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On the Structures of Some Adducts of Biotin with Electrophiles: Does Sulfur Transannular Interaction with the Carbonyl Group Play a Role in the Chemistry or Biochemistry of Biotin?

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Biotin methyl ester (**1**), 1′-*N*-carbomethoxy biotin methyl ester (**2**) and 1′-*N*-trifluoroacetylbiotin methyl ester (**3**) were treated with boron trifluoride etherate and triethyloxonium tetrafluoroborate. Whereas no reaction could be observed in the case of **3**, coordination of the electrophiles to the urea oxygen could be deduced from the IR, ^{1}H-NMR, and ^{13}C-NMR spectra of the adducts obtained from **1** and **2**. X-Ray analysis of the **1**/BF_3-adduct (**4**) confirmed the O-coordination, but showed no transannular sulfur–carbonyl interaction. From comparison of the spectral data obtained for all addition products it is concluded that none of them shows a significant transannular sulfur–carbonyl interaction. Reaction of **3** with SbF_5 also formed an O-coordinated adduct (**10**) without sulfur transannular bonding. In magic acid ($FSO_3H/SbF_5/SO_2$) both **1** and **3** added a proton to the urea oxygen, to the sulfur atom, and to the ester group, as reported previously for biotin itself. The relationship of these findings to the kinetics of acid-catalyzed NH exchange in biotin, and to possible mechanisms of biochemical biotin-catalyzed reactions, are discussed.

INTRODUCTION

Biotin (**1**, but R = $(CH_2)_4CO_2H$) covalently attached to an enzyme through its carboxyl group, plays the central role in carboxylation reactions that utilize bicarbonate and ATP (*1a*). In an intermediate step, ATP is converted to ADP and phosphate, and the bicarbonate becomes a carboxyl group on the 1′-*N* (cf. structure **e** of Fig. 2) (*1b*). In a later step this carboxyl group is transferred to a substrate. There is still some dispute as to the precise mechanism by which this sequence occurs (*2, 3*), and no agreement at all on the relationship of biotin's structure to its function. In particular, the sulfur atom of biotin is added at the last stages of its biosynthesis, and presumably is important to its chemistry (*4*), but it is not clear why.

One possible function of the sulfur would be a transannular interaction with the carbon of the urea group (*5*). Such an interaction has been suggested previously (*6*), but X-ray studies (*7*) on biotin show that the distance is too large. However,

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in most of the mechanisms one can invoke for the action of biotin there is attack on it by an electrophilic species. In some mechanisms (e.g., that of Fig. 2, presented later) the electrophile is a phosphate group attacking the carbonyl oxygen, while in other mechanisms (*3*) it is a carboxyl group of a carbonic-phosphoric anhydride species that attacks, or even an enzyme catalytic group. One would expect that a possible transannular interaction of the sulfur atom with the urea carbon could be increased if the urea oxygen were attacked by an electrophile. This possibility is made especially intriguing by the recent finding by Mildvan (*8*) that hydrogen exchange on 1′-*N* in biotin can be second order in hydrogen ion. However, in O-heterobiotin or in desthiobiotin, both lacking the sulfur, only first-order kinetics are observed. A possible explanation for this finding involves transannular interaction of the sulfur atom with a protonated biotin urea group. For all these reasons it seemed desirable to determine the structure of adducts of electrophiles with biotin. The only previous relevant study was the NMR work by Olah (*9*) using biotin in $FSO_3H/SbF_5/SO_2$. He observed triprotonation of biotin: at the urea oxygen, at the carboxyl group, and at the sulfur atom.

We were able to prepare five relevant adducts. In one case good crystals were obtained that permitted us to determine the X-ray structure, and the other four adducts (detected in solution) had similar spectroscopic properties and thus presumably similar structures. As it turned out, no evidence could be obtained for the hypothesized transannular sulfur interaction.

RESULTS

D-biotin methyl ester (**1**) and 1′-*N*-carbomethoxybiotin methyl ester (**2**) were prepared according to literature procedures (*10*). Treatment of biotin methyl ester (**1**) with trifluoroacetic anhydride produced the 1′-*N*-trifluoroacetyl derivative **3**. Only the 1′-*N* isomer was detected. In the synthesis of **2**, 5% of the hindered 3′-*N* isomer were formed and removed by chromatography. Each of the compounds

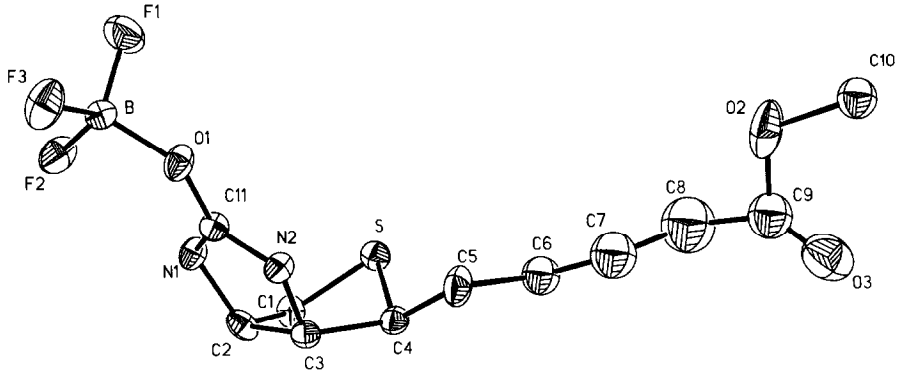
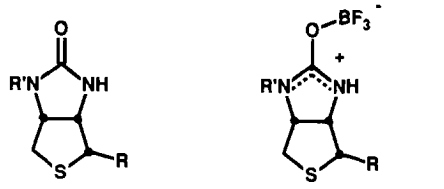
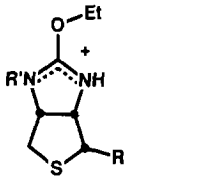
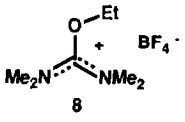
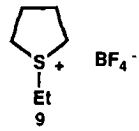


