imidazole nitrogens and histidine protons is shown. Conditions: d_6-DMSO, 20 °C, 400.13 MHz for ^1H and 40.55 MHz for ^{15}N; δ (ppm) relative to external NH_3 (liq).

phenyl (Dnp) group to be on the τ nitrogen,[14] and this is confirmed by our measurements on compound **3**. Acyl groups such as tosyl (Tos) and Boc were assumed to be τ-located because *N*-acylation of imidazole rings occurs regioselectively on the least hindered τ nitrogen.[15] On the other hand, random *N*-alkylation normally results in a variable mixture of regioisomers,[15] one exception being represented by the very bulky trityl (Trt) group,[16] which exclusively is introduced on the τ-position. Indeed, we found that these τ-substituted histidine derivatives (**1**−**4**) had a cross-peak between the β-methylene protons and the higher frequency resonating nitrogen (the unsubstituted one). On the contrary, in the established π-substituted compound **6**, the cross-peak corresponded to the lower frequency resonating nitrogen (the substituted one). By this means we were also able to unambiguously confirm the previously chemically assigned substitution pattern of the 2,6-Dmbz-protected regioisomers (**5** and **7**).

To our best knowledge, this is the first report on the application of ^{1}H−^{15}N correlation NMR for establishing the N^{im}-position of protecting groups of histidine derivatives. Consequently, we propose it as a general and facile method for fully characterizing protected imidazole derivatives for synthetic applications.

Experimental Section

Fmoc-His(π-2,6-Dmbz)-OH and Fmoc-His(τ-2,6-Dmbz)-OH were prepared as previously described.[10] All other amino acid derivatives were commercially available compounds. In a typical NMR experiment, approximately 50 mg of compound was dissolved with d_6-DMSO (0.5 mL) in a 5-mm-diameter NMR tube, and the data collection was performed at 20 °C. NMR spectra were recorded at 400.13 and 40.55 MHz frequencies for ^{1}H and ^{15}N, respectively, with a tunable 5-mm multinuclear probe. The chemical shifts (δ) of ^{1}H are reported in ppm as positive to higher frequency from TMS and were corrected by using the signal of the residual partially nondeuterated DMSO as internal standard. The chemical shifts of ^{15}N are reported in ppm as positive to higher frequency from liquid ammonia and were corrected by using DMF as an external standard (103.2 ppm). Standard Bruker gs-HMBC pulse programs were used and spectra were processed with Bruker XWIN NMR software. In ^{1}H−^{15}N gs-HMBC correlation experiments, the proton spectral window was sampled between 0 and 8.5 ppm in *F*2, and the *F*1 window was sampled over a sweep window of up to 300 ppm. Multiple-bond correlation experiments were optimized for long-range couplings of 7.7 Hz, with no decoupling applied during acquisition. Data were acquired with 2048 points in *t*2, and the number of increments for time evolution was 128. The number of scans per increment was 232, and the delay between transients was set to 1.5 s. The total acquisition time was 15 h and 30 min. Data sets were zero-filled and Fourier transformed to give a final matrix of 2048 × 256 points. The linear prediction algorithm was used in *F*1 with appropriate parameters depending on the case.

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Supporting Information Available: ^{1}H−^{15}N HMBC 2D spectra of all compounds reported in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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