

Intramolecular hydrogen bonding of (+)-biotin and biotin derivatives in organic solvents

Geoffrey T. Crisp* and Yu-Lin Jiang

Department of Chemistry, Adelaide University, Adelaide, South Australia, 5005 Australia

E-mail: geoffrey.crisp@adelaide.edu.au

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Abstract

The intramolecular hydrogen bonding of (+)-biotin and biotin derivatives in mixtures of CDCl₃ and DMSO-d₆ was investigated by ¹H NMR spectroscopy. The dynamic changes observed in the chemical shifts for the 1-NH and 3-NH protons with changes in solvent composition and temperature confirmed the presence of an intramolecular hydrogen bond between the valeryl side chain hydrogen bond acceptor and the 3-NH proton in a range of biotin derivatives.

Keywords: Biotin, intramolecular, hydrogen bonding, NMR

Introduction

(+)-Biotin, **1**, is involved as an enzyme cofactor in a variety of carboxylase, decarboxylase and transcarboxylase reactions.^{1,2} The specificity inherent in the reversible carboxylation of biotin involving the electrophilic substitution of the 1-NH proton by the carboxylate group has long been known.^{3,4} Previous reports have demonstrated the more rapid rate of exchange for the 1-NH proton compared to the 3-NH proton in both biotin itself and biotin methyl ester by measurements involving the rate of D₂O exchange in DMSO-d₆ and NMR saturation transfer experiments.^{4,5,6,7} This increased rate of exchange was attributed to an intramolecular hydrogen bond formed between the carboxylate group in the valeryl side chain and the 3-NH proton and NOESY measurements confirmed the presence of a conformation similar to that shown in Figure 1.⁵ A number of ¹H and ¹³C NMR studies on biotin has been reported and a complete assignment for the observed resonances is available.^{5,8,9}

Previous NMR studies on biotin have been conducted at concentrations = 17 mM⁵ and so both inter- and intramolecular hydrogen bonding might be expected in solution, depending on the

solvent.¹⁰ In addition, we were not aware of any published data for simple biotin derivatives at low concentration that demonstrated temperature dependent NMR chemical shifts supporting the proposed intramolecular hydrogen bonding between 3-NH and the carboxyl carbonyl on the valeryl side chain. In this paper we report on the effect of solvent composition in the mixed solvent $\text{CDCl}_3/\text{DMSO-d}_6$ on the chemical shifts of the 1-NH and 3-NH protons and on the temperature dependence of the 1-NH and 3-NH protons in biotin and its derivatives in dilute solutions.

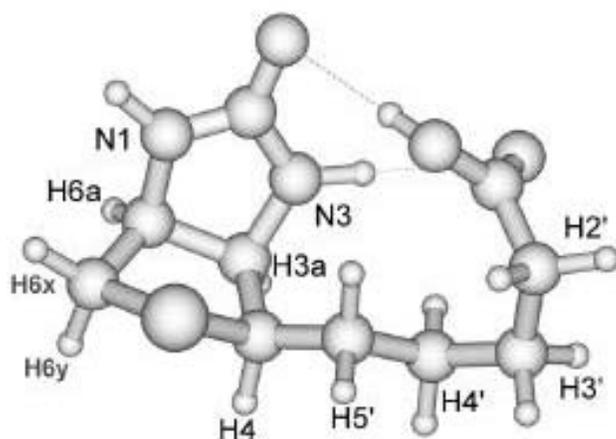
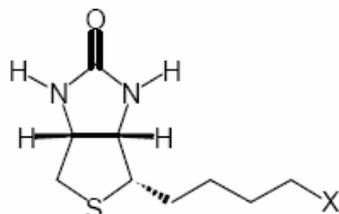


Figure 1. Folded conformation of biotin with intramolecular hydrogen bonding.

Results and Discussion

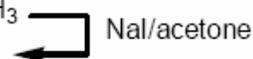
Preparation of biotin derivatives

In order to investigate the intramolecular hydrogen bonding of biotin a series of derivatives, **2-8**, was prepared using literature procedures.