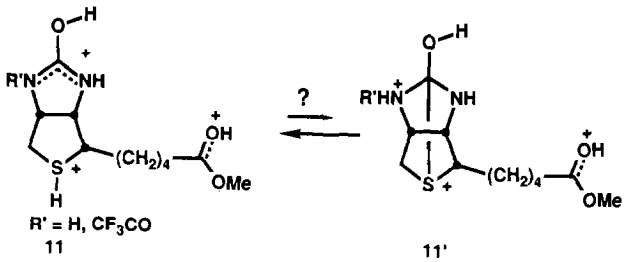
FIG. 1. ORTEP diagram of the BF_3-adduct **4** showing 30% probability ellipsoids, with selected structural data. Selected bond lengths (Å): B–O1 1.449 (10); O1–C11 1.283 (10); N1–C11 1.309 (9); N2–C11 1.286 (10); S–C11 3.680 (10). No *intermolecular* S–C11 distances <5.87 Å could be observed.











(**1–3**) was examined in its reaction with BF_3 etherate and with triethyloxonium tetrafluoroborate. The trifluoroacetyl derivative **3** was not converted to an adduct by either of these reagents, but the other two biotin derivatives reacted.

Reaction of biotin methyl ester (**1**) with BF_3 etherate afforded a crystalline material **4** whose structure was determined by X-ray crystallography. The structure (Fig. 1) shows that the BF_3 is coordinated to the biotin urea oxygen (on the unhindered side away from the sidechain) and that there is no detectable transannular interaction of the sulfur atom with the carbonyl group. The distance between

sulfur and carbon in the BF_3 adduct is 3.65 Å, compared with 3.68 Å in biotin itself (*7*). This length is well beyond any sensible bonding distance, since typical S—C bonds are ca. 1.8 Å long. Indeed, the sum of the van der Waals radii of S and C is 3.55 Å, so the sulfur and carbon are just in van der Waals contact (*6*). The lack of significant shortening with BF_3 coordination also indicates that there is no transannular interaction.

Further evidence on this could be seen in the ^{1}H-NMR, the ^{13}C-NMR, and the IR spectra of the BF_3 adducts **4** and **5**. The ethylated biotin derivatives **6** and **7** had analogous spectral properties. ^{1}H-NMR spectroscopic assignments were based on the coupling patterns: the 5-H_{endo} as a doublet, the 5-H_{exo} as a doublet of doublets, etc. In the ^{13}C-NMR, the only significant (larger than 1 ppm) shifts from the spectrum of biotin methyl ester (**1**) were observed for the two doublet signals of C-3 and C-4 in **4** and **6**, both of which shift by ca. 4 ppm to lower field. The data are listed in the Experimental section and in Table 1. For comparison to the spectroscopic data on compounds **4–7**, we have prepared compounds **8** (*11*) and **9** (*12*). Their NMR data are included under Experimental.

The data all together show that in **4–7** the electrophile is bonded to the oxygen of the urea group, as in the characterized adduct **4** whose X-ray structure was determined. In the infrared, all four adducts show a C=O shift to longer wavelengths and a C—N shift to shorter wavelengths, as expected for oxygen coordination (*13a*). Furthermore the ^{13}C-NMR of the ethyl group in **6** shows the methylene carbon in the region characteristic of oxygen coordination (as in **8**), not sulfur coordination (as in **9**). The data show that there is no significant transfer of positive charge to the sulfur atom by transannular bonding, since the NMR shifts are just those expected if an electrophile is coordinated to the carbonyl group (cf. the data on an O-ethylated imidazolone in (*13b*), we thank Professor Kohn for calling this work to our attention). As Table 1 shows, the shifts occurring when electrophiles are coordinated to **1** or **2** are similar in magnitude.

The trifluoroacetyl derivative **3** did form an adduct (**10**) with the stronger Lewis acid SbF_5 in SO_2 at −20°C. The IR data appear under Experimental; the NMR summary in Table 1 shows that this adduct also involves urea coordination without sulfur participation. With a large excess of SbF_5 there is apparently additional coordination to the sulfur atom, leading to large downfield shifts of 2-H and 5-H.

