# Syntheses and Spectral Properties of 2-Thiobiotin and Biotin Derivatives



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A series of methylated derivatives of 2-thiobiotin (2-4) have been prepared as potential aids in elucidation of the mechanism of biotin catalysis. The syntheses and mass, infrared, $^1H$ nmr, and $^{13}C$ nmr spectral properties for these substrates, their 2-thiobiotin precursors (6, 10-14) and the corresponding biotin analogs (1, 15, 31-34) are described. A series of selective proton-proton and proton-carbon decoupling experiments were employed to secure a number of the $^1H$ and $^{13}C$ nmr assignments. Consistent patterns noted throughout the data set proved helpful in structure determination.

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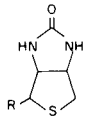
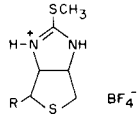
In naturally occurring systems, biotin (**1**) functions as an important carbon dioxide transfer reagent (2). The overall biochemical process proceeds with carboxylation occurring at an activated position on the biological substrate. The detailed chemical mechanisms of these carboxylation reactions, however, are not well understood.

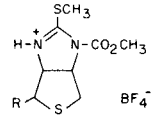


**1** R = $(CH_2)_4CO_2H$

In an effort to gain information concerning these mechanisms, we undertook the synthesis and characterization of three simple, potential affinity labels (**2-4**). Substrates of this type should serve as sensitive probes in determining the active site for the two half-reactions in the biotin-dependent enzyme, acetyl-CoA carboxylase from *E. coli B* (3). In conjunction with the preparation of **2-4**, we have also conducted a careful survey of the spectroscopic properties of biotin and 2-thiobiotin derivatives. Despite the importance of this vitamin (**1**) many of these properties have not been previously recorded for biotin analogs. Moreover, the present compilation leads us to suggest a revision of an earlier assignment (4) of the $^{13}C$ nmr spectra of two 1'-*N*-carbomethoxy derivatives of biotin (**1**) itself.



**2** R = $(CH_2)_4CO_2H$



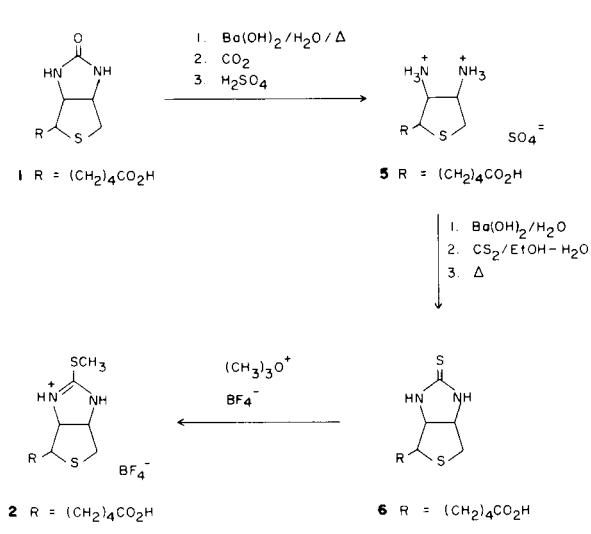
**3** R = $(CH_2)_4CO_2CH_3$

**4** R = $(CH_2)_5OC(O)CH_3$

## Synthesis.

The first substrate **2** is readily accessible in three steps from *d*-biotin (**1**) in 70% overall yield (Scheme I). A procedure comparable to that used by Hofmann, Melville, and du Vigneaud was utilized for the conversion of **1** to the diaminocarboxylic acid sulfate **5** (77% yield) (5). Treatment of the free base of **5** with carbon disulfide gave 2-thiobiotin (**6**). A similar route was previously employed by Green (6). Noteworthy, the yield for this reaction was increased from 31% (6) to 94% by simply: 1) doubling the amount of carbon disulfide used in the reaction, 2) maintaining the reaction temperature (40°) below the boiling point of carbon disulfide (46°) prior to sealing the reactants in a closed vessel, 3) increasing both the temperature and length of time for the sealed tube step of the reaction, and 4) avoiding the use of hydrochloric acid in the work-up procedure (7). The last step in the synthesis of **2** involved the addition of trimethyloxonium fluoroborate to **6**. This reaction proceeded in 97% yield and gave no evidence for the formation of the isomeric *N*-methyl derivatives or the corresponding product in which alkylation had occurred at the tetrahydrothiophene sulfur atom.

Scheme I




**1** R = $(CH_2)_4CO_2H$ — **5** R = $(CH_2)_4CO_2H$

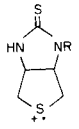
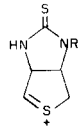
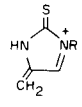
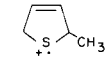
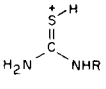
**2** R = $(CH_2)_4CO_2H$ — **6** R = $(CH_2)_4CO_2H$

This result is supported by a competition reaction in which equimolar amounts of imidazolidinethione (**7**) and tetrahydrothiophene (**8**) were treated with one equivalent

0022-152X/81/071425-12$02.25

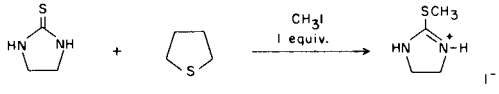
Table I

Molecular Ions in Mass Spectra of Thiobiotin Derivatives
(data expressed as m/e)

| Compound No. | 6 | 12 | 10 | 13 | 11 | 14 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|---|
| Molecular Ion | 260 | 246 | 274 | 288 | 332 (a-c) | 346 (a,c) | 274 (d) | 346 (d) | 360 (d) |
| 17  | (e) | 170 | 198 | 212 | 198 | 212 | (e) | 198 | (e) |
| 18  | (e) | 152 | 166 | (c) | 166 | (e) | (e) | 166 | (e) |
| 19  | 160 | 160 | 160 | 160 | 218 (b) | 218 | 174 (f) | 232 (b,c,f) | 232 (b,c,f) |
| 20  | (e) | 159 | 159 | 159 | 217 (c) | 217 | 173 (f) | 231 (b,f) | 231 (b,f) |
| 21  | 113 | 113 | 113 | 113 | 171 (b,c) | 171 (b) | 127 (f) | 185 (b,c,f) | 185 (b,c,f) |
| 22  | 101 | 101 | 101 | 101 | 159 (a-c) | 159 (a-c) | 115 (f) | 173 (c,f) | 173 (c,f) |
| 23  | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 24  | 87 | 87 | 87 | 87 | 87 | 87 | 87 | 87 | 87 |
| 25  | 85 | 85 | 85 | 85 | 85 | 85 | 85 | 85 | 85 |
| 26  | 77 | 77 | 77 | 77 | 135 (a-c) | 135 (a-c) | 91 (f) | 149 (f) | 149 (b,f) |

(a) Elemental composition verified by high-field mass spectroscopy. (b) A significant peak (>5% of base ion) corresponding to the I-58 fragment was also detected. (c) A. significant peak (75% of base ion) corresponding to the I-59 fragment was also detected. (d) Ion observed corresponded to the P-$HBF_4$ fragment. (e) No significant ion was observed corresponding to this fragment. (f) Value reported is for the *methyl* derivative of the ion drawn.

of methyl iodide. A near quantitative yield of only 2-methylthio-2-imidazoline hydriodide (**9**) was obtained.

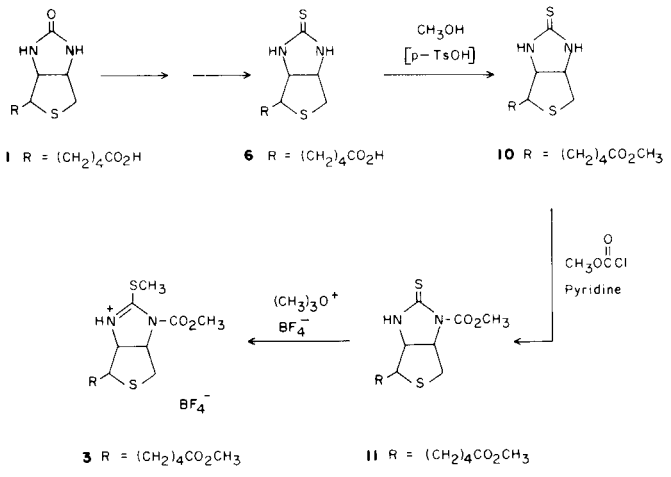


**7** **8** **9**

The synthesis of **3** is outlined in Scheme II. Each step proceeded in high yield except the carbomethoxylation of 2-thiobiotin methyl ester (**10**→**11**). The best results (36% yield) for this conversion were obtained by using a four-fold excess of methyl chloroformate and pyridine. Lower amounts of either the acylating agent or the base led to significant amounts of unreacted starting material (tlc analysis). A 400.1 MHz $^1H$ nmr spectrum of **11** indicated the presence of only the desired *N*1′-isomer (**9**). Methylation at the 2-thione group in **11** with trimethyloxonium fluoroborate gave **3** in excellent yield (98%).

