Pergamon

Tetrahedron Letters 39 (1998) 7499–7502

TETRAHEDRON LETTERS

Desulfurization of Biotin and Epibiotin Sulfoxides with Nickel Boride: Analysis of the Stereoselectivity through [^{2}H] NMR in a Polypeptide Liquid Crystal

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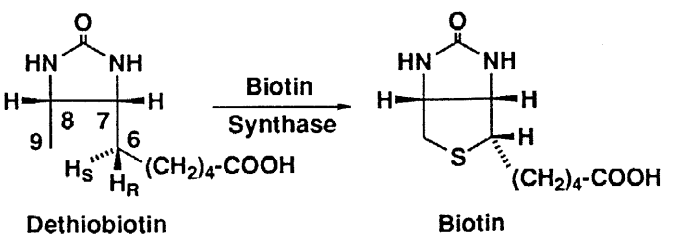
Received 26 June 1998; accepted 28 July 1998

Abstract: Reduction of biotin or epibiotin sulfoxides with nickel boride and deuterated reagents affords as major products bideuterated dethiobiotin. Deuterium localisation by [^{2}H] NMR in a chiral liquid crystal allows the analysis of the stereoselectivity of the reaction.

In the course of mechanistic studies of biotin synthase [1,2], the enzyme responsible for the last step of biotin biosynthesis (scheme 1) we needed two dethiobiotin (DTB) samples deuterated at the 6pro-R and 6pro-S positions.

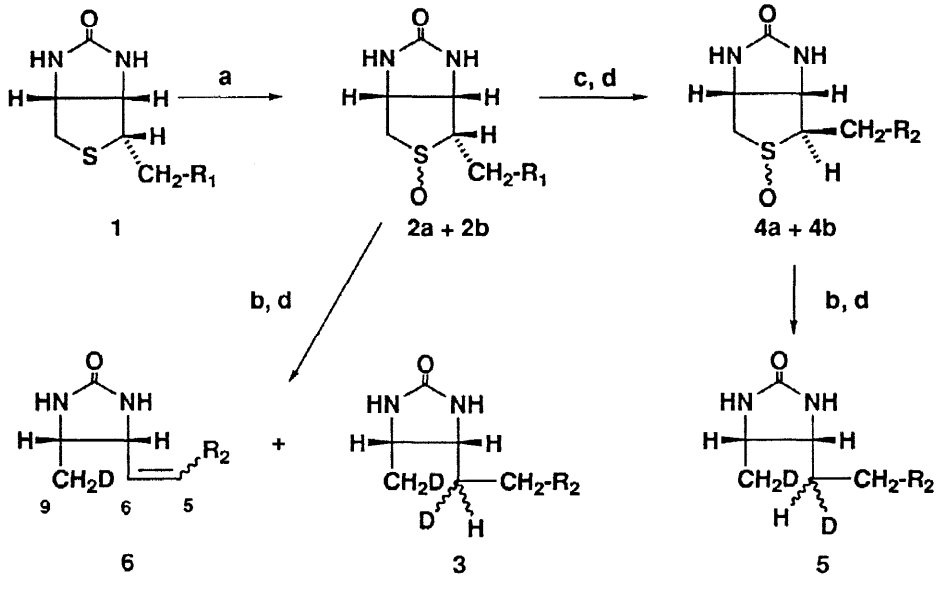


Scheme 1

The corresponding 6-[^{3}H] labeled molecules have already been synthesized by a rather long route [3]. Instead of repeating this strategy we checked the very straightforward method described by T.G. Back et al. [4] for the hydrogenolysis of C-S bonds in thiols and dialkylsulfides or sulfoxides, reported to proceed

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01625-6

stereoselectively with retention of configuration. Assuming this hypothesis, reduction of biotin or biotin sulfoxides **2a+2b** should produce the 6(R) deuterated product whereas reduction of epibiotin or epibiotin sulfoxides **4a+4b** should lead to the 6(S) isomer (Scheme 2). Of course this reaction introduces an additional deuterium at C-9 but this will not prevent the interpretation of the biochemical results [2].



Scheme 2

Reaction of biotin sulfoxides **2a+2b** with $NiCl_2$, $NaBD_4$ in CH_3OD followed by esterification and purification gave **3** in 36% overall yield. A secondary product was isolated with a very low yield (1%). According to its ^1H NMR spectrum, it is a 1/4 mixture of *Z* and *E* 5,6-dehydrodethiobiotin **6**. Epibiotin sulfoxides **4a+4b** were obtained by epimerisation of **2a+2b** in NaOH 1N [5]. The major diastereoisomer was isolated and converted into **5**, as described for **2a+2b**.

The determination of the stereoselectivity of the reaction raised however a problem since the two hydrogens at C-6 are not differentiated by ^1H NMR. This difficulty could be solved by using the method described by one of us namely [^2H] NMR in polypeptide liquid crystal [6]. This technique relies on the fact that enantiotopic or diastereotopic nuclei have different orientations when dissolved in poly-γ-benzyl-L-glutamate (PBLG) / dimethyl formamide (DMF) chiral liquid crystal mixtures. This

orientation difference affects the order dependent NMR interaction such as the deuterium quadrupolar couplings. The proton decoupled deuterium spectrum of compounds **3** and **5** dissolved in the chiral liquid crystal is represented respectively in Figures 1a and 1b.