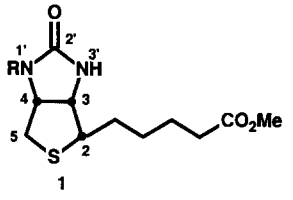
Olah had reported the NMR spectrum of biotin in magic acid (*9*). We examined biotin methyl ester (**1**) and the trifluoroacetyl derivative **3** in the same medium. We find the same pattern, with three protons added one each to the urea carbonyl, the sulfur, and the ester carbonyl group (cf. **11**). As in Olah's study, the proton on sulfur shows a clear six-line pattern as a doublet of triplets. The other two protons appear as sharp singlets in the expected region, as in Olah's work.

DISCUSSION

Coordination of Lewis acids like BF_3 and SbF_5 or alkylation on oxygen of biotin furnish species that are related to some of the intermediates that one can invoke in

TABLE 1

SUMMARY OF ^{1}H-NMR CHEMICAL SHIFT DIFFERENCES OBSERVED UPON REACTING **1**, **2**, AND **3** WITH ELECTROPHILES



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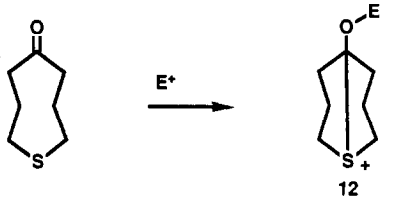
Electrophile: Proton	BF_3		$Et_3O^+BF_4^-$		SbF_5
	x = H[a]	x = —CO_2Me[b]	x = —H[c]	x = —CO_2Me[d]	x = $COCF_3$[e]
5-H_{endo}	−0.21	−0.05	−0.24	−0.07– −0.14	−0.12
5-H_{exo}	−0.10	−0.06	−0.25		−0.10– −0.12
2-H	−0.14	−0.12– −0.18	−0.18– −0.24	−0.23– −0.24	−0.19
4-H	−0.30– −0.31	−0.19– −0.20	−0.42– −0.45	−0.30– −0.35	−0.22
3-H	−0.25– −0.26	−0.39 +0.15	−0.39– −0.43 −2.31	−0.54– −0.62 −1.43	−0.28 −1.24
NH	−1.40– −1.53				
NH			−2.25		

[a] δ(**1**)-δ(**4**).
[b] δ(**2**)-δ(**5**).
[c] δ(**1**)-δ(**6**).
[d] δ(**2**)-δ(**7**).
[e] δ(**3**)-δ(**10**).

biotin biochemistry. They are also related to the O-protonated biotin that one can invoke in the acid catalyzed H/D-exchange reaction of biotin (*8*). However, our data indicate that no sulfur coordination to the carbonyl group occurs, and instead the positive charge is entirely stabilized by conjugative interaction within the urea unit. Such conjugative stabilization is the likely reason that transannular sulfur interaction was not observed, since it is well known that 1-thiacyclooctane-5-ones show strong transannular interaction (*5*), especially on reaction of the carbonyl oxygen with electrophiles (*12*, Eq. [1]).



[1]

We thought that we might diminish such conjugative interaction by examining *N*-carbomethoxybiotin methyl ester (**2**), in which the carbomethoxy group that deactivates one nitrogen resembles the substituent in 1′-*N*-carboxybiotin, the apparent biological intermediate (structure **e** in Fig. 2). However, the spectroscopic data on reaction products **5** and **7** show also that reaction occurs on the urea oxygen and that there is no detectable sulfur interaction as judged from the spectroscopic parameters. Thus even here coordination of an electrophile to the urea oxygen leads to a cation whose charge is delocalized essentially within the urea group.

One might expect even less conjugative stabilization of such a species if the nitrogen carried a stronger electron attracting group; for this reason we have prepared 1′-*N*-trifluoroacetyl biotin methyl ester (**3**). In this compound the urea group is so deactivated that it no longer gave detectable adduct formation with BF_3 etherate or with triethyloxonium tetrafluoroborate. However, with SbF_5 it did form an adduct (**10**) at the urea carbonyl, but again no sulfur participation was observed.

It had seemed to us that an attractive mechanism could be written for *N*-carboxylation of biotin (Fig. 2) in which transannular sulfur coordination is critical to make the nitrogen-1′ basic enough to permit attack on the carboxyl. However, no support for such a mechanism can be invoked from our current data.

Our findings leave unclear the explanation of the unusual kinetics of deuterium

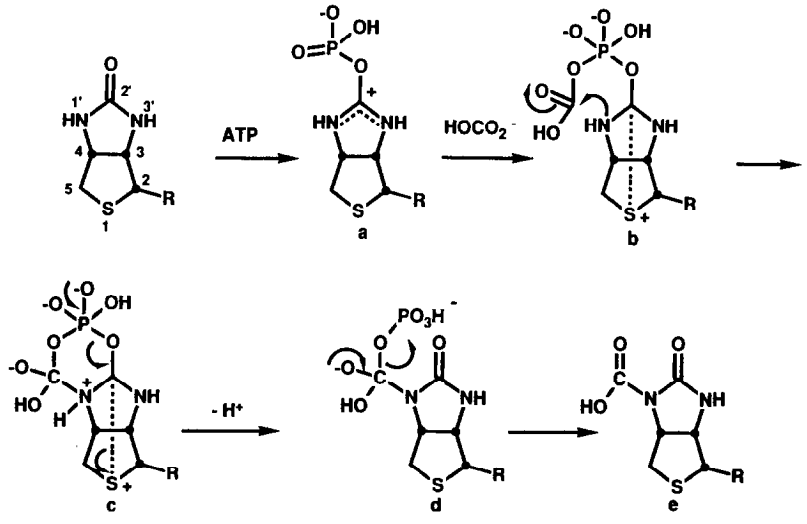


FIG. 2. A possible mechanism of biochemical biotin carboxylation.