Compound **4** (Scheme III) required the preparation of 2-thiobiotinol (**12**). This substrate was accessible by two independent routes. The pathway generally utilized was conversion of biotin (**1**) to 2-thiobiotin (**6**), followed by LAH

Scheme II

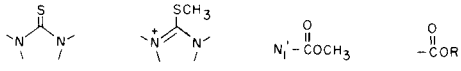



1 R = $(CH_2)_4CO_2H$ 6 R = $(CH_2)_4CO_2H$ 10 R = $(CH_2)_4CO_2CH_3$

3 R = $(CH_2)_4CO_2CH_3$ 11 R = $(CH_2)_4CO_2CH_3$

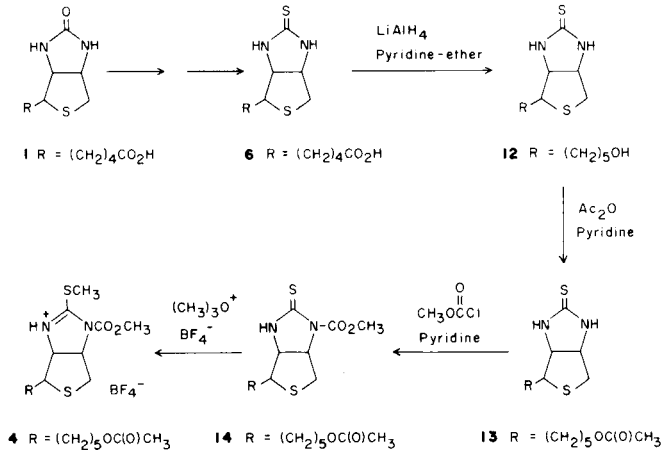
Table II

Summary of Selected Infrared Spectral Properties of Thiobiotin Derivatives.



| No. | Phase | | | | | Other |
|---|---|---|---|---|---|---|
| **6** | KBr | 1535, 1510 | | | 1720 | 1690 |
| **12** | KBr | 1540, 1490 | | | | |
| **10** | KBr | 1545, 1490 | | | 1745 | |
| **13** | KBr | 1535, 1485 | | | 1740 | |
| **11** | $CHCl_3$ | 1515 (sh), 1495 | | 1765 | 1735 | |
| **14** | $CHCl_3$ | 1510 (sh), 1495 | | 1765 | 1730 | |
| **2** | KBr | | 1550 | | 1710 | |
| **3** | $CHCl_3$ | | 1570 | 1765 | 1735 | |
| **4** | $CDCl_3$ | | 1565 | 1765 | 1730 | |

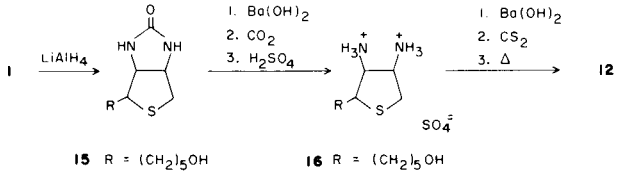
Scheme III




1 R = $(CH_2)_4CO_2H$ 6 R = $(CH_2)_4CO_2H$ 12 R = $(CH_2)_5OH$

4 R = $(CH_2)_5OC(O)CH_3$ 14 R = $(CH_2)_5OC(O)CH_3$ 13 R = $(CH_2)_5OC(O)CH_3$

reduction of the carboxylic acid group to give **12**. Alternatively, **12** could be prepared by initial reduction of biotin (**1**) to biotinol (**15**) with LAH using a slightly modified procedure of Lane and co-workers (4). This compound was then ring-opened with barium hydroxide to give **16**. In this case, the diamino sulfate **16** (10) was not extensively purified but was converted back to the free amine with barium hydroxide and then recyclized with carbon disulfide to yield **12**. The overall conversion of biotin (**1**) to thiobiotinol (**12**) was higher using the first procedure (62% *vs.* 26%). Acetylation of **12** with acetic anhydride gave 2-thiobiotinyl acetate (**13**), which was then treated with methyl chloroformate and pyridine to give **14**. High-field $^1H$ nmr analysis of the carefully purified product indicated only the presence of the desired isomer (9). Completion of the synthesis was accomplished by addition of 1.1 equivalents of trimethyloxonium fluoroborate to a nitromethane solution of **14** to yield **4** (86% yield).




1 12

15 R = $(CH_2)_5OH$ 16 R = $(CH_2)_5OH$

Mass Spectral Data.

Consistent fragmentation patterns were observed in the mass spectra of thiobiotin derivatives (**2-4, 6, 10-14**) and are summarized in Table I. This listing of ions correlates well with the findings previously observed for biotin compounds (see Table III in reference 4). The composite set of data also provided helpful structural evidence for all newly prepared 2-thiobiotin derivatives.

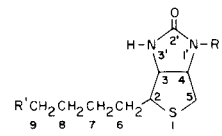
The assignments of the precise elemental composition for the ions reported in Table I are tentative and are primarily based on trends observed throughout the data set. In a few select cases, high-resolution measurements were utilized to distinguish between different fragments. Furthermore, alternative structures for the ions listed in Table I are conceivable and have not been excluded.

Each neutral 2-thiobiotin derivative (**6, 10-14**) gave a discernible parent ion in the mass spectrum (ionization voltage 70 eV). Correspondingly, a prominent peak corresponding to the P-$HBF_4$ ion was observed in the spectrum of each of the *S*-methylated salts **2-4**. Moreover, weak signals were detected in these three spectra for the loss of a methyl group from the initial ion.

Examination of the data led to the conclusion that a significant fragment in most of the mass spectra were ions **17** and **18**. These ions can be attributed to the initial cleavage of the thioureido or substituted thioureido moiety. Another noticeable breakdown pattern corresponded to the loss of the side chain giving ion **20**. An additional peak

TableIII

$^1$H NMR Data for Biotin Derivatives (a)

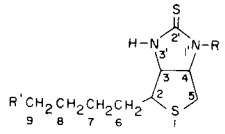


| No. | **1** | **15** | **31** | **33** (b) | **32** | **34** (b) |
|---|---|---|---|---|---|---|
| R | H | H | H | H | $CO_2CH_3$ | $CO_2CH_3$ |
| R′ | $CO_2H$ | $CH_2OH$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ |
| Solvent | DMSO-$d_6$ | $CD_3OD$ | DMSO-$d_6$ | $CDCl_3$ | $CDCl_3$ | $CDCl_3$ |
| $C_2$-H | 2.94-3.32, m | 3.00-3.20, m | 2.92-3.30, m | 3.10 | 3.06-3.28, m | 3.20 |
| $C_3$-H | | | | 4.31 | 4.10-4.30, m | 4.30 |
| | 3.97-4.50, m | 4.10-4.64, m | 4.00-4.47, m | | | |
| $C_4$-H | | | | 4.46 | 4.70-4.92, m | 4.88 |
| $C_5$-$H_2$ | 2.64-2.90, m | 2.72-2.90, m | 2.60-2.86, m | 2.80 | 2.92-3.08, m | 3.10 |
| $C_6$-$H_2$ | | | | (c) | | (c) |
| $C_7$-$H_2$ | 1.17-1.97, m | 1.20-2.00, m | 1.18-1.94, m | (c) | 1.20-1.95, m | (c) |
| $C_8$-$H_2$ | | | | (c) | | (c) |
| $C_9$-$H_2$ | 1.97-2.40, m | | 2.10-2.44, m | (c) | 2.12-2.42, m | (c) |
| $C_{10}$ | (d) | 3.34-3.75, m (e) | (f) | (c,g) | (h) | (c,g) |
| R = $CO_2CH_3$ | | | | | 3.82, s | (c) |
| N-H (s) | 6.23-6.57, br s | (i) | 5.20-5.60, br s | 5.99<br>6.33 | 7.00-7.24, br s | 6.91 |

(a) The initial number in each entry in the table is the chemical shift value ($\delta$) observed in ppm relative to TMS, which is followed by the multiplicity observed for the signal. (b) Data taken from reference 4. (c) The chemical shift value for this proton was not given in reference 4. (d) The carboxylic acid proton was not detected in the spectrum. (e) The chemical shift value for the hydroxyl proton could not be assigned. (f) $^1$H nmr data for $CO_2CH_3$ signal: $\delta$ 3.58, s. (g) The chemical shift value for the acetyl methyl protons was not given in reference 4. (h) $^1$H nmr data for $CO_2CH_3$ signal: $\delta$ 3.62, s. (i) The chemical shift value for this proton could not be assigned.