- 1 X = COOH
- 2 X = COOCH₃
- 3 X = COOC₃H₇
- 4 X = COOC₆H₁₃
- 5 X = COOC₁₁H₂₃

- 6 X = COO(4-NO₂Ph)
- 7 X = CH₂OH
- 8 X = CH₂OSO₂CH₃
- 9 X = CH₂I



Nmr spectra of (+)-biotin

(+)-Biotin, **1**, does not dissolve significantly in CDCl_3 and so DMSO-d_6 was used for the preparation of homogeneous and stable solutions. The assignments for the ^1H NMR spectrum for biotin were $\text{H4}'$ (1.465, m, 2H); $\text{H5}'\text{b}+\text{H3}'\text{a}$ (1.642, m, 3H); $\text{H5}'\text{a}$ (1.737, m, 1H); $\text{H3}'\text{b}$ (2.292, t, $J=7.2\text{Hz}$, 2H); H6x (2.756, d, $J=13.2\text{Hz}$, 1H); H6y (2.888, dd, $J=13.2\text{Hz}$, 4.8Hz, 1H); H4 (3.148, m, 1H); H3a (4.275, m, 1H); H6a (4.466, m, 1H); 3NH (5.586, s, 1H); 1-NH (5.681, s, 1H), with $\text{H2}'$ under the solvent resonance (Figure 2).

Previous ^1H NMR data for biotin in DMSO-d_6 showed that the 3-NH proton occurred further downfield compared to the 1-NH proton.^{5,8,9} The 2D-COSY spectrum for a 5mM solution of biotin in 10% DMSO-d_6 and 90% CDCl_3 at rt, however, indicated that the 1NH proton was further downfield, rather than the expected 3-NH (Figure 3). The 2DROESY NMR spectrum of biotin under these condition displayed cross peaks between H3a and methylene protons $\text{H4}'$, between H4 and $\text{H4}'$ and between 3-NH and $\text{H4}'$ (Figure 4).

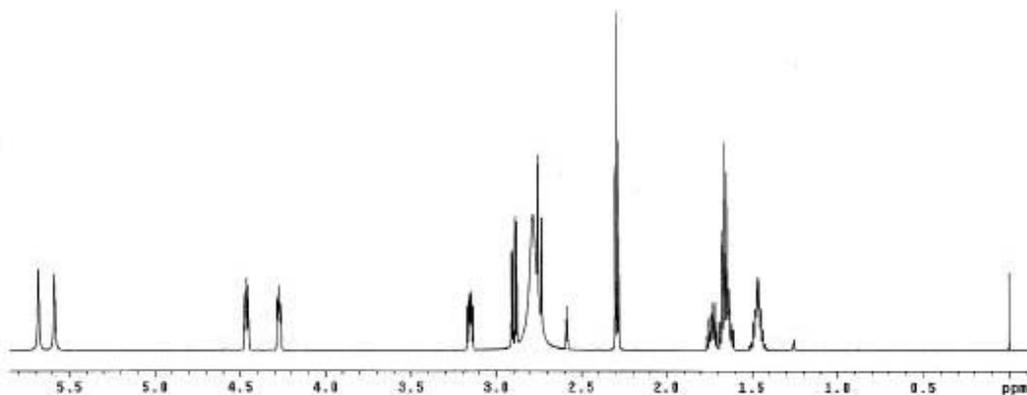


Figure 2. ^1H NMR spectrum of (+)-biotin in 10% DMSO-d_6 and 90% CDCl_3 .