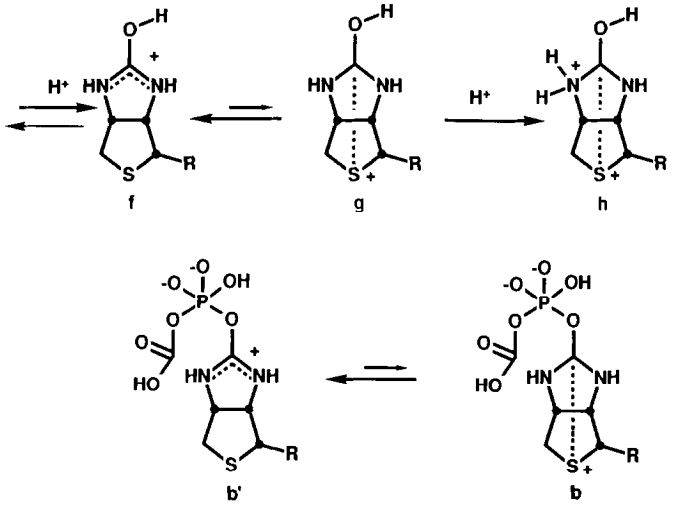


FIG. 3. Equilibria between isomers with transannular sulfur bonding and those without such interaction.

exchange observed by Mildvan (*8*), unless extensive sulfur interaction occurs only when *two* protons are added to the urea unit. In the proposed mechanism of Fig. 2, this would have an analog if the sulfur participation were complete *only* in structure **c**, a biotin derivative with *two* attached electrophiles. The urea-group conjugation is blocked at one nitrogen, so sulfur participation could become important. In both the Mildvan experiment (*8*) and in the mechanism of Fig. 2 there could be equilibria (Fig. 3) between a major isomer without sulfur interaction (**f** or **b'**) and a minor isomer with it (**g** or **b**). Then in the Mildvan exchange experiment (*8*) protonation of the basic nitrogen in the cyclized isomer (**g**) would lead to the observed second order kinetics in $[H^+]$. In our mechanism (Fig. 2) the cyclization of **b** to **c** would occur only with the sulfur-participating isomer **b**, not with an open-chain analog **b'** (Fig. 3).

It is usually considered that carboxylation of the biotin nitrogen occurs through an iminol structure (related to an enol) in which the urea nitrogen has lost its proton. As structure **b** of Fig. 2 shows, transannular bonding by sulfur would make this a basic nitrogen even *without* deprotonation, since such interaction removes the deactivating carbonyl group. In its biochemical functions, the sulfur of biotin might be playing only the geometric role in enforcing planarity that has been suggested for it previously (*7*). Alternatively, it is still possible that in the enzymatic reactions some transannular sulfur interaction indeed occurs (as in Fig. 2), possibly favored by the conformation in the enzyme pocket.

To explain his second order kinetics in $[H^+]$ for proton exchange at 1'-*N*, Mildvan (in part at our suggestion) (*14*) invoked a species **h** (Fig. 3) with one proton on the carbonyl oxygen, one proton on 1'-*N*, and an S—C bond. Of course Olah had examined biotin in magic acid (*9*), and found that triprotonated biotin has one proton on the urea oxygen and one on the *sulfur*, in addition to a proton on the carboxyl group. The assignment of a protonated sulfur is based on the coupling

pattern of this proton, and seems unambiguous. Furthermore, we have confirmed this protonation pattern both for biotin methyl ester (**1**) and for the trifluoroacetyl derivative (**3**). That is, triprotonation produces structure **11**, not **11′**. If **11′** is formed at all, it must be in small amount. Thus if the species **h** postulated in the Mildvan paper is a kinetic intermediate in acid-catalyzed exchange, it must be a minor isomer. The general possibility that minor isomers are involved in the biochemical mechanisms as well means that one cannot exclude mechanisms such as that of Fig. 2. We simply have as yet no direct evidence in support of such mechanisms.

EXPERIMENTAL

Chemicals were purchased from Aldrich. Triethyloxonium tetrafluoroborate was freshly prepared prior to use (*15*). Melting points are uncorrected. Elemental analyses were carried out by Schwarzkopf analytical labs, New York. IR spectra were recorded on a Perkin–Elmer 1420 instrument. ^{1}H-NMR spectra were measured at 200 MHz on a Varian XL-200 spectrometer or at 300 MHz on a Bruker WM-300 spectrometer. ^{13}C-NMR spectra were taken at 75.5 MHz on a Bruker WM-300 instrument. All manipulations on the compounds **4–7** were carried out in a drybox under argon atmosphere.