Table IV

$^1$H NMR Data for Thiobiotin Derivatives (a)

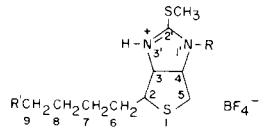


| No. | **6** | **12** | **10** | **13** | **11** | **14** |
|---|---|---|---|---|---|---|
| R | H | H | H | H | $CO_2CH_3$ | $CO_2CH_3$ |
| R′ | $CO_2H$ | $CH_2OH$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ |
| Solvent | DMSO-$d_6$ | DMSO-$d_6$ | DMSO-$d_6$ | DMSO-$d_6$ | $CDCl_3$ | $CDCl_3$ |
| $C_2$-H | 3.00-3.30, m | 2.97-3.17, m (b) | 2.97-3.42, m | 2.90-3.33, m | 3.23-3.50, m | 3.23-3.47, m |
| $C_3$-H | | | | | 4.17-4.60, m | 4.20-4.57, m |
| | 4.24-4.64, m | 4.08-4.63, m | 4.14-4.77, m | 4.17-4.70, m | | |
| $C_4$-H | | | | | 4.83-5.20, m | 4.97-5.30, m |
| $C_5$-$H_2$ | 2.52-3.00, m | 2.60-2.83, m | 2.67-2.97, m | 2.63-2.90, m | 2.93-3.23, m | 2.97-3.23, m |
| $C_6$-$H_2$ | | | | | | |
| $C_7$-$H_2$ | 1.16-1.93, m | 1.13-1.80, m | 1.15-2.00, m | 1.07-1.83, m | 1.33-2.07, m | 1.20-1.96, m |
| $C_8$-$H_2$ | | | | | | |
| $C_9$-$H_2$ | 2.02-2.32, m | | 2.13-2.60, m | | 2.07-2.60, m | |
| C10 | (c) | 2.97-3.17, m (b,d) | (e) | 3.77-4.13, m (f) | (g) | 3.96-4.20, m (h) |
| R = $CO_2CH_3$ | | | | | 3.84, s | 3.88, s |
| N-H (s) | (i) | (i) | 7.97-8.30, br s | 7.90-8.23, br s | 7.90-8.20, br s | 7.73-7.97, br s |

(a) The initial number in each entry in the table is the chemical shift value ($\delta$) observed in ppm relative to TMS, which is followed by the multiplicity observed for the signal. (b) The overlapping of peaks for the C2-H, C10-$H_2$ and exchangeable protons did not permit a definitive chemical shift assignment for these protons. (c) The chemical shift value for the carboxylic acid proton could not be assigned. (d) The chemical shift value for the hydroxyl proton could not be assigned. (e) $^1$H nmr data for $CO_2CH_3$ signal: $\delta$ 3.60, s. (f) $^1$H nmr data for $CH_2OC(O)CH_3$ signal: $\delta$ 1.99, s. (g) $^1$H nmr data for the $CO_2CH_3$ signal: $\delta$ 3.67, s. (h) $^1$H nmr data for $CH_2OC(O)CH_3$ signal: $\delta$ 2.06, s. (i) The chemical shift value for this proton could not be assigned.

Table V

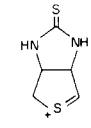
$^1$H NMR Data for Derivatives of
2'-Thiobiotin-2'-*S*-methyl Fluoroborate (a)



| No. | **2** | **3** | **4** |
|---|---|---|---|
| R | H | $CO_2CH_3$ | $CO_2CH_3$ |
| R' | $CO_2H$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ |
| Solvent | $CD_3NO_2$ | $CD_3NO_2$ | $CDCl_3$ |
| $C_2$-H | 3.24-3.60, m | 3.40-3.66, m | 3.20-3.64, m |
| $C_3$-H | 4.83-5.40, m | 4.73-5.18, m | 4.84-5.16, m |
| $C_4$-H | | 5.22-5.53, m | 5.20-5.54, m |
| $C_5$-H2 | 3.03-3.17, m | 3.13-3.40, m | 3.00-3.24, m |
| $C_6$-H2, $C_7$-H2, $C_8$-H2 | 1.40-1.93, m | 1.23-2.10, m | 1.20-1.82, m (b) |
| $C_9$-H2 | 2.20-2.57, m | 2.17-2.56, m (c) | |
| R = $CO_2CH_3$ | | 4.00, s | 4.00, s |
| S-C$H_3$ | 2.70, s | 2.77, s | 2.70, s |
| N-H (s) | (d) | 8.57-9.00, br s | (e) |

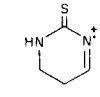
(a) The initial number in each entry in the table is the chemical shift value (δ) observed in ppm relative to TMS, which is followed by the multiplicity of the signal. (b) $^1$H nmr data for $C_{10}H_2$ signal: δ 3.74-4.20, m; $^1$H nmr data for $C_{10}H_2OC(O)CH_3$ signal: δ 2.00, s. (c) $^1$H nmr data for $C_{10}O_2CH_3$ signal: δ 3.65, s. (d) The chemical shift value for this proton could not be assigned. (e) The N-H proton was not detected in the spectrum.

for the protonated analog of fragment **20**, **19**, was also observed throughout the series. Fragmentation of the bicyclic ring structure occurred in all compounds examined resulting in the formation of ions **21** and **22**, as well as **23-25**. In the case of compounds **11** and **14**, the elemental composition of the signals at m/e 159 (ion **22**) was verified by high-resolution mass spectrometry. This result removed the ambiguity that this signal corresponded to the $C_5H_7N_2S_2$ fragment **27**. Peaks at m/e 100, 87 and 85 were uniformly seen throughout this set of compounds. These



**27**

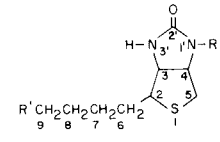
signals can be attributed to ions **23-25**, respectively, arising from the tetrahydrothiophene ring. In addition to these ring cleavage ions, a significant ion at m/e 114 was noted in the mass spectra of all the neutral derivatives (**6**, **10-14**). High resolution measurements of this signal in the spectra of compounds **11** and **14** confirmed the elemental composition corresponding to ion **28** (11). An analogous oxygen-containing fragment was previously identified (4).



**28**

Table VI

$^{13}$C NMR Data for Biotin Derivatives (a)

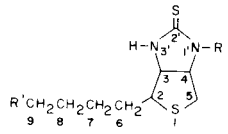


| No. | **1** | **15** | **31** | **33** | **32** | **34** (b) |
|---|---|---|---|---|---|---|
| R | H | H | H | H | $CO_2CH_3$ | $CO_2CH_3$ |
| R' | $CO_2H$ | $CH_2OH$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ |
| Solvent | Pyridine-$d_5$ | Pyridine-$d_5$ | Pyridine-$d_5$ | Pyridine-$d_5$ | $CDCl_3$ | $CDCl_3$ |
| C-2' | 164.4 δ, s | 164.7 δ, s | 164.5 δ, s | 164.6 δ, s | 156.0 δ, s | 156.3 δ |
| C-2 | 56.3 δ, d, 144 | 56.5 δ, d, 138 | 56.2 δ, d, 136 | 56.4 δ, d, 142 | 55.3 δ, d, 139 | 55.6 δ |
| C-3 | 62.4 δ, d, 144 | 62.5 δ, d, 141 | 62.5 δ, d, 147 | 62.5 δ, d, 140 | 57.8 δ, d, 147 | 57.9 δ |
| C-4 | 60.6 δ, d, 148 | 60.6 δ, d, 149 | 60.6 δ, d, 152 | 60.6 δ, d, 148 | 62.9 δ, d, 153 | 62.8 δ |
| C-5 | 41.1 δ, t, 141 | 41.0 δ, t, 141 | 41.0 δ, t, 141 | 41.0 δ, t, 141 | 38.7 δ, t, 143 | 38.7 δ |
| C-6 | 25.7 δ, t, 124 | 26.6 δ, t, 122 | 25.2 δ, t, 124 | 26.2 δ, t, 125 | 24.6 δ, t, 126 | 25.8 δ |
| C-7 | 29.1 δ (c), t, 128 | 29.4 δ, t, 132 | 28.9 δ, t, 127 | 29.1 δ, t, 131 | 28.1 δ, t, 126 | 28.3 δ |
| C-8 | 29.1 δ (c), t, 128 | 29.5 δ, t, 132 | 29.0 δ, t, 127 | 29.2 δ, t, 131 | 28.3 δ, t, 126 | 28.3 δ |
| C-9 | 35.0 δ, t, 126 | 33.4 δ, t, 126 | 33.9 δ, t, 124 | 28.7 δ, t, 129 | 33.6 δ, t, 129 | 28.6 δ |
| C-10 | 176.4 δ, s | 61.9 δ, t, 139 | 173.7 δ, s (d) | 64.4 δ, t, 145 (e) | 174.0 δ, s (f) | 64.4 δ (g) |
| R = $CO_2CH_3$ | | | | | 152.4 δ, s | 152.4 δ |
| R = $CO_2CH_3$ | | | | | 53.5 δ, q, 153 | 53.4 δ |

(a) The initial number in each entry in the table is the chemical shift value (δ) observed in ppm relative to TMS, which is succeeded by the multiplicity of the signal in the corresponding $^{13}$C-H coupled spectrum, followed by the $^{13}$C-H coupling constant in Hz (± 4 Hz). (b) Data taken from reference 4. (c) This signal is approximately double the intensity of the neighboring peaks. (d) $^{13}$C nmr data for $CO_2CH_3$ signal: 51.3 δ, q, 146. (e) $^{13}$C nmr data for the $CH_2OC(O)CH_3$ signal: 170.7 δ, s; $^{13}$C nmr data for $CH_2OC(O)CH_3$ signal: 20.8 δ, q, 129. (f) $^{13}$ C nmr data for $CO_2CH_3$ signal: 51.5 δ, q, 150. (g) $^{13}$C nmr data for $CH_2OC(O)CH_3$ signal: 171.3 δ, s; $^{13}$C nmr data for $CH_2OC(O)CH_3$ signal: 21.0 δ.