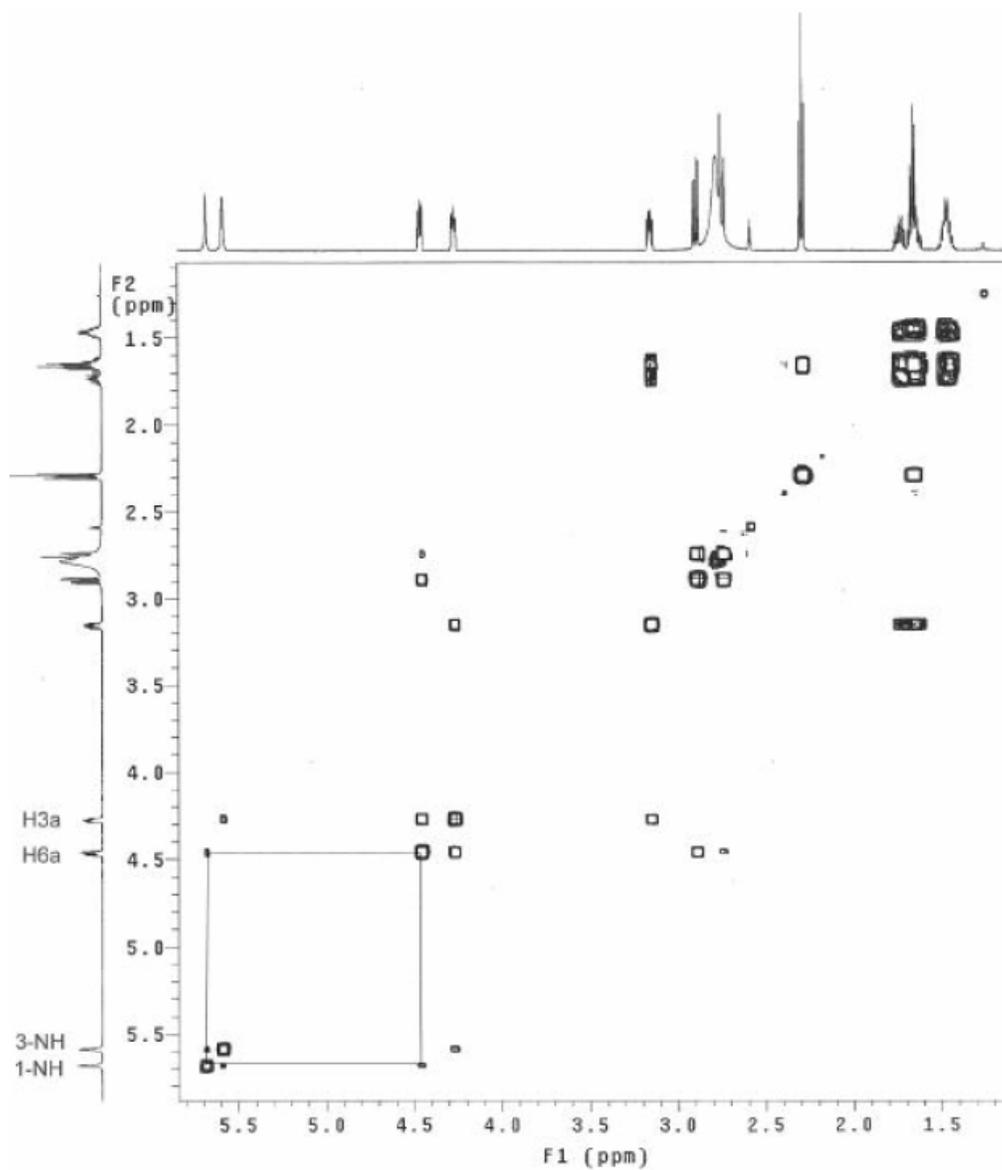


Figure 3. COSY NMR spectrum of biotin 5mM 10%DMSO-d₆ and 90% CDCl₃.