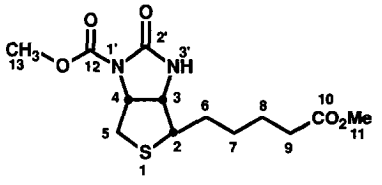
1′-N-Carbomethoxybiotin Methyl Ester (**2**)

Biotin methyl ester (**1**) was acylated with methyl chloroformate as described by Lynen *et al.* (*10*). The crude product mixture obtained from 1.50 g (5.81 mmol) **1** was subjected to flash-chromatography on silica gel (90 g, eluting with chloroform/methanol 95 : 5) to afford 1.11 g (57%) of the pure 1′-*N*-carbomethoxy isomer **2** as a colorless oil besides 458 mg (24%) of a mixture of the 1′-*N* and 3′-*N* isomers. The pure isomer **2** was crystallized from aqueous methanol to afford colorless needles, mp 131–132°C (lit. (*10*), 131–132°C).

IR (Nujol): 1749, 1732 (ester C=O), 1710, 1698 (urea C=O) cm^{-1}.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 1.28–1.76 (m; 6H, 6,7,8-H), 2.30 (br. t, J = 7.6 Hz; 2H, 9-H), 2.94 (dd, J = 13.6 Hz, 4.9 Hz; 1H, 5-H_{exo}), 3.09 (d, J = 13.6 Hz; 1H, 5-H_{endo}), 3.07–3.22 (m; 1H, 2-H), 3.60 (s; 3H, 11-H), 3.80 (s; 3H, 13-H), 4.17–4.21 (m; 1H, 3-H), 4.81–4.84 (m; 1H, 4-H), 7.64 (br. s; 1H, NH).

^{13}C-NMR (CD_3CN): δ = 25.5 (t; C-6), 28.9 (t; C-7), 29.1 (t; C-8), 34.2 (t; C-9), 39.2 (t; C-5), 51.9 (q; C-11), 53.5 (q; C-13), 56.1 (d; C-2), 58.5 (d; C-3), 63.7 (d; C-4), 153.1 (s; C-12), 156.3 (s; C-2′), 174.6 (s; C-10).



2

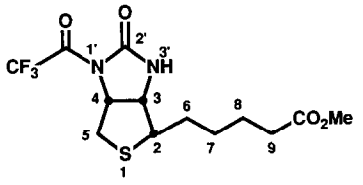
1'-N-Trifluoroacetylbiotin Methyl Ester (3)

Biotin methyl ester (**1**) (350 mg, 1.36 mmol) was dissolved in 7 ml abs chloroform, 2.0 ml (2.97 g, 14.2 mmol) trifluoroacetic anhydride was added and the solution refluxed under exclusion of moisture for 22 h. The solvent and excess trifluoroacetic anhydride were pumped off and the remaining colorless oil crystallized from hexane/benzene, affording 443 mg (92%) colorless needles, mp 97°C.

IR (Nujol): 1761 (ester C=O); 1715, 1701 (urea, amid C=O) cm^{-1}.

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 1.41–1.83 (m; 6H, 6,7,8-H), 2.34 (br.t, *J* = 7 Hz; 2H, 9-H), 3.07–3.13 (m; 2H, 5-H), 3.20–3.33 (m; 1H, 2-H), 3.67 (s; 3H, 11-H), 4.30–4.40 (m; 1H, 3-H), 4.92–5.03 (m; 1H, 4-H), 7.01 (br. s; 1H, NH).

Anal. Calcd for $C_{13}H_{17}F_3N_2O_4S$: C, 44.07; H, 4.84; N, 7.91. Found: C, 44.02; H, 4.86; N, 7.91.



3

Addition of $BF_3 \cdot Et_2O$ to Biotin Methyl Ester (1)

Biotin methyl ester (**1**) (250 mg, 0.967 mmol) was placed into a Schlenck tube under nitrogen and dissolved in 15 ml abs chloroform. A solution of 142 mg (1.00 mmol) freshly distilled $BF_3 \cdot Et_2O$ in 2 ml abs chloroform was syringed into the stirred solution of **1** at ca. 20°C. After ca. 1 min, a colorless solid began to precipitate. After completion of the precipitation (ca. 15 min), the solid was filtered off, washed with abs $CHCl_3$ (2 × 5 ml), and dried at 20°C and 0.1 Torr; 210 mg (67%) of a colorless crystalline material was obtained. Final purification was achieved by recrystallization from benzene/acetonitrile, affording the adduct **4** as colorless plates, mp 138°C (dec.).

IR (Nujol): 1736, 1718 (ester C=O), 1689 (urea C=O), 1559 (C—N) cm^{-1}.

^{1}H-NMR (CD_3CN, 200 MHz): δ = 1.38–1.79 (m; 6H, 6,7,8-H), 2.33 (br. t, *J* = 7.2 Hz; 2H, 9-H), 2.86 (d, *J* = 12.7 Hz; 1H, 5-H_{endo}), 3.00 (dd, *J* = 12.7 Hz, 4.8 Hz; 1 H, 5-H_{exo}), 3.27–3.36 (m; 1H, 2-H), 3.63 (s; 3H, 11-H), 4.47–4.54 (m; 1H, 3-H), 4.70–4.77 (m; 1H, 4-H), 6.74 (br. s; 2H, NH).

