Differentiation between N(im)-Substituted Histidines by NOE Difference Spectroscopy

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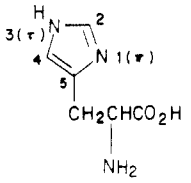
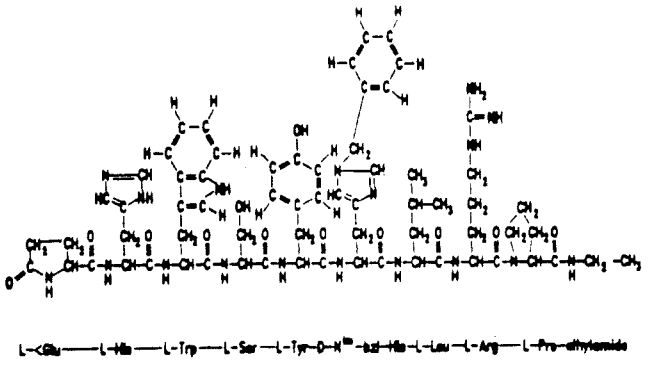
Assigning the position of nitrogen protecting groups on the imidazole ring of histidine has historically been a difficult problem. Early work on the protection of imidazole nitrogens generally makes no distinction between substitution at N-1 (the π nitrogen) and N-3 (the τ nitrogen).[1] In a few recent cases, the position of substitution



has been unambiguously assigned by crystallographic analysis or by chemical degradation.[2] An NMR approach to this problem relies on an empirical rule developed by Matthews and Rapoport based on the observed cross-ring coupling constants between imidazole protons.[3] For N-3-protected histidines, the observed coupling constant for the imidazole protons is in the range of 1.1–1.5 Hz, and for N-1-protected histidines the range is 0.9–1.0 Hz.

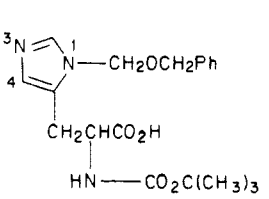
The use of this empirical rule to assign the position of the histidine substituent may not be clear-cut in many cases because of the very narrow range that the coupling constants fall within, the contiguous nature of the cutoff values, and the difficulty in accurately measuring coupling constants of that magnitude. In complex structures the coupling constants are often not resolvable due to overlapping signals, line-broadening effects, and/or solvent effects. As a result, we have developed an unambiguous method that enabled us to easily distinguish between the alternative imidazole structures even when the histidine was part of a complex structure such as a peptide.

During studies with the LH/RH agonist histrelin (1),[4]



1

it was important to verify the position of the N(im)-benzyl protecting group on the D-histidine residue of this nonapeptide: ΔGlu-His-Trp-Ser-Tyr-D-N(im)-Bzl-His-Leu-Arg-Pro-ethylamide. Overlapping signals in the aromatic region of the proton NMR spectrum made it difficult to resolve the imidazole proton cross-ring J coupling constants even at 300 MHz. Instead, NOE difference spectroscopy was used to differentiate between N-1(π) and N-3(τ) substitution on the histidine residue. To validate the assignments in the peptide, the model compounds N-BOC-N(im)-[(benzyloxy)methyl]-D-histidine (2) and N-BOC-N(im)-benzyl-D-histidine (3) were prepared according to literature procedures[5] and studied by using the empirical rule and by NOE difference.



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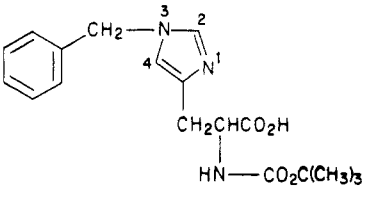

3

If N-1 was substituted (2), an NOE effect would be expected between the H-2 imidazole proton and the methylene protons of the benzyl group, whereas if N-3 was substituted (3) an NOE effect would be expected between both the H-2 and H-4 imidazole protons and the benzyl methylene.

Figure 1. NOE difference spectrum for histrelin. Irradiation of the methylene protons of the benzyl protecting group (5.1 ppm) results in the enhancement of both imidazole protons H-2 (7.6 ppm) and H-4 (6.9 ppm) along with the ortho protons of the benzyl ring (7.2 ppm).

Discussion

In the case of N-BOC-N(im)-benzyl-D-histidine (3), the signal intensity of the benzyl methylene protons was enhanced when both imidazole protons H-2 and H-4 were irradiated, indicating that the benzyl group was attached to N-3.[6] The cross-ring coupling constant between H-2 and H-4 could not be resolved. An NOE effect was observed with imidazole proton H-2 but not to proton H-4 when the benzyloxy methylene protons of N-BOC-N-(im)-[(benzyloxy)methyl]-D-histidine (2) were irradiated, indicating substitution at N-1. Again the cross-ring coupling could not be resolved, and therefore the empirical rule could not be used to determine the position of substitution. However, the NOE studies performed on model compounds showed that difference spectroscopy could be used to easily and accurately differentiate between the two possible sites of N substitution on the imidazole ring of histidine in the peptide 1.