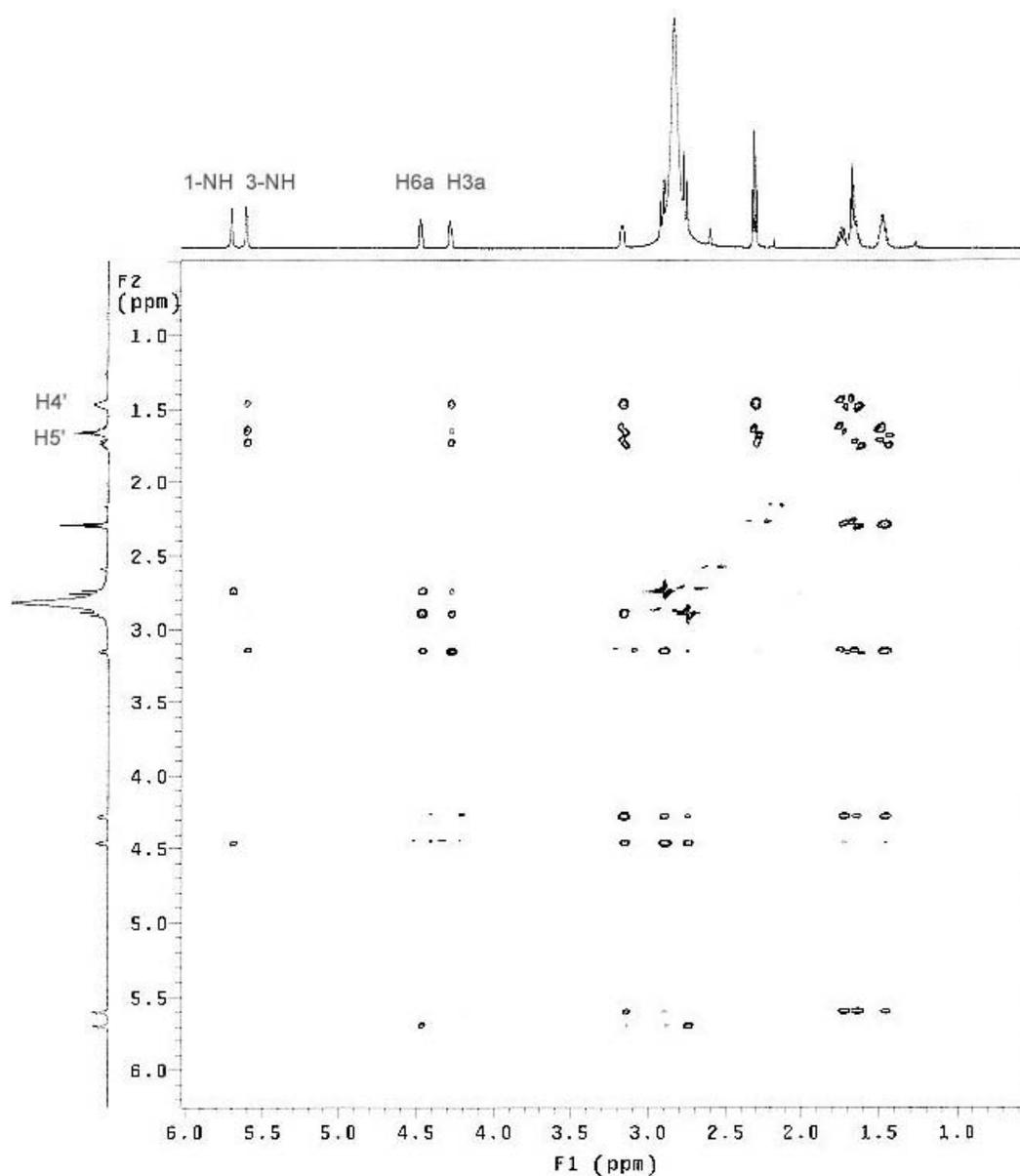


Figure 4. ROESY NMR spectrum of biotin 5mM 10%DMSO-d₆ and 90% CDCl₃.

Given the apparent reversal in the assignments for the 3-NH and 1-NH protons in 10% DMSO-d₆ and 90% CDCl₃, we investigated the effect of solvent composition on biotin ¹H NMR signals and the results for 3-NH and 1-NH are summarized in Figure 5. It is apparent that the addition of increasing proportions of DMSO-d₆ to CDCl₃ causes a dramatic downfield shift in the ureido protons. When the proportion of DMSO-d₆ in the mixed solvent was 4.0%, the 3-NH proton was downfield from 1-NH, suggesting that 3NH was involved in intramolecular hydrogen bonding. As the proportion of DMSO-d₆ was increased the resonance for 3-NH moved upfield,

whereas the resonance for 1-NH moved downfield, consistent with a reduction in intramolecular hydrogen bonding for 3NH. At 6.0% DMSO- d_6 content the chemical shifts of both 1-NH and 3-NH became coincident and as the proportion of DMSO- d_6 was increased further, both 1-NH and 3NH moved downfield. Figure 5 shows that the resonance for 1-NH moved further downfield than that of 3-NH for DMSO- d_6 content between 6.6% and 20.0%. When the content of DMSO- d_6 was 26.7%, the chemical shifts for 1-NH and 3-NH again became coincident, and increasing the content of DMSO- d_6 above 33.3% caused 3-NH to be further downfield than 1-NH. Previous NMR assignments for biotin have usually assumed that 3-NH resonated further downfield compared to 1-NH and the present results have highlighted the need to determine the effect of solvent composition on the chemical shift carefully for protons capable of hydrogen bonding.

At room temperature, no intermolecular amide-amide hydrogen bonding was detected in a 1mM solution of peptide in halogenated solvents, such as CD_2Cl_2 and $CDCl_3$.¹⁰ When polar organic solvents were used, such as CH_3CN , the concentration of peptide could be raised to 10mM before significant intermolecular hydrogen bonding was detected.¹⁰ Therefore, the solution of biotin at 5mM in a mixed solvent of $CDCl_3$ and DMSO- d_6 would be expected to be substantially free of intermolecular hydrogen bonding.