^{13}C-NMR (CD_3CN): δ = 25.6 (t; C-6), 28.9 (t; C-7), 29.2 (t; C-8), 34.3 (t; C-9), 40.2 (t; C-5), 52.0 (q; C-11), 56.0 (d; C-2), 63.5 (d; C-4), 64.3 (d; C-3), 163.9 (s; C-2'), 174.8 (s; C-10).

Anal. Calcd. for $C_{11}H_{18}BF_3N_2O_3S$: C, 40.51; H, 5.56; N, 8.59. Found: C, 40.66; H, 5.83; N, 8.87.

For comparison, the spectral data of biotin methyl ester (**1**) are given here, too:

IR (Nujol): 1741 (ester C=O), 1705 (urea C=O) cm^{-1}.

^{1}H-NMR (CD_3CN, 200 MH): δ = 1.36–1.74 (m; 6H, 6,7,8-H), 2.32 (br. t, *J* = 7.2 Hz; 2H, 9-H), 2.65 (d, *J* = 12.7 Hz; 1 H, 5-H_{endo}), 2.90 (dd, *J* = 12.7 Hz, 4.8 Hz;

1H, 5-H_{exo}), 3.13–3.22 (m; 1H, 2-H), 3.63 (s; 3H, 11-H), 4.22–4.28 (m; 1H, 3-H), 4.40–4.46 (m; 1H, 4-H), 5.21 (br. s; 1H, NH), 5.34 (br.s; 1H, NH).

^{13}C-NMR ($CDCl_3$, 67.9 MHz) (*16*): δ = 24.8 (t; C-6), 28.3 (t; C-7), 28.4 (t; C-8), 33.7 (t; C-9), 40.6 (t; C-5), 51.6 (q; C-11), 55.5 (d; C-2), 60.2 (d; C-4), 62.0 (d; C-3), 163.7 (s; C-2'), 174.1 (s; C-10).

X-Ray Analysis of the BF_3-Adduct 4

The BF_3-adduct **4** crystallizes orthorhombically in the space group $P2_12_12_1$ with a = 4.991(1) Å, b = 8.585(3) Å, c = 34.380(8) Å. The unit cell contains Z = 4 formula units and the density was calculated to be 1.47 g · cm^{-3}. The orientation matrix and the cell parameters were determined from a clear colorless crystal of dimensions 0.75 × 0.72 × 0.25 mm on a Nicolet *R3m* diffractometer. The intensities of 2116 reflections were measured, 1268 of them with $I > 3\sigma(I)$ were applied for structure determination, R = 0.083. For details see supplementary material paragraph at the end of this section.

Addition of $Et_3O^+BF_4^-$ to Biotin Methyl Ester (I)

$Et_3O^+BF_4^-$ (350 mg, 1.84 mmol) and biotin methyl ester (**1**) (380 mg, 1.47 mmol) were placed into a Schlenck tube under nitrogen. Ten milliliters abs chloroform was added and the mixture stirred at ca. 20°C. After ca. 30 min, a homogenous solution was obtained. The solvent was then pumped off to afford the adduct **6** as a highly viscous colorless oil.

IR (Nujol): 1724 (ester C=O), 1635 (urea C=O), 1599 (C—N) cm^{-1}.

^{1}H-NMR (CD_3CN, 200 MHz): δ = 1.39–1.86 (m; 6H, 6,7,8-H), 1.41 (t, J = 7.0 Hz; 3H, CH_3—CH_2—O), 2.34 (br. t, J = 7.3 Hz; 2H, 9-H), 2.89 (d, J = 13.6 Hz; 1H, 5-H_{endo}), 3.15 (dd, J = 13.6 Hz, 4.7 Hz; 1H, 5-H_{exo}), 3.31–3.46 (m; 1H, 2-H), 3.63 (s; 3H, 11-H), 4.43 (q, J = 7.0 Hz; 2H, CH_3—CH_2—O), 4.61–4.71 (m; 1H, 3-H), 4.82–4.91 (m; 1H, 4-H), 7.52 (br. s; 1H, NH), 7.59 (br.s; 1H, NH).

^{13}C-NMR (CD_3CN): δ = 14.4 (q; CH_3—CH_2—O), 25.5 (t; C-6), 28.8 (t; C-7), 29.1 (t; C-8), 34.2 (t; C-9), 39.8 (t; C-5), 51.9 (q; C-11), 55.9 (d; C-2), 64.5 (d; C-4), 66.0 (d; C-3), 72.3 (t; CH_3—CH_2—O), 164.9 (s; C-2'), 174.7 (s; C-10).

Addition of $BF_3 \cdot Et_2O$ to 1'-N-Carbomethoxybiotin Methyl Ester (2)

A solution of 18.5 mg (58.5 μmol) 1'-*N*-carbomethoxy biotin methyl ester (**2**) in 0.6 ml abs $CDCl_3$ was placed into an NMR tube and cooled to −40°C. Eighty microliters of a 0.81 M solution of $BF_3 \cdot Et_2O$ in abs $CDCl_3$ were syringed in. ^{1}H-NMR and IR spectra were taken before and after the $BF_3 \cdot Et_2O$ addition at −40°C and during warmup to ca. 20°C.

IR (neat):[3] 1683 (urea C=O), 1547 (C—N) cm^{-1}.