Table VII

$^{13}C$ NMR Data for Thiobiotin Derivatives (a)



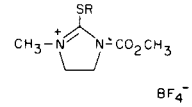
| No. | **6** | **12** | **10** | **13** | **11** | **14** |
|---|---|---|---|---|---|---|
| R | H | H | H | H | $CO_2CH_3$ | $CO_2CH_3$ |
| R′ | $CO_2H$ | $CH_2OH$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ |
| Solvent | Pyridine-$d_5$ | DMSO-$d_6$ | DMSO-$d_6$ | $CH_3OD$ | Pyridine-$d_5$ | Pyridine-$d_5$ | $CDCl_3$ | $CDCl_3$ |
| C-2′ | 185.4 δ, s | 182.5 δ, s | 182.4 δ, s | (b) | 185.2 δ, s | 185.4 δ, s | 181.0 δ, s | 181.0 δ, s |
| C-2 | 56.8 δ, d, 137 | 55.6 δ, d, 140 | 55.8 δ, d, 136 | 56 δ, d, 140 | 56.8 δ, d, 141 | 56.9 δ, d, 139 | 55.6 δ, d, 140 | 55.7 δ, d, 138 |
| C-3 | 67.6 δ, d, 149 | 66.0 δ, d, 152 | 66.0 δ, d, 149 | 67.4, δ, d, 150 | 67.6 δ, d, 154 | 67.7 δ, d, 152 | 63.1 δ, d, 150 | 63.1 δ, d, 150 |
| C-4 | 65.4 δ, d, 149 | 64.1 δ, d, 151 | 64.1 δ, d, 149 | 65.6 δ, d, 150 | 65.4 δ, d, 149 | 65.5 δ, d, 150 | 67.4 δ, d, 153 | 67.4 δ, d, 152 |
| C-5 | 40.8 δ, t, 142 | 39.9 δ, t, 145 (c) | (d) | 39.8 δ, t, 140 | 40.8 δ, t, 146 | 40.8 δ, t, 142 | 39.3 δ, t, 144 | 39.3 δ, t, 140 |
| C-6 | 25.5 δ, t, 124 | 24.5 δ, t, 126 | 25.4 δ, t, 125 | 26.0 δ, t, 123 | 25.1 δ, t, 132 | 26.2 δ, t, 126 | 24.6 δ, t, 127 | 25.8 δ, t, 128 |
| C-7 | 29.2 δ, t, 132 | 27.9 δ, t, 127 | 28.2 δ, t, 130 | 28.9 δ, t, 130 | 29.1 δ, t, 124 | 29.3 δ, t, 131 | 28.4 δ (e), t, 128 | 28.8 δ (e), t, 129 |
| C-8 | 29.3 δ, t, 132 | 28.1 δ, t, 126 | 28.5 δ, t, 130 | 29.1 δ, t, 130 | 29.2 δ, t, 124 | 29.4 δ, t, 131 | 28.4 δ (e), t, 128 | 28.8 δ (e), t, 129 |
| C-9 | 34.8 δ, t, 124 | 33.4 δ, t, 127 | 32.2 δ, t, 120 | 32.5 δ, t, 120 | 33.9 δ, t, 128 | 28.8 δ, t, 123 | 33.6 δ, t (f) | 28.4 δ, t, 126 |
| C-10 | 176.1 δ, s | 174.3 δ, s | 60.6 δ, t, 139 | 62.1 δ, t, 140 | 173. 8 δ, s (g) | 64.4 δ, t, 145 (h) | 174.0 δ, s (i) | 64.2 δ, t, 149 (j) |
| R = $CO_2CH_3$ | | | | | | | 152.1 δ, s | 152.0 δ, s |
| R = $CO_2CH_3$ | | | | | | | 53.7 δ, q, 148 | 53.7 δ, q, 149 |

(a) The initial number in each entry in the table is the chemical shift value (δ) observed in ppm relative to TMS, which is succeeded by the multiplicity of the signal in the corresponding $^{13}C$-H coupled spectrum, followed by the $^{13}C$-H coupling constant in Hz (± 4 Hz). (b) Signal was not observed. (c) Peak buried under the solvent signal, value obtained from coupled spectrum. (d) Peak buried under the solvent signals. (e) This signal is approximately double the intensity of neighboring peaks. (f) Overlapping of the neighboring peaks prevented the determination of the $^{13}C$-H coupling constant. (g) $^{13}C$ nmr data for $CO_2CH_3$ signal: 51.4 δ, q, 146. (h) $^{13}C$ nmr data for $CH_2OC(O)CH_3$ signal: 170.8 δ, s; $^{13}C$ nmr data for $CH_2OC(O)CH_3$ signal: 20.9 δ, q, 129. (i) $^{13}C$ nmr data for $CO_2CH_3$ signal: 51.6 δ, q, 147. (j) $^{13}C$ nmr data for $CH_2OC(O)CH_3$ signal: 171.2 δ, s; $^{13}C$ nmr data for $CH_2OC(O)CH_3$ signal: 21.0 δ, q, 128.

In the case of compound **11**, a trace peak at m/e 114.0677 (Calcd. for $C_6H_{10}O_2$, 114.0681) was also detected. The last ion listed in Table I correlates with fragment **26**. The basic composition of this ion in the mass spectra of compounds **11** and **14** was once again verified by high-resolution mass spectrometry. The detection of this fragment in the breakdown pattern in compounds **2-4** was consistent with other spectral data indicating that alkylation has proceeded at the 2-thione position of the thiobiotin ring system.

Infrared Spectral Data.

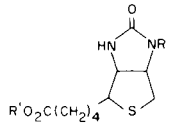
The high-frequency absorption band in the infrared spectra for the thiourea moiety was consistently observed in all neutral 2-thiobiotin derivatives (**6, 10-14**) (Table II). These values correlate well with data previously obtained by ourselves for related compounds (12). In both studies, however, we were unable to confidently assign the associated lower-frequency bands for the thiourea functional group (13). Salts **2-4** exhibited a strong absorption between 1550-1570 $cm^{-1}$ for the cyclic iminium group (13). The appearance of this signal supported other spectroscopic data which indicated that methylation of the neutral precursors (**6, 11** and **14**) had occurred at the 2-thione position. The absorption for the iminium group in the fully alkylated derivatives **29** and **30** was observed at 1595 $cm^{-1}$ (14,15). Finally, the values reported in the table for the various carbonyl groups present in compounds **2-4, 6, 10, 11, 13** and **14** agree with expectation (13,16).



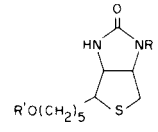
**29** R = $CH_3$
**30** R = $CH_2C(O)Ph$

Magnetic Resonance Data. $^1H$ NMR.

The $^1H$ nmr data for the biotin derivatives (**1, 15, 31-34**), 2-thiobiotin compounds (**6, 10-14**), and the *S*-methylated affinity labels (**2-4**) are recorded in Tables III-V, respectively. The chemical shifts for the various protons present in these compounds can readily be assigned by the use of correlation charts (12,18,19).



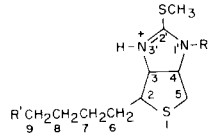
**1** R = H, R′ = H
**31** R = H, R′ = $CH_3$ [17]
**32** R = $CO_2CH_3$, R′ = $CH_3$ [17]



**15** R = H, R′ = H
**33** R = H, R′ = $C(O)CH_3$ [4]
**34** R = $CO_2CH_3$, R′ = $C(O)CH_3$ [4]

Table VIII

$^{13}C$ NMR Data for Derivatives of
2'-Thiobiotin-2'-*S*-methyl Fluoroborate (a)



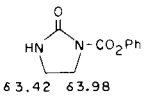
| No. | 2 | 3 | 4 |
|---|---|---|---|
| R | H | $CO_2CH_3$ | $CO_2CH_3$ |
| R' | $CO_2H$ | $CO_2CH_3$ | $CH_2OC(O)CH_3$ |
| Solvent | Pyridine-$d_5$ | $CD_3CN$ | $CDCl_3$ |
| C-2' | 171.5 δ, s | 176.7 δ (b), s | 175.3 δ, s |
| C-2 | 56.7 δ, d, 142 | 56.6 δ, d, 136 | 56.4 δ, d, 141 |
| C-3 | 69.7 δ, d, 152 | 69.8 δ (b), d, 159 | 68.7 δ (b), d, 159 |
| C-4 | 67.9 δ, d, 156 | 70.0 δ (6), d, 159 | 69.0 δ (b), d, 159 |
| C-5 | 40.3 δ, t, 148 | 39.1 δ, t, 144 | 38.5 δ, t, 145 |
| C-6 | 25.3 δ, t, 120 | 25.4 δ, t, 125 | 25.4 δ, t (c) |
| C-7 | 29.1 δ, t, 124 | 28.8 δ, t, 125 | 28.2 δ, t, 127 |
| C-8 | 29.2 δ, t, 124 | 29.4 δ, t, 125 | 29.0 δ, t, 127 |
| C-9 | 34.5 δ, t, 124 | 34.2 δ, t, 123 | 27.9 δ, t, 126 |
| C-10 | 175.6 δ, s | 174.8 δ, (b,d), s | 64.3 δ, t, 146 (e) |
| R = $CO_2CH_3$ | | 151.5 δ, s | 150.3 δ, s |
| R = $CO_2CH_3$ | | 56.1 δ, q, 151 | 55.4 δ, q, 150 |
| S-$CH_3$ | 14.2 δ, q, 144 | 16.3 δ, q, 144 | 15.1 δ, q, 144 |

(a) The initial number in each entry in the table is the chemical shift value (δ) observed in ppm relative to TMS, which is succeeded by the multiplicity of the signal in the corresponding $^{13}C$-H coupled spectrum, followed by the $^{13}C$-H coupling constant in Hz (± 4 Hz). (b) The assignment of these peaks may be reversed. (c) Overlapping of neighboring peaks prevented determination of $^{13}C$-H coupling constants. (d) $^{13}C$ nmr data for $CO_2CH_3$ signal: 52.0 δ, q, 147. (e) $^{13}C$ nmr data for $CH_2OC(O)CH_3$ signal: 171.3 δ, s; $^{13}C$ nmr data for $CH_2OC(O)CH_3$ signal: 20.9 δ, q, 129.