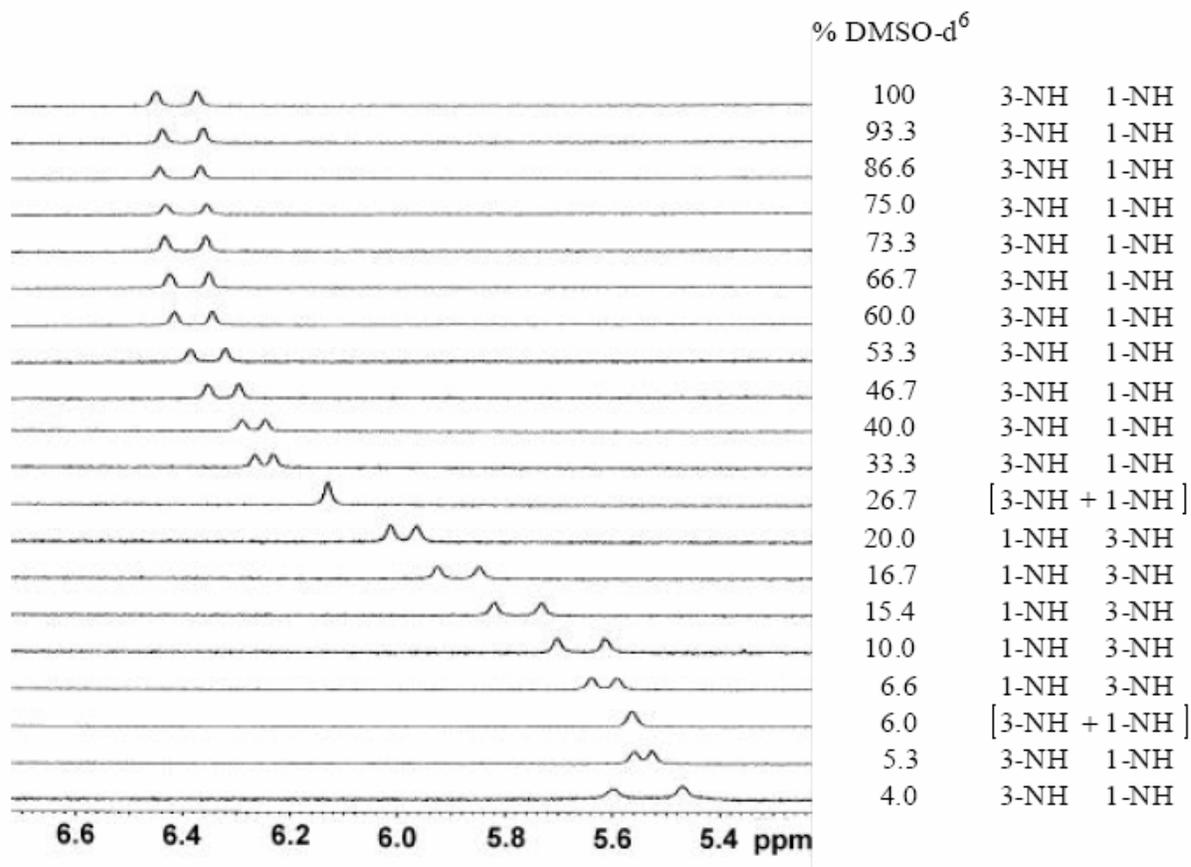


Figure 5. ¹H NMR of 3-NH and 1-NH of biotin in mixtures of DMSO-d₆ and CDCl₃.

Variable temperature ¹H NMR of biotin derivatives

The temperature dependence of amide protons, the $\Delta\delta_{\text{NH}}/\Delta T$ value, provides an indication of the efficacy of intramolecular hydrogen bonding. Non-hydrogen bonded amide protons generally show a small temperature dependence, < 3 ppb/K.^{10,11} In order to confirm the degree of intramolecular hydrogen bonding of biotin and its derivatives (**1-9**), the temperature dependence was determined at 1 mM in CDCl₃ and shown in Table 1 (2 % DMSO-d₆ and 98% CDCl₃ for biotin).

The $\Delta\delta_{\text{NH}}/\Delta T$ value for both 1-NH and 3-NH of compound **9** was 3.8 ppb/K, indicating insignificant intramolecular hydrogen bonding and confirming that **9** would be a suitable standard for comparing the remaining biotin derivatives. As the data in Table 1 shows, for compounds **2-8**, the $\Delta\delta_{\text{NH}}/\Delta T$ values for 3-NH were much higher than 3 ppb/K, indicating that in each case 3-NH was involved in intramolecular hydrogen bonding.