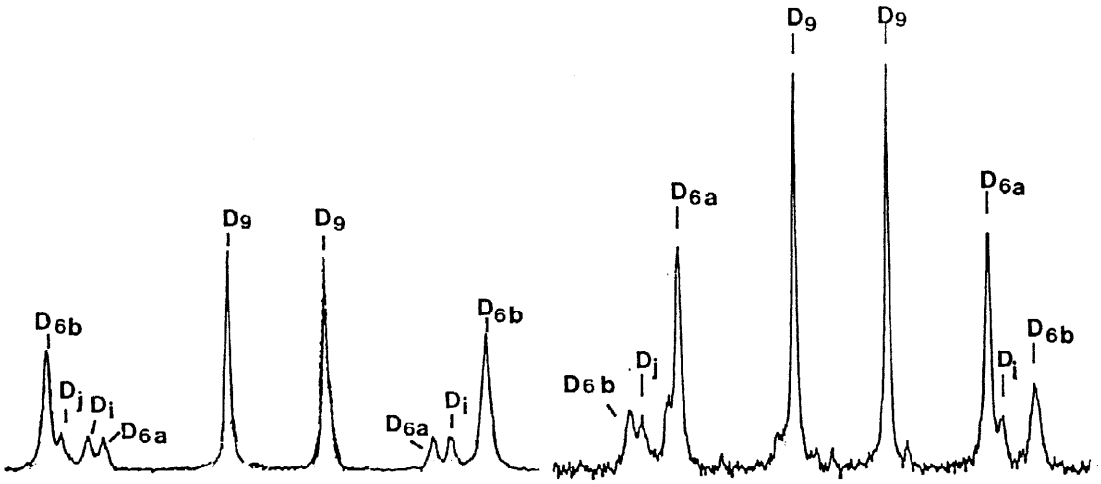
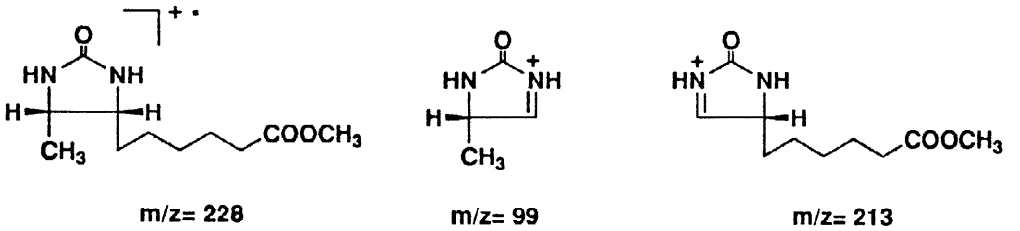


Fig. 1a Fig. 1b

Figure 1. Proton decoupled [^{2}H] NMR spectra of **3** (Fig.1a) and **5** (Fig.1b) dissolved in PBLG/DMF liquid crystal

It is clear from the chemical shifts that the central doublet corresponds to the deuterium at C-9. Two other quadrupolar doublets designed as D6a and D6b with reversed intensity in **3** and **5** are attributed to the two epimeric deuterons at C-6. Of course, the spectrum does not allow the stereochemical assignment of D6a and D6b [7]. The spectrum shows two additional doublets designed as Di and Dj revealing the presence of two other deuterons. The ^{1}H NMR spectra of **3** and **5** show a single coupling figure for H_7 and H_8 showing that the impurity is not due to isomerisation at these positions. The presence of both D6a and D6b on the spectra of **3** and **5** may indicate that the hydrogenolysis of the C-S bond is not completely stereoselective. Another explanation, relying on the isolation of the unsaturated compound **6**, is that the hydrogenolysis could be stereoselective, the extra peaks corresponding to the deuteration of an intermediate double bond on both faces.

The electron impact mass spectrum of DTB taken as reference, revealed two characteristic fragments at m/z= 99 and m/z= 213 (Scheme 3) identified in a previous work [8]. Mass spectrometry analysis of these fragments in **3** and **5** (Table 1) indeed confirms the presence of extra deuterium atoms localised on the side chain.



Scheme 3

	m/z= 99	m/z= 100	m/z= 213	m/z= 214	m/z= 215
3	15%	85%	17%	59%	13%
5	12.5%	87.5%	11%	66%	13%

Table 1. Percentage of deuteriation of characteristic fragments of **3** and **5** obtained from EIMS [9]

These results emphasize the power of the [^{2}H] NMR technique, since it would have been otherwise difficult to localise the deuterium atoms. It brings light on the mechanism of the reduction which should be further studied.

Acknowledgement: We thank F. Fournier and D. Lesage for mass spectrometry analysis.

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