The most significant change observed in the $^1H$ nmr spectra for the biotin series (Table III) was the downfield shift of one of the two imidazolidone methine protons ($C_3$-H and $C_4$-H) upon introduction of the carbomethoxy group (**31**→**32**, **33**→**34**). The most deshielded proton in **32** [a compound structurally verified by X-ray crystallography (20)] and **34** was tentatively assigned by Lane and co-workers as $C_4$-H (4). This assignment was supported by a comparable shift in the $^1H$ nmr spectra for one set of the ring protons of 2-imidazolidone (**35**) upon acylation to give **36**. We, likewise, have noticed similar effects in a large variety of substituted 2-imidazolidones and 2-imidazolidinethiones (12). In line with this observation, we also noted a small downfield shift for the $C_5$-$H_2$ methylene protons in **32** and **34** *versus* **31** and **33**, while no significant change was seen in the chemical shift for the $C_2$-H methine proton. Significantly, although the logic employed in the previous study for the assignments of $C_3$-H and $C_4$-H in **32** and **34** is sound (4), the evidence provided is not conclusive. The reverse assignment is conceivable. Related information bearing on this point is provided in the 2-thiobiotin series.

δ3.52

**35**



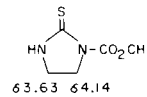
δ3.42 δ3.98

**36**

Comparable observations were noted for the 2-thiobiotin derivatives (Table IV). Introduction of the carbomethoxy group (**10**→**11**, **13**→**14**) led to a distinct separation of the imidazolidinethione methine protons ($C_3$-H and $C_4$-H). These changes are paralleled once again in the $^1H$ nmr spectra of imidazolidinethione (**7**) and *N*-carbomethoxyimidazolidinethione (**37**) (12). In the latter case, confirmation of the assignment was secured by a high-field proton-proton decoupling experiment. Double irradiation of the N-H proton led to a sharpening of the upfield multiplet (δ 3.63). This experiment together with a complete high-field $^1H$ nmr analysis of **11** and **14** provided convincing evidence for the assignments listed in Tables III and IV (9,21).

δ3.52

**7**



δ3.63 δ4.14

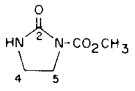
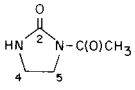
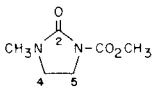
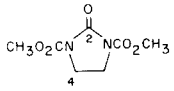
**37**

Comparison of the $^1H$ nmr spectral data for the biotin and 2-thiobiotin compounds indicated that the C-3 and C-4 proton resonances in the latter series consistently appeared at lower field than in the biotin derivatives. Analogous trends were detected in the simpler imidazolidone and imidazolidinethione systems (12,22), and an explanation has been offered (22). All the remaining chemical shift values in these two series of compounds are reasonably similar.

Each of the three salts (**2-4**) contained a characteristic absorption at approximately δ 2.70 for the *S*-methyl group (14,23). The appearance of this signal confirmed that alkylation of the thione precursors (**6**, **11**, **14**) had occurred at sulfur rather than either of the two nitrogen atoms. Methylation at these positions would have led to a proton resonance at approximately δ 3.50 (*i.e.* the *N*-methyl signal for **29** and **30** appeared at δ 3.49 (14) and δ 3.52 (15), respectively). Furthermore, comparison of $^1H$ nmr spectra of these three substrates (**2-4**) with their precursors (**6**, **11**, **14**) revealed a number of interesting patterns. Alkylation typically led to a downfield shift in the $C_3$-H and $C_4$-H protons. In the *N*-carbomethoxy compounds (**3** and **4**) the $C_3$-H proton appeared to be effected the most (24). Smaller downfield shifts were also observed for the $C_2$-H and $C_5$-$H_2$ protons and for the N-$CO_2CH_3$ group. No significant changes were noted for the remaining protons. These trends are in agreement with our conclusion that alkylation had proceeded at the 2-thione position rather than at the sulfur atom of the tetrahydrothiophene ring. If the lat-

Table IX

$^{13}C$ NMR Data for Substituted Imidazolidones

| Compound | | No. | Solvent | C-2 | C-4 | C-5 | N-C(=O)-R | N-C(=O)-R |
|---|---|---|---|---|---|---|---|---|
|  | | **35** | DMSO-$d_6$ | 165 | 40.5 | | | |
|  | (a,b) | **38** | $CDCl_3$ | 163.8 | 46.4 | 37.5 | | |
|  | (c) | **39** | $CDCl_3$ | 156.4 | 36.7 | 43.1 | 152.4 | 53.2 |
|  | (d) | **40** | $CDCl_3$ | 157.0 | 36.4 | 42.1 | 170.7 | 23.2 |
|  | (e,f) | **41** | $CDCl_3$ | 154.5 | 44.0 | 39.8 | 152.2 | 53.6 |
|  | (e) | **42** | $CDCl_3$ | 148.2 | 39.7 | | 152.2 | 53.7 |

(a) J. G. Frick, B. A. Kottis, and J. D. Reid, *Text. Res. J.*, **29**, 314 (1959). (b) $^{13}C$ nmr data for $N_3$-$CH_3$ signal: 31.2 $\delta$. (c) H. J. Schaeffer and P. S. Bhargava, *J. Pharm. Sci.*, **51** 1116 (1962); *ibid.*, **53**, 137 (1964). (d) H. K. Hall and A. K. Schneider, *J. Am. Chem. Soc.*, **80**, 6409 (1958). (e) Refeece 12. (f) $^{13}C$ nmr data for $N_3$-$CH_3$ signal 31.2 $\delta$.

ter possibility had occurred, the greatest deshielding effect should have been observed for the $C_2$-H and $C_5$-$H_2$ protons.

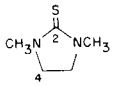
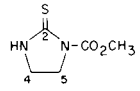
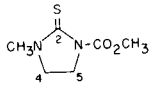
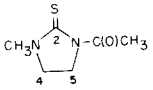
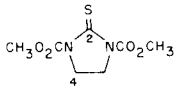
## $^{13}C$ NMR.

The composite set of $^{13}C$ nmr data for the biotin and the 2-thiobiotin derivatives (Table VI-VIII) gave a consistent set of information which proved helpful in structure confirmation. A majority of the ring carbon atoms could be readily identified from correlation charts (12,19,25,26). No attempt was made to identify the insulated methylene carbon atoms of the side chain. All assignments listed are in agreement with the multiplicity observed for the signals in the corresponding proton coupled spectrum.

Examination of the $^{13}C$ nmr spectra for the biotin compounds (Table VI) revealed three prominent effects upon introduction of the N1'-$CO_2CH_3$ group: upfield shifts in the adjacent $sp^2$ carbon (C-2' [$\Delta$ ppm $\sim$ 8.4]) and the $sp^3$ carbons beta to the N-carbomethoxy group (C-3 [$\Delta$ ppm $\sim$ 4.8], and C-5 [$\Delta$ ppm $\sim$ 2.3]), and a downfield shift in the $sp^3$ carbon alpha to the nitrogen site of carbomethoxylation (C-4 [$\Delta$ ppm $\sim$ 2.3]). These effects are mirrored in the simpler imidazolidones (Table IX). We noted in this series an approximate 8 ppm upfield shift for the C-2 carbonyl carbon when an acyl group is attached to N-1 (**35**→**39** or **40**, **38**→**41**). Introduction of a second acyl group (**39**→**42**) led to an additional 8.2 ppm upfield shift. Furthermore, in agreement with the patterns observed in the biotin compounds, the signal for the $sp^3$ carbon (C-5) adjacent to the nitrogen undergoing acylation moved downfield ($\Delta$ ppm $\sim$ 2.2), while the furthest $sp^3$ carbon (C-4) moved upfield ($\Delta$ ppm $\sim$ 3.4). These nearly equal but opposite perturbations are essentially cancelled out by the introduction of a second carbomethoxy group (**39**→**42**). The ring methylene carbons in **42** appeared at approximately the same chemical shift value as the totally unsubstituted compound **35**. It is important to note that no convenient observation assured the paired assignment given in Tables VI and IX for C-3 and C-4. The values for these two carbons may be reversed. Related information, however, is