Table 1. Temperature dependence, $\Delta\delta_{\text{NH}}/\Delta T$ in ppb/K, of **1-9** at 1mM in CDCl_3

| Compound | 1 ^a | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|----------|-----------------------|----------|----------|----------|----------|----------|----------|----------|----------|
| 1-NH | 7.9 | 5.7 | 6.2 | 7.0 | 6.6 | 4.6 | 7.6 | 5.8 | 3.8 |
| 3-NH | 11.7 | 9.1 | 10.2 | 9.9 | 10.2 | 9.2 | 21.1 | 11.4 | 3.8 |

^a 2 % DMSO- d_6 and 98% CDCl_3

The $\Delta\delta_{\text{NH}}/\Delta T$ values for 1-NH were higher than expected for **1-8**, as previous studies had indicated that favourable conformations involving intramolecular hydrogen bonding between 3-NH and the valeryl carbonyl were possible but no suggestion of intramolecular hydrogen bonding involving 1-NH.⁵ The observed chemical shifts are averaged over the numerous conformations, including those involving extended chains and intramolecular hydrogen bonding. These data do indicate that weak intramolecular bonding is possible between acceptors in the valeryl side chain and the donor on 1-NH.

The geometry of biotin ester **6** in CDCl_3 at 20mM was probed with a homonuclear NOESY experiment. Irradiation of H3a revealed the following NOE enhancements, H3' (0.46%) and H4' (0.48%); irradiation of H4 revealed the following NOE enhancements H3' (1.94%), H4' (2.10%) and H5' (1.31%); suggesting a conformation analogous to that shown for biotin in Figure 1. The 2D-ROESY spectrum of biotin ester **6** in CDCl_3 at room temperature is shown in Figure 6 with cross peaks observed between 3-NH and H3', H4' and H5'.