In the case of the nonapeptide histrelin (1), selective irradiation of the benzyl methylene protons (δ 5.15), which were well-resolved in the spectrum in contrast to the imidazole protons in the crowded aromatic region, resulted in enhancement of both the H-2 (δ 7.6) and H-4 (δ 6.9) imidazole protons along with the ortho protons of the benzyl ring (Figure 1). This allowed the assignment of the N-benzyl protecting group in 1 to N-3, the τ nitrogen of the D-histidine residue. NOE difference spectroscopy has been proven to be a satisfactory method for deter-

(1) DuVigneaud, V.; Behrens, O. K. *J. Biol. Chem.* **1937**, *117*, 27.
(2) Brown, T.; Jones, J. H.; Wallis, J. D. *J. Chem. Soc., Perkin Trans.* **1982**, *3045*.
(3) Matthews, H. R.; Rapoport, H. *J. Am. Chem. Soc.* **1973**, *95*, 2297.
(4) Cao, Y.-Q.; Sundaram, K.; Bardin, C. W.; Rivier, J.; Vale, W. *Int. J. Andrology* **1982**, *5*, 158.
(5) See ref 1 and: Brown, T.; Jones, J. H.; Richards, J. D. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1553.
(6) The magnitude of the NOE enhancements was on the order of 10% for the model compounds and 5% for histrelin.

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mining the position of N(im)-benzylation in compounds containing a histidine moiety. This is especially valuable in cases where the cross-ring coupling information is not available.

Experimental Section

Proton NMR Spectra were measured at 300 MHz. All spectra were recorded as methanol-d_4 solutions (10 mg/0.5 mL) containing Me_4Si as an internal reference. The solutions were degassed. Nuclear Overhauser enhanced difference spectra were obtained using standard software. The spectra were obtained at a spectral width of 4 kHz, using 16k data points resulting in a spectral resolution of 0.49 Hz per point. All experiments were performed at ambient temperature (298 K).

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Registry No. 1, 76712-82-8; 2, 99310-01-7; 3, 65717-64-8; histidine, 71-00-1; imidazole, 288-32-4.

Novel Route from Thiocarbamate to Isocyanate: 2,2,2-Trinitroethyl Isocyanate

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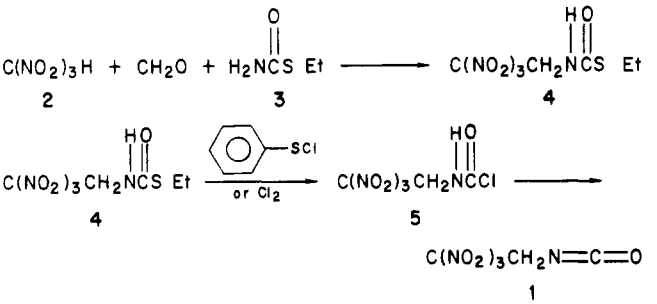
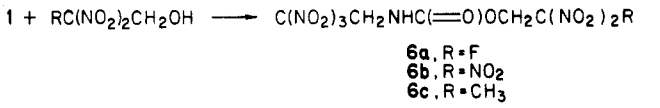
Standard methods for isocyanate synthesis are not suitable for the preparation of 2,2,2-trinitroethyl isocyanate (**1**), a compound that is of interest as an intermediate for energetic materials. The method employing an amine with phosgene[1] cannot be used because the required precursor, 2,2,2-trinitroethylamine, is not available.[2] An alternate method via the rearrangement of an acyl azide[3] is impractical since the required 3,3,3-trinitropropanoic acid is quite unstable and is only available in low yield from a relatively scarce starting material.[4] Therefore we have devised a route (Scheme I) to **1** starting from the readily available trinitromethane (**2**), *S*-ethyl thiocarbamate (**3**), and formaldehyde.

Finding a method to condense **2** with **3** and formaldehyde in good yield proved to be difficult. Although **2** has been shown to readily condense with *N*-methylol amides,[5] there has been only very limited success in similar reactions with carbamates[6] and there is no literature report

Scheme I



Scheme II



of a corresponding thiocarbamate condensation. Insignificant yields of **4** were obtained when base catalysis was used to attempt to form the *N*-methylol derivative of **3** before **2** was added.[7] Attempts to trap the *N*-methylol derivative of **3** as the trifluoroacetate[8] by treating **3** with paraformaldehyde in trifluoroacetic acid followed by later addition of trifluoroacetic anhydride gave instead the *N*-trifluoroacetyl derivative of **3**. Fusion of 2,2,2-trinitroethanol and **3** gave decomposition with only insignificant amounts of **4** being produced.