^{1}H-NMR ($CDCl_3$, 300 MHz); δ = 1.30–1.72 (m; 6H, 6,7,8-H), 2.31 (br. t, J = 7.3 Hz; 2H, 9-H), 3.00 (dd, J = 13.8 Hz, 4.4 Hz; 1H, 5-H_{exo}), 3.14 (d, J = 14.1 Hz; 1H, 5-H_{endo}), 3.25–3.34 (m; 1H, 2-H), 3.62 (s; 3H, 11-H), 3.85 (s; 3H, 13-H), 4.56–4.60 (m; 1H, 3-H), 5.00–5.04 (m; 1H, 4-H), 7.49 (br. s; 1H, NH).

[3] Complex pattern in the C=O region.

Addition of $Et_3O^+BF_4^-$ to 1'-N-Carbomethoxy biotin methyl ester (2)

A solution of 20.0 mg (105 μmol) $Et_3O^+BF_4^-$ in 0.5 ml dry CD_2Cl_2 was placed into an NMR tube and cooled to −40°C. 1'-*N*-Carbomethoxy biotin methyl ester (**2**) (30.0 mg, 94.7 μmol) was dissolved in ca. 0.5 ml dry CD_2Cl_2 and syringed into the NMR tube. ^{1}H-NMR and IR spectra were taken before and after the $Et_3O^+BF_4^-$ addition at −40°C and during warmup to ca. 20°C.

IR (neat):[3] 1646 (urea C=O), 1531 (C—N) cm^{-1}.

^{1}H-NMR (CD_2Cl_2, 300 MHz): δ = 1.39–1.78 (m; 6H, 6,7,8-H), 1.51 (t, *J* = 7.0 Hz; 3H, CH_3—CH_2—O), 2.31 (br. t, *J* = 7.4 Hz; 2H, 9-H), 3.08–3.16 (m; 2H, 5-H), 3.31–3.45 (m; 1H, 2-H), 3.61 (s; 3H, 11-H), 3.81 (s; 3H, 13-H), 4.60–4.83 (m; 3H, 3-H, CH_3—CH_2—O), 5.11–5.19 (m; 1H, 4-H), 9.07 (br. s; 1H, NH).

Treatment of 1'-N-Trifluoroacetylbiotin Methyl Ester (3) with BF_3-$Et_3O^+BF_4^-$

No reaction could be observed by IR or ^{1}H-NMR when 1'-*N*-trifluoroacetylbiotin methyl ester (**3**) was treated with ca. 1.2 molar amounts of $BF_3 \cdot Et_2O$ or $Et_3O^+BF_4^-$ in dry $CDCl_3$ or CD_2Cl_2 at ca. 20°C for up to 17 h.

^{1}H-NMR of Biotin Methyl Ester (1) and 1'-N-Trifluoroacetylbiotin Methyl Ester (3) in Magic Acid (11)

Molar mixtures (1 : 1) of fluorosulfonic acid and antimony pentafluoride were prepared under N_2 and diluted with an equal volume of liquid SO_2. Solutions of biotin methyl ester (**1**) or 1'-*N*-trifluoroacetylbiotin methyl ester (**3**) in liquid SO_2 were added at −78°C to afford ca. 5% (by weight) of the substrate in the magic acid solvent. CD_2Cl_2 in an internal capillary was used for the lock signal and as the reference.

Biotin methyl ester (1)/Magic Acid

^{1}H-NMR ($FSO_3H/SbF_5/SO_2$, 300 MHz, −60°C): δ = 1.94–2.48 (m; 6H, 6,7,8-H), 3.30 (t, *J* = 7.0 Hz; 2H, 9-H), 4.10 (ddd, *J* = 5.5 Hz, 14.8 Hz, 15.6 Hz; 1H, 5-H_{exo}), 4.28 (dd, *J* = 3.9 Hz, 15.6 Hz; 1H, 5-H_{endo}), 4.47–4.55 (m; 1H, 2-H), 4.70 (s; 3H, 11-H), 5.58–5.62 (m; 1H, 3-H), 5.74–5.79 (m; 1H, 4-H), 6.44 (dt, *J* = 3.9 Hz, 14.8 Hz; 1H, S^+-H), 7.13 (br.s; 1H, NH), 7.30 (br.s; 1H, NH), 9.73 (s; 1H, $=O^+$—H), 12.58 (s; 1H, —CO^+—H (OMe)).

1'-N-Trifluoroacetylbiotin methyl ester (3)/Magic acid

^{1}H-NMR ($FSO_3H/SbF_5/SO_2$, 300 MHz, −20°C): δ = 1.95–2.65(m; 6H, 6,7,8-H), 3.30 (t, *J* = 7.3 Hz; 2H, 9-H), 4.40–4.49 (m; 2H, 5-H), 4.66–4.77 (m; 1H, 2-H), 4.71 (s; 3H, 11-H), 5.80–5.84 (m; 1H, 3-H), 6.15–6.25 (m; 1H, 4-H), 6.61–6.73 (m; 1H, S^+—H), 8.55 (br. s; 1H, NH), 10.24 (s; 1H, $=O^+$—H, 12.51 (s; 1H, —CO^+—H(OMe)).