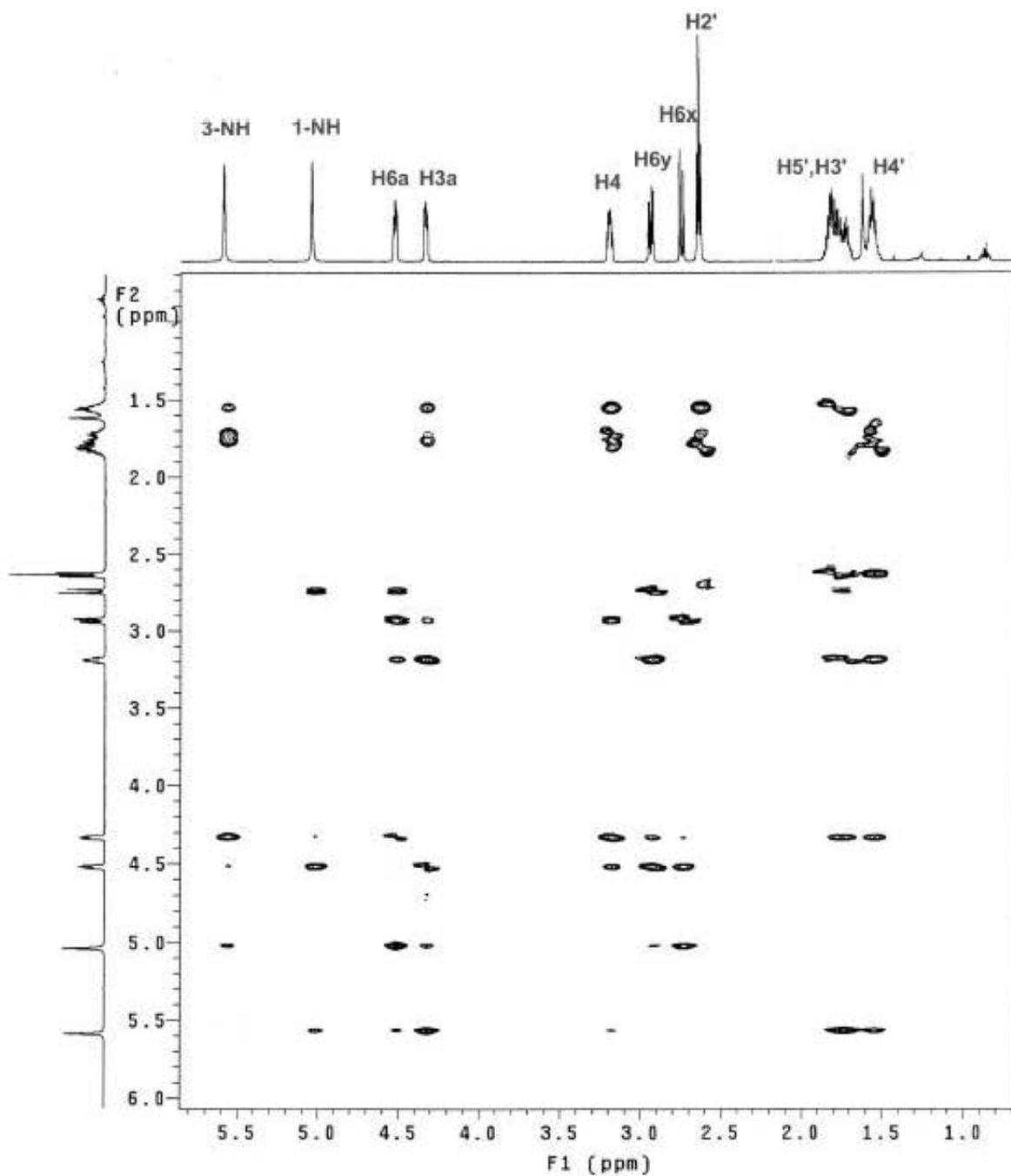


Figure 6. ROESY ^1H NMR spectrum of **6** in CDCl_3 at room temperature.

In conclusion, we have shown that there is a need to be cautious in the assignment of ^1H NMR spectra for compounds in different solvents when intramolecular hydrogen bonding is possible and confirmed that intramolecular hydrogen bonding between 3NH and the valeryl side chain is possible in a range of biotin derivatives. In addition, the 1-NH of biotin and its derivatives is also capable of intramolecular hydrogen bonding but to a lesser extent than the previously reported 3-NH.

Experimental Section

General Procedures. Melting points were recorded on a Reichert hot stage apparatus. ^1H and ^{13}C NMR spectra were recorded on a Varian 600 spectrometer in CDCl_3 and DMSO-d_6 as solvent with TMS as an internal standard. Chemical shifts were reproducible within ± 0.002 ppm. CDCl_3 was dried over 4\AA molecular sieves for at least one month before use. 2D-COSY, ROESY and NOESY NMR were recorded on a Varian 600 NMR spectrometer. Variable temperature NMR experiments were performed under the automatic control of the attachments on the Bruker ACP-300 and Varian 600 spectrometers and the temperature was maintained within a $\pm 0.02^\circ\text{C}$.

Compounds **2**¹², **3**¹³, **4**¹³, **5**¹³, **6**¹⁴, **7**¹⁵ and **8**¹⁵ were prepared by literature procedures.

[3a S-(3a , 4b , 6a)]-Tetrahydro-4-(5-iodopentyl)-1H-thieno[3,4-d]imidazol-2(3H)-one (9)
[3aS-(3a, 4b, 6a)]-Tetrahydro-4-(5-(methylsulfonyl)pentyl)-1H-thieno[3,4-d]imidazol-2(3H)-one (8) (0.010g, 0.032mmol) was dissolved in acetone (2mL) and anhydrous sodium iodide (0.096g, 0.64mmol) was added. The mixture was stirred overnight at room temperature. The solvent was removed with a stream of nitrogen and the residue was submitted to flash column separation with 8% MeOH in CH_2Cl_2 as an eluant to give **9** as a light yellow solid in 74% (0.008g). Mp $173-176^\circ\text{C}$. ^1H NMR(CDCl_3 , 1mM, rt): δ (ppm) 1.460-1.859(m, 8H, CH_2); 2.768(d, 1H, $J=12.6$ Hz, H6x); 2.984(dd, 1H, $J=12.6\text{Hz}, 5.4\text{Hz}$, H6y); 3.149(m, 1H, H4), 3.172(t, 2H, $J=13.8\text{Hz}$, CH_2), 4.340(m, 1H, H3a); 4.535(m, 1H, H6a); 4.442(s, 2H, 1-NH, 3-NH). Calc. for $[\text{M}+\text{H}]^+$ $\text{C}_{10}\text{H}_{18}\text{NOSI}$: 2341.0203. Found: 341.0192.

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