Table X

$^{13}C$ NMR Data for Substituted Imidazolidinethiones

| Compound | | No. | Solvent | C-2 | C-4 | C-5 | N-C(=O)R | N-C(=O)R |
|---|---|---|---|---|---|---|---|---|
|  | | **7** | DMSO-$d_6$ | 183.5 | 44.0 | | | |
|  | (a,b) | **43** | $CDCl_3$ | 183.4 | 48.2 | | | |
|  | (c) | **37** | $CDCl_3$ | 180.9 | 41.3 | 47.6 | 152.3 | 53.6 |
|  | (d,e) | **44** | $CDCl_3$ | 177.8 | 43.9 | 47.9 | 152.0 | 52.9 |
|  | (d,f) | **45** | $CDCl_3$ | 178.5 | 43.4 | 47.5 | 171.3 | 26.1 |
|  | (d) | **46** | $CDCl_3$ | (e) | 44.7 | | 152.2 | 53.6 |

(a) L. Maier, *Helv. Chim. Acta*, **53**, 1417 (1970). (b) $^{13}C$ nmr data for N-$CH_3$ signal: 35.0 $\delta$. (c) Reference 15. (d) Reference 12. (e) $^{13}C$ nmr data for $N_3$-$CH_3$ signal: 34.6 $\delta$. (f) $^{13}C$ nmr data for $N_3$-$CH_3$ signal: 35.2 $\delta$. (e) Signal was not observed.

presented in the 2-thiobiotin series which substantiated these assignments. Finally, we suggest that a number of the assignments reported by Lane and co-workers for compounds **32** and **34** be changed (4,27).

The $^{13}C$ nmr chemical shift values recorded in Table VII for the 2-thiobiotin derivatives paralleled the results obtained for the biotin compounds. The inability to accurately determine all the $^{13}C$ nmr parameters for **6** and **12** prompted the use of two different solvents for the acquisition of the data.

Similar shielding and deshielding effects were noted in this set of compounds upon introduction of the N1'-$CO_2CH_3$ group. The C-2', C-3, and C-5 resonances moved upfield by approximately 3, 4.6, and 1.5 ppm while the signal for C-4 appeared at lower field ($\Delta$ ppm $\sim$ 2.0). We have again compiled a list of $^{13}C$ nmr chemical shift values for the simpler imidazolidinethiones (Table X). Similar effects were observed.

Significantly, for compound **37** confirmatory evidence for the C-3 and C-4 paired assignments was obtained. Selective proton-carbon decoupling experiments interrelated the $^1H$ nmr resonance ($\delta$ 4.12-4.16) with the most deshielded $^{13}C$ nmr signal (47.6 ppm), and the high-field $^1H$ nmr peak ($\delta$ 3.60-3.65) with the $^{13}C$ nmr signal at 41.3 ppm. This information along with the previously mentioned N-H double irradiation study made the C-3 and C-4 assignments for **37** certain, and provided support for the assignments listed in Tables VI, VII, IX and X.

The $^{13}C$ nmr data for compounds **2-4** are recorded in Table VIII. The appearance of signals in close proximity to one another did not permit definitive assignment for a number of carbon atoms (*i.e.* compound **3**: C-2' and C-10, C-3 and C-4; compound **4**: C-3 and C-4).

Each compound exhibited a characteristic upfield signal at approximately 15 ppm, which appeared as a quartet (J = 144 Hz) in the corresponding coupled spectrum. The chemical shift value for this methyl group further supported other spectral observations which indicated that alkylation had proceeded at sulfur rather

than at nitrogen (25,26). Moreover, the relative insensitivity of the chemical shift values for carbons 2 and 5 upon methylation (**6**→**2**, **11**→**3** and **14**→**4**) provided evidence suggesting that alkylation had proceeded at the thione sulfur atom rather than on the tetrahydrothiophene ring. Finally, we noted an upfield shift for the C-2′ signal of the salts **2-4** when compared to their neutral precursors (**6**, **11** and **14**), while the reverse was true for the C-3 and C-4 carbon resonances.

## EXPERIMENTAL

General.

Melting points were determined with a Thomas Hoover Capillary melting point apparatus and are uncorrected. Infrared (ir) spectra were run on a Beckman Model IR 4250 spectrophotometer. The ir absorption intensities are indicated by the symbols s (strong), m (medium), w (weak), b (broad) and sh (shoulder). All ir absorption values are expressed in wave numbers ($cm^{-1}$). Proton nuclear magnetic resonance ($^1H$ nmr) spectra were recorded on a Varian Associates Model T-60 instrument. Carbon-13 nuclear magnetic resonance ($^{13}C$ nmr) spectra were run by Mr. Steven Silber on a Varian Associates Model XL-100-15 spectrometer, equipped with a Nicolet Technology Corporation TT-100 data system. The selective proton-carbon decoupling experiments were run by Professor M. R. Willcott, III. High-field $^1H$ nmr (400.1 MHz) decoupling experiments were performed by Dr. Ruth Inners at the NSF sponsored nmr facility at the University of South Carolina on a Bruker WH-400 nmr spectrometer. The nmr chemical shifts are expressed in parts per million ($\delta$ values) relative to an internal standard of TMS unless otherwise noted. Coupling constants (J values) are expressed in hertz (Hz). Spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet) and m (multiplet). Mass spectral (ms) data was collected using a Hewlett-Packard 5930 Gas Chromatograph-Mass Spectrometer equipped with a Hewlett-Packard 5933A data system. High-resolution mass spectra were performed by Dr. James Hudson at the Department of Chemistry, University of Texas at Austin on a CEC21-110B double focusing magnetic sector spectrometer at 70eV. Elemental Analyses were obtained at Spang Microanalytical Laboratories, Eagle Harbor, Michigan.

(+) Biotin was purchased from Calbiochem-Behring Corporation, La Jolla, California. Trimethyloxonium fluoroborate was purchased from Willow Brook Labs, Incorporated, Waukesha, Wisconsin. All solvents and reactants were of the best commercial grade available and were used without purification unless otherwise noted. When dry solvents were necessary, methylene chloride was distilled from phosphorus pentoxide, nitromethane was predistilled, anhydrous ether was dried and stored over sodium metal ribbon, and pyridine was stored over potassium hydroxide.

Preparation of Thiobiotin (**6**).

A modified version of the procedure described by Green (6) was adopted for the synthesis of **6**. Compound **5** (5) (4.00 g, 12.60 mmoles) was dissolved in 80 ml warm water and added to a warm aqueous solution (120 ml) of barium hydroxide octahydrate (3.98 g, 12.60 mmoles). After cooling, the barium sulfate precipitate was removed by filtration through a Celite bed. The filtrate was evaporated *in vacuo* to give a white solid which was redissolved in a 1:1 (v:v) solution of ethanol-water and transferred to a 250 ml round bottomed flask fitted with a condensor and drying tube. Carbon disulfide (2 ml, 33.2 mmoles) was then added, and the reaction stirred (1 hour) at 40°. The resulting yellow solution was transferred to two heavy wall glass tubes (250 × 40 mm), sealed with a torch, and heated in an oil bath overnight at 100-120°. The tubes were then opened, the contents melted with a hot oil bath (~ 100°), recombined, and the malodorous material heated until the smell of hydrogen sulfide was no longer evident. The mixture was then filtered hot, and the filtrate refrigerated overnight to give a copious precipitate. The solid material was filtered and washed successively with water (50 ml), ethanol (50 ml) and ether (50 ml). The crystals were then dried to give 2.61 g of **6**, mp 230-232° (lit (6) mp 234-235°). A second crop of 0.47 g (mp 229-231°) was obtained by concentration of the mother liquor to give an overall yield of 3.08 g (94%) of **6**. The product was generally used without further purification, however, it could be recrystallized from water if desired: mp 232-235°; ms: m/e (relative %) 260 (36), 201 (13), 127 (36), 115 (37), 114 (100), 113 (75), 112 (23), 101 (77), 100 (68), 98 (27), 97 (30), 87 (24), 85 (46), 81 (49).

Preparation of 2′-Thiobiotin-2′-*S*-methyl Fluoroborate (**2**).

2′-Thiobiotin (**6**) (0.25 g, 0.96 mmole) was predried (vacuum, phosphorus pentoxide) and suspended in freshly distilled nitromethane (15 ml). A solution of trimethyloxonium fluoroborate (0.14 g, 0.96 mmole) in nitromethane (3 ml) was then added dropwise, with stirring, under positive nitrogen pressure. Upon addition of the alkylating agent, another 10 ml of nitromethane was added to insure complete dissolution, and the reaction was stirred overnight. The reaction was then concentrated to dryness to give a solid. The white solid was purified by reprecipitation (3×) from a 1:1 dichloromethane-acetonitrile solution with ether to give 0.38 g (97%) of the desired compound, mp 151-154°; ir (potassium bromide): 3100 (s, broad), 1710 (s), 1550 (s), 1100 (s) $cm^{-1}$; ms: m/e (relative %) 275 (2), 274 (15), 259 (3), 215 (17), 174 (7), 141 (25), 128 (62), 127 (100), 115 (49), 113 (17), 112 (13), 100 (18), 95 (21), 91 (9), 90 (7), 87 (11), 85 (14).

*Anal.* Calcd. for $C_{11}H_{19}N_2O_2S_2BF_4$: C, 36.47; H, 5.29; N, 7.73. Found: C, 36.26; H, 5.23; N, 7.80.