We did find that reasonable yields of **4** could be obtained if **2** was heated with **3** at 70–75 °C for 5 h in aqueous formaldehyde solution buffered with potassium acetate and acetic acid to control the pH. The elevated temperatures were necessary for the formation of **4** and increased reaction times gave lowered yields. When the reagents were heated without pH control (starting pH 4), only very low yields (<5%) of **4** were obtained. The pH fell during the reaction presumably due to decomposition of **2** at elevated temperatures.

The thiocarbamate **4** can be readily converted to the carbamyl chloride **5** and eventually to isocyanate **1** with either benzenesulfenyl chloride[9] or gaseous chlorine. When **4** was treated with chlorine in carbon tetrachloride, workup gave a mixture of **5** and **1**. By contrast, **4** with benzenesulfenyl chloride gave pure **5** and was the reagent of choice when isolation of the intermediate **5** was desired.[10]

Several derivatives (carbamates) were prepared from **1** by the addition of polynitro alcohols (Scheme II).

(1) (a) Roberts, J. D.; Caserio, M. C. "Basic Principles of Organic Chemistry"; W. A. Benjamin, Inc.: New York, 1964; p 685. (b) Adolph, H. G. *J. Org. Chem.* **1972**, *37*, 747.

(2) The only isolable and reasonable stable 2-substituted 2,2-dinitroethylamine is 2-fluoro-2,2-dinitroethylamine. The other dinitroethylamines of this type cannot be isolated because the reverse Mannich reaction predominates: $ZC(NO_2)_2CH_2NH_2 \rightleftharpoons ZC(NO_2)_2^- + [CH_2{=}NH_2]^+$. Where Z = F, this equilibrium is shifted to the left because fluorodinitromethide ion is less stable and more nucleophilic than the other dinitro carbanions. (Adolph, H. G.; Kamlet, M. J. *J. Org. Chem.* **1969**, *34*, 45).

(3) (a) Reference 1a, p 685. (b) Gold, M. H.; Frankel, M. B.; Linden, G. B.; Klager, K. *J. Org. Chem.* **1962**, *27*, 334.

(4) The trinitropropanoic acid is prepared in 33% yield from 1,1,1,3-tetranitropropane. The hydrogen of the methylene group of the acid exhibit a very high reactivity. For example, the acid reacts with water even at room temperature to form 2-hydroxy-3,3-dinitropropanoic acid. (Golod, E. L.; Novatskii, G. N.; Bagal, L. I. *Zh. Org. Khim.* **1973**, *9*, 1111.)

(5) (a) Feuer, H.; Lynch-Hart, U. E. *J. Org. Chem.* **1961**, *26*, 391. (b) Kranyuskin, M. M.; Andreeva, T. G.; Shvarts, I. Sh.; Sevost'yanova V. V.; Yarovenko, V. N.; Novikov, S. S. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1980**, *3*, 642.

(6) The only literature report is: Klager, K.; Frankel, M. B. "A Review of Nitroform and 2,2,2-Trinitroethanol"; Aerojet Report No. 494, 13 Feb, 1951, p 36, 52. It is stated that methyl *N*-(trinitroethyl)carbamate and ethyl *N*-(trinitroethyl)carbamate were prepared from 2,2,2-trinitroethanol and the corresponding alkoxy carbamate by fusion at 120–130 °C. Yields were not given. It is also stated that condensation of **2** with ethyl *N*-(hydroxymethyl)carbamate was unsuccessful.

(7) A procedure analogous to that in ref 5b was followed.

(8) The thiocarbamate–formaldehyde reaction should be reversible as is the reaction of an amide and formaldehyde. (Lamberton, A. H.; Lindley, C.; Owston, P. G.; Speakman, J. C. *J. Chem. Soc.* **1949**, 1641). Trapping the methylol derivative as the trifluoroacetate might allow reaction of it with the potassium salt of **2** to give **4**.

(9) The conversion of **4** to **5** by benzenesulfonyl chloride is analogous to the reaction: $CH_3C(=O)SCH_3 + CH_3SCl \rightarrow CH_3C(=O)Cl + CH_3SSCH_3$. (Douglass, I. B. *J. Org. Chem.* **1959**, *24*, 2004.) Benzenesulfenyl chloride was chosen for our studies because of convenience but obviously any sulfenyl chloride (RSCl, R = alkyl, aryl) of similar reactivity could be used.

(10) The loss of HCl from **5** is very facile and is presumably due to the electron-withdrawing trinitroethyl group which increases the acidity of the hydrogen attached to nitrogen. Thus, removal of the volatiles from the reaction mixture is sufficient to sweep away HCl with formation of **1**. The reaction with benzenesulfenyl chloride can be run conveniently at high concentration and thus considerable less volatiles have to be removed during workup.

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