*^{1}H-NMR and IR of the 1′-N-Trifluoroacetylbiotin methyl ester (**3**)/SbF_5 Complex (**10**)*

A 0.24 M solution of SbF_5 in liquid SO_2 was prepared at −78°C and a molar amount of 1′-*N*-trifluoroacetylbiotin methyl ester (**3**) in liquid SO_2 was added. At −30°C, the slow formation of a new set of ^{1}H-NMR signals could be observed. The chemical shift differences caused by SbF_5-coordination are given in Table 1.

After completion of the reaction the SO_2 was evaporated and the ^{1}H-NMR spectrum of the residue retaken in d_6-DMSO. Only the signals of 1′-*N*-trifluoroacetylbiotin methyl ester (**3**) and no decomposition products were observed.

When 1′-*N*-trifluoroacetylbiotin methyl ester (**3**) was treated with a 25-fold excess of SbF_5 in liquid SO_2 at −30°C a rather complex spectrum was obtained. It could clearly be seen, however, that the 2,5-H pattern had shifted downfield by 0.6–0.8 ppm.

IR (neat): 1736 (ester C=O), 1673 (amid C=O), 1624 (urea C=O), 1561 (C—N) cm^{-1}.

*Bis(Dimethylamino)Ethoxycarbenium Tetrafluoroborate (**8**) (11).*

^{1}H-NMR (CD_3CN, 200 MHz): δ = 1.41 (t, *J* = 7.0 Hz; 3H, C*H*$_3$—CH_2-O), 3.04 (s; 12H, CH_3-N), 4.37 (q, *J* = 7.0 Hz; 2H, CH_3—C*H*$_2$—O).

^{13}C-NMR (CD_3CN): δ = 15.2 (q; *C*H_3—CH_2—O); 40.4 (q; *C*H_3—N), 72.9 (t; CH_3—*C*H_2—O), 164.9 (s; *C*O).

*1-Ethyltetrahydrothiophenium Tetrafluoroborate (**9**) (12).*

^{1}H-NMR (CD_3CN, 200 MHz): δ = 1.39 (t, *J* = 7.0 Hz; 3H, C*H*$_3$-CH_2-S), 2.14–2.41 (m; 4H, —CH_2—CH_2—), 3.13 (q, *J* = 7.0 Hz; 2H, CH_3—C*H*$_2$—S), 3.24–3.63 (m; 4H, —CH_2—S—CH_2—).

^{13}C-NMR (CD_3CN): δ = 10.2 (q; *C*H_3—CH_2—S), 29.1 (t; —*C*H_2—*C*H_2), 37.4 (t; CH_3—*C*H_2—S), 43.6 (t; *C*H_2—S—*C*H_2).

Supplementary Material Available

Crystallographic data for the BF_3-adduct **4** can be obtained from Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Requests should include a complete citation of this publication.

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REFERENCES

1. (a) WOOD, H. G., AND BARDEN, R. E. (1977) *Ann. Rev. Biochem.* **46,** 385; (b) GUCHHAIT, R. B., POLAKIS, S. E., HOLLIS, D., FENSELAU, C., AND LANE, M.D. (1974) *J. Biol. Chem.* **249,** 6646.
2. KLUGER, R., DAVIS, P. P., AND ADAWADKAR, P. D. (1979) *J. Amer. Chem. Soc.* **101,** 5995.
3. WALSH, C. (1979) "Enzymatic Reaction Mechanisms," p. 119, Freeman, San Francisco.
4. LANE, M. D., YOUNG, D. L., AND LYNEN, F. (1964) *J. Biol. Chem.* **239,** 2858.
5. LEONARD, N. J., MILLIGAN, T. W., AND BROWN, T. L. (1960) *J. Amer. Chem. Soc.* **82,** 4075.
6. BOWEN, C. E., RAUSCHER, E., AND INGRAHAM, L. L. (1968) *Arch. Biochem. Biophys.* **125,** 865.
7. DE TITTA, G. T., EDMONDS, J. W., STALLINGS, W., AND DONOHUE, J. (1976) *J. Amer. Chem. Soc.* **98,** 1920.
8. FRY, D. C., FOX, T. L., LANE, M. D., AND MILDVAN, A. S. (1985) *J. Amer. Chem. Soc.* **107,** 7659.
9. OLAH, G. A., AND WHITE, A. M. (1968) *J. Amer. Chem. Soc.* **90,** 6087.
10. KNAPPE, J., RINGELMANN, E., AND LYNEN, F. (1961) *Biochem. Z.* **335,** 168.
11. MEERWEIN, H., FLORIAN, W., SCHOEN, N., AND STOPP, G. (1961) *Ann. Chem.* **641,** 1.
12. UMEHARA, M., KANAI, K., KITANO, H., AND FUKUI, K. (1962) *Nippon Kagaku Zasshi* **83,** 1060.
13. (a) HARTMAN, J. S., AND SCHROBILGEN, G. J. (1972) *Canad. J. Chem.* **50,** 713; (b) CRAVEY, M. J., AND KOHN, H. (1980) *J. Amer. Chem. Soc.* **102,** 3928.
14. cf. Acknowledgments of Ref. (*8*).
15. MEERWEIN, H. (1973) *Org. Synth. Coll. Vol. V,* 1080.
16. BRADBURY, J. H., AND JOHNSON, R. N. (1979) *J. Magn. Reson.* **35,** 217.