Preparation of 2′-Thiobiotin Methyl Ester (**10**).

2′-Thiobiotin (**6**) (1.00 g, 3.80 mmoles), *p*-toluenesulfonic acid monohydrate (0.15 g, 0.79 mmoles) and methanol (125 ml) were combined and the suspension was heated to reflux (18 hours). The clear, colorless solution was then allowed to cool to room temperature during which time the desired compound (**10**) precipitated. The flask was refrigerated (4 hours) and the crystals collected and dried to give 0.73 g (70%) of the desired ester, mp 215-217°. A second crop of **10** (0.07 g, 7%, mp 206-210°) was obtained by concentration of the mother liquor to give an overall yield of 77%. An analytical sample of the desired ester was prepared by recrystallization (3×) from methanol; ms: m/e (relative %) 274 (68), 243 (19), 201 (20), 166 (22), 127 (21), 115 (43), 114 (100), 113 (90), 101 (98), 100 (83), 98 (30), 97 (39), 77 (70).

*Anal.* Calcd. for $C_{11}H_{18}N_2O_2S_2$: C, 48.14; H, 6.61; N, 10.21. Found: C, 48.23; H, 6.55; N, 10.13.

Preparation of 1′-*N*-Carbomethoxy-2′-thiobiotin Methyl Ester (**11**).

Compound **10** (0.86 g, 3.13 mmoles) was dissolved in freshly distilled dichloromethane (250 ml) and pyridine (0.76 ml, 9.43 mmoles) was added. Methylchloroformate (0.73 ml, 9.44 mmoles) was then carefully added. The yellow solution was heated to reflux (18 hours) under positive nitrogen pressure. The solution was extracted with water (2 × 25 ml), the organic layer dried (sodium sulfate), filtered, and evaporated *in vacuo* to give a crude yellow solid. Purification of **11** was achieved by reprecipitation with ethyl acetate-hexanes (1×) and with methanol-water (2×) to give 0.37 g (36%) of the desired compound as a white powder, mp 168-169°; ms: m/e (relative %) 332 (20), 301 (8), 273 (10), 259 (8), 199 (18), 198 (17), 166 (40), 159 (37), 135 (45), 134 (51), 133 (43), 114 (27), 113 (74), 101 (38), 100 (82), 98 (38), 97 (43), 85 (62), 76 (40), 74 (41), 67 (33), 59 (100); molecular weight of parent ion and fragments: 332.0864 (Calcd. for $C_{13}H_{20}N_2O_4S_2$: 332.0864); 159.0230 (Calcd. for $C_5H_7N_2O_2S$: 159.0228); 135.0231 (Calcd. for $C_5H_7N_2O_2S$: 135.0228); 114.0254 (Calcd. for$C_4H_6N_2S$: 114.0252); 114.0677 (Calcd. for $C_6H_{10}O_2$: 114.0681). (The last reported ion was observed only in trace amounts.)

*Anal.* Calcd. for $C_{13}H_{20}N_2O_4S_2$: C, 46.97; H, 6.06; N, 8.43. Found: C, 47.00; H, 6.13; N, 8.74.

Preparation of 1′-*N*-Carbomethoxy-2′-thiobiotin Methyl Ester-2′-*S*-methyl Fluoroborate (**3**).

Compound **11** (0.37 g, 1.11 mmoles) was dissolved in freshly distilled nitromethane (20 ml). A solution of trimethyloxonium fluoroborate (0.18 g, 1.22 mmoles) in nitromethane (4 ml) was added dropwise with stirring, under positive nitrogen pressure. The reaction was stirred at room temperature overnight and then concentrated *in vacuo* to approximately one third the orginial volume. Ether (100 ml) was then added until the solution turned turbid, and the mixture was stored at -10° (18 hours). The desired compound oiled out. The mother liquor was decanted and the oil was washed with ether (100 ml) and placed under vacuum to yield 0.47 g (98%) of the desired salt as a hygroscopic solid. The salt was purified by oiling out (3×) from a 1:1 dichloromethane-acetonitrile solution with ether; ir (chloroform): 3250 (s, broad), 1765 (s), 1735 (s), 1570 (s), 1450 (s), 1050 (s, broad) $cm^{-1}$; ms: m/e (relative %) 348 (4), 347 (5), 346 (30), 331 (28), 315 (34), 273 (100), 232 (23), 185 (37), 173 (75), 149 (33), 148 (36), 127 (77).

*Anal.* Calcd. for $C_{14}H_{23}N_2O_4S_2BF_4$: C, 38.71; H, 5.34; N, 6.45. Found: C, 38.31; H, 5.40; N, 6.52.

Preparation of 2′-Thiobiotinol (**12**).

To a stirred suspension of LAH (1.48 g, 39.00 mmoles) in anhydrous ether (250 ml), a pyridine (50 ml) solution of vacuum dried (over phosphorus pentoxide) **6** (1.56 g, 5.99 mmoles) was added. Addition of the 2′-thiobiotin (**6**) led to an evolution of gas and the formation of a cloudy white suspension. The reaction mixture was stirred at room temperature (0.5 hour) and then heated to reflux for an additional 0.5 hour. The excess LAH was then destroyed by careful dropwise addition of water. The organic solvents were removed by steam distillation. The pot residue was cooled to room temperature and carefully acidified (*p*H ~ 2) with 6*N* hydrochloric acid. The suspension was continuously extracted with chloroform (10 days) and then the chloroform layer concentrated *in vacuo* to give 1.27 g (86%) of the desired product as a white powder. This material was generally pure enough to be used without further purification, however **12** could be purified by reprecipitation from hot methanol by the addition of ether until a persistantly turbid suspension resulted, followed by refrigeration at -10° (18 hours) (3×), mp 210-211°; ms: m/e (relative %) 246 (53), 170 (13), 113 (72), 101 (99), 77 (100), 31 (69).

*Anal.* Calcd. for $C_{10}H_{18}N_2O_2S_2$: C, 48.74; H, 7.36; N, 11.37. Found: C, 48.57; H, 7.30; N, 11.22.

Preparation of 2′-Thiobiotinyl Acetate (**13**).

Compound **12** (0.77 g, 3.13 mmoles) was dissolved in dry pyridine (25 ml), and then acetic anhydride (2.1 ml, 22.2 mmoles) was added, and the solution was stirred overnight. The solvent was evaporated *in vacuo* and dried to give 0.88 g (98%) of the desired product as a brown solid. Reprecipitation of the residue from methanol-ether and storing at -10° (3×) gave purified **13**, mp 169-170°; ms: m/e (relative %) 288 (100), 229 (44), 114 (40), 113 (50), 101 (75), 100 (52), 77 (46), 59 (27).

*Anal.* Calcd. for $C_{12}H_{20}N_2O_2S_2$: C, 49.97; H, 6.99; N, 9.71. Found: C, 49.75; H, 6.82; N, 9.65.

Preparation of 1′-*N*-Carbomethoxy-2′-thiobiotinyl Acetate (**14**).

Compound **13** (0.48 g, 1.66 mmoles) was dissolved in freshly distilled dichloromethane (50 ml) and pyridine (0.54 ml, 6.70 mmoles) was added. Methyl chloroformate (0.5 ml, 6.66 mmoles) was then carefully added and the yellow solution was heated to reflux (18 hours). The solution was extracted with water (2 × 25 ml), the organic layer dried (sodium sulfate), filtered, and evaporated *in vacuo* to give a clear yellow oil which solidified when placed under vacuum (18 hours). Purification was achieved by reprecipitation of the yellow solid with ethyl acetate-hexanes (1×) and with methanol-water (2×) to give 0.25 g (43%) of the desired product as a white powder, mp 132-135°; ms: m/e (relative %) 346 (6), 287 (10), 173 (13), 159 (24), 152 (22), 135 (12), 134 (15), 133 (33), 127 (25), 113 (30), 100 (56), 97 (32), 85 (20), 76 (25), 59 (53), 43 (100); molecular weight of parent ion and fragments: 346.1014 (Calcd. for $C_{14}H_{22}N_2O_4S_2$: 346.1021); 159.0224 (Calcd. for $C_5H_7N_2O_2S$: 159.0228); 135.0235 (Calcd. for $C_3H_7N_2O_2S$: 135.0228); 114.0254 (Calcd. for $C_4H_6N_2S$: 114.0252).

*Anal.* Calcd. for $C_{14}H_{22}N_2O_4S_2$: C, 48.53; H, 6.40; N, 8.09. Found: C, 48.49; H, 6.26; N, 7.93.

Preparation of 1′-*N*-Carbomethoxy-2′-thiobiotinyl Acetate-2′-*S*-methyl Fluoroborate (**4**).

To a nitromethane (10 ml) solution of **14** (0.11 g, 0.32 mmole), a nitromethane (2 ml) solution of trimethyloxonium fluoroborate (0.05 g, 0.34 mmole) was added. The reaction was stirred overnight, and the solvent evaporated to give **4** as a thick oil. The desired product was purified by successively oiling out the salt from a 1:1 dichloromethane-acetonitrile solution of **4** with ether, followed by the addition of ether to a dichloromethane solution of **4**, yield 0.10 g (86%); ir (deuteriochloroform): 3225 (s, broad), 1765 (s), 1730 (s), 1565 (s), 1080-1020 (s) $cm^{-1}$; ms: m/e (relative %) 362 (4), 361 (6), 360 (35), 345 (11), 232 (62), 186 (22), 185 (40), 173 (76), 149 (47), 148 (84), 127 (100). Molecular weight 360.1187 (Calcd. for $C_{15}H_{24}N_2O_4S_2$, 360.1177).

*Anal.* Calcd. for $C_{15}H_{25}N_2O_4S_2BF_4$: C, 40.18; H, 5.62; N, 6.25. Found: C, 39.72; H, 5.45; N, 6.48.

Preparation of *d*-Biotinol (**15**).

The procedure described by Lane and coworkers was modified for the preparation of **15** (4). To a stirred suspension of LAH (1.96 g, 51.64 mmoles) in anhydrous ether (250 ml), a hot pyridine (50 ml) solution of vacuum dried (over phosphorus pentoxide) **1** (1.96 g, 8.02 mmoles) was added dropwise. After addition, the reaction mixture was stirred at room temperature (0.5 hour) and then heated to reflux for an additional 0.5 hour. The excess LAH was then destroyed by careful dropwise addition of water. An additional 100 ml of water was added and the organic solvents were removed by steam distillation. The pot residue was cooled to room temperature and carefully acidified (*p*H ~ 2) with 6*N* hydrochloric acid. The suspension was continuously extracted with chloroform (2 days) and the chloroform layer evaporated *in vacuo* to give a white solid. Recrystallization from methanol-water gave 1.05 g (60%) of the reduced product (**15**), mp 165-169° (lit (4) mp 173-174°).

Decarbonylation of *d*-Biotinol (**15**), Preparation of **16**.

*d*-Biotinol (**15**) (0.40 g, 1.74 mmoles) was combined with water (12 ml) and anhydrous barium hydroxide (5.00 g, 29.17 mmoles) in a heavy wall glass tube (200 × 15 mm), sealed with a torch, and then heated with an oil bath at 140° (18 hours). The tube was then opened and the contents transferred (with water rinsing when necessary) to a 125 ml Erlenmeyer flask. Carbon dioxide gas was bubbled into the basic reaction mixture until a *p*H of 7 was reached. The mixture was then filtered through a Celite bed and the filtrate was acidified with 1*N* sulfuric acid until it was faintly acidic to Congo Red paper. The mixture was then refiltered through Celite to remove the barium sulfate that formed. The filtrate was evaporated *in vacuo* to an oil. Methanol (~ 25 ml) was added and a white solid formed. The solid was washed with ether and dried to give 0.46 g (87%) of salt **16**, mp 203-211° dec, which was used without further purification; ir (potassium bromide): 3300 (s), 2900 (s), 1580 (m), 1490 (m), 1100 (s) $cm^{-1}$.

Preparation of 2′-Thiobiotinol (**12**) from **16**.

Salt **16** (0.39 g, 1.29 mmoles) was dissolved in water (10 ml) and added to a warm aqueous solution (12 ml) of barium hydroxide octahydrate (0.41 g, 1.29 mmoles). The barium sulfate precipitate was removed by filtration through a Celite bed. The filtrate was evaporated *in vacuo* to a volume of 2 ml. An equal volume of ethanol was added followed by carbon disulfide (0.1 ml, 1.7 mmoles) and the solution was stirred (1 hour) at 40°. The solution was transferred to a heavy walled glass tube (200 × 15 mm), sealed with a torch, and heated at 100° for 3 hours. The tube was then opened and the reaction mixture was heated until the smell of hydrogen sulfide was no longer evident. The reaction mixture was transferred to a flask and evaporated *in vacuo* to give 0.16 g (50%) of **12** as a white powder, mp 210°; nmr (DMSO-$d_6$): $\delta$ 1.13-1.80 (m, 8H), 2.60-2.83 (d, J = 6 Hz, 2H), 2.97-3.17 (m, 5H), 4.03-4.63 (m, 2H), 7.83-8.10 (1H).

Competative Alkylation Study Between 2-Imidazolidinethione (**7**) and Tetrahydrothiophene (**8**).

Tetrahydrothiophene (**8**) (2.00 ml, 22.68 mmoles) and 2-imidazolidinethione (**7**) (2.32 g, 22.68 mmoles) were combined with methanol (50 ml). Iodomethane (1.40 ml, 22.68 mmoles) was added and the solution brought to reflux (18 hours). The solution was evaporated *in vacuo* to give a solid residue which was triturated with ether (125 ml) and then vacuum dried to give a quantitative yield of 2-methylthio-2-imidazoline hydriodide (**9**) (4.32 g), mp 145-147° (8); nmr (DMSO-$d_6$): $\delta$ 2.67 (s, 3H), 3.87 (s, 4H). (The NH protons were not detected.)

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## REFERENCES AND NOTES

(1) Alfred P. Sloan Foundation Fellow, 1977-1981. Camille and Henry Dreyfus Teacher-Scholar Grant Recipient, 1977-1982.

(2) Review: J. Moss and M. D. Lane, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **35**, 321 (1971); J. Knappe, *Annu. Rev. Biochem.*, **39**, 757 (1970); H. G. Wood, *Trends Biochem.*, **1**, 4 (1976); H. G. Wood and R. E. Barden, *Annu. Rev. Biochem.*, **46**, 385 (1977); T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms", Vol. 2, W. A. Benjamin, New York, 1966, Chapter 11. See also references therein for a review of the earlier literature.

(3) R. B. Guchhait, S. E. Polakis, P. Dimroth, E. Stoll, J. Moss and M. D. Lane, *J. Biol. Chem.*, **249**, 6633 (1974); S. E. Polakis, R. B. Guchhait, E. E. Zwergel and M. D. Lane, *ibid.*, **249**, 6657 (1974).

(4) R. B. Guchhait, S. E. Polakis, D. Hollis, C. Fenselau and M. D. Lane, *J. Biol. Chem.*, **249**, 6646 (1974).

(5) K. Hoffman, D. B. Melville and V. duVigneaud, *ibid.*, **141**, 207 (1941).

(6) N. M. Green *Biochem. J.*, **101**, 774 (1966).

(7) $^1$H nmr analysis of the initial reaction product obtained by the method of Green (6) indicated the presence of both 2'-thiobiotin (**6**) and the corresponding ethyl ester. The presence of the latter compound necessitated the subsequent use of base in the work-up.

(8) The melting point and spectral properties obtained for this compound in this reaction were identical to a commercial sample (Aldrich Chemical Co.).

(9) A full discussion of the $^1$H nmr spectrum of this compound along with its corresponding simulated spectrum will appear elsewhere, H. Kohn, S. Silber, R. Inners, and H. Flaster, manuscript in preparation.

(10) The corresponding dihydrochloride derivative has previously been prepared: R. M. Baxter and J. H. Quastel, *J. Biol. Chem.*, **201**, 751 (1953) and references therein.

(11) Significant signals were detected in the mass spectra of **2-4** at m/e 128 corresponding to the *S*-methyl derivative of ion **28**.

(12) H. Kohn, M. J. Cravey, J. H. Arceneaux, R. L. Cravey and M. R. Willcott, III, *J. Org. Chem.*, **42**, 941 (1977).

(13) L. J. Bellamy, "Advances in Infra-red Group Frequencies", Methuen, London, 1968 and references cited therein.

(14) M. J. Cravey and H. Kohn, *J. Am. Chem. Soc.*, **102**, 3928 (1980).

(15) H. Kohn, *ibid.*, **98**, 3690 (1976).

(16) K. Nakanishi and P. H. Solomon, "Infra-red Absorption Spectroscopy", 2nd ed., Holden-Day, San Francisco, 1977.

(17) J. Knappe, E. Ringelmann and F. Lynen, *Biochem. Z.*, **335**, 168 (1961).

(18) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed., Pergamon Press, Elmsford, N. Y., 1969. See in particular Part 3, p. 159.

(19) For a thorough study of the $^1$H and $^{13}$C nmr spectral properties of biotin methyl ester and the corresponding sulfoxides, see: F.-T. Liu and N. J. Leonard, *J. Am. Chem. Soc.*, **101**, 996 (1976).

(20) W. C. Stallings, C. T. Monti, M. D. Lane and G. T. DeTitta, *Proc. Natl. Acad. Sci. USA*, **77**, 1260 (1980).

(21) In the 400.1 MHz $^1$H nmr spectra of **11** and **14**, the proton-proton coupling patterns observed for the C3-H and C4-H ring methine protons indicated that the most deshielded proton (C4-H) is flanked by one methine hydrogen (C3-H) and one methylene group (C5-H2), while the upfield hydrogen (C3-H) is adjacent to two other methine hydrogens (C2-H and C4-H).

(22) R. Greenhalgh and M. A. Weinberger, *Can. J. Chem.*, **43**, 3340 (1965).

(23) The S-C$H_3$ resonance for compound **29** appeared at $\delta$ 2.74.

(24) The assignments for C3-H and C4-H are tentative; these values may be reversed.

(25) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972, and references cited therein.

(26) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, 1972, and references therein.

(27) A number of the assignments originally made (4) are inconsistent with the data obtained from the corresponding proton coupled $^{13}$C nmr data.