lonsky, G. Ferreol, R. Toubiana, and E. Lederer, *Bull. Soc. Chim. Fr.*, 1471 (1956); T. Gendre and E. Lederer, *ibid.*, 1478 (1956); G. Brochére-Ferréol and J. Polonsky, *ibid.*, 714 (1958).
(9) H. Bredereck, *Chem. Ber.*, **63**, 959 (1930).
(10) Cf. R. L. Edwards and N. Kale, *J. Chem. Soc.*, 4084 (1964); A. F. Kluge, K. G. Untch, and J. H. Fried, *J. Am. Chem. Soc.*, **94**, 7827 (1972).
(11) Closely related processes were encouraging. Cf. A. G. McInnes, *Can. J. Chem.*, **43**, 1998 (1965); D. T. Hurst and A. G. McInnes, *ibid.*, **43**, 2004 (1965); E.-F. Fuchs and J. Lehmann, *Chem. Ber.*, **107**, 721 (1974).
(12) A similar removal of silyl groups with fluoride ion has been observed. Cf. E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, **94**, 6190 (1972); G. H. Dodd, B. T. Golding, and P. N. Ioannou, *J. Chem. Soc., Chem. Commun.*, 249 (1975).
(13) J. H. Brewster and C. J. Ciotti, Jr., *J. Am. Chem. Soc.*, **77**, 6214 (1955). The earlier implied conclusion that the symmetrical acid anhydride is the actual acylating agent may be questioned. The mixed carboxylic–sulfonic anhydride appears to be a more likely candidate.
(14) T. L. Goodfriend, L. Levine, and G. D. Fasmann, *Science*, **144**, 1344 (1964); D. G. Hoare and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **88**, 2057 (1966); *J. Biol. Chem.*, **242**, 2447 (1967); F. S. Chu and E. Crary, *Biochem. Biophys. Acta*, **194**, 287 (1969); K. J. Kramer and J. A. Rupley, *Arch. Biochem. Biophys.*, **156**, 414 (1973).
(15) G. Birch, *J. Chem. Soc.*, 3489 (1965).
(16) **Note Added in Proof.** After this paper was submitted for publication, we became aware of work by R. Toubiana, B. C. Das, J. Defaye, and B. Mompon, *Carbohydr. Res.*, **44**, 308 (1975), in which the same hexasilylated compound **4** was prepared by selective desilylation of octasilylated trehalose **3** with potassium carbonate in methanol. The reported melting was 115–118 °C in agreement with our value.
(17) H. O. Chaplin and L. Hunter, *J. Chem. Soc.*, 375 (1938).

# A Stereospcific Synthesis of Biotin via Thiophene Intermediates[1a]

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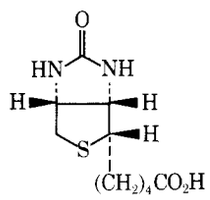
A total synthesis of the vitamin biotin (**1**) is described. Catalytic hydrogenation of the easily prepared thiophene **22** was found to occur stereospecifically and proceed in excellent yield. This approach features a selective ring closure of the amino diacid **6** to the eight-membered lactam **7**. A number of interesting rearrangements were discovered during the course of a modified Curtius reaction involving the mixed anhydride **16**, which led to the key aromatic substrate for reduction. A novel and efficient ring closure of the mixed diurethane **24** to the imidazolidone moiety of biotin was used to complete the synthesis.

Initially, the biological activity of biotin (**1**), a member of the B vitamin complex, was confined to its prevention of dermatitis and other degenerative effects in experimental animals.[1b] In recent years, however, researchers have discovered many new applications of this natural product in the areas of nutrition and growth promotion.[2] These findings have generated a renewed interest in the total synthesis of biotin, and this has led to the development of several new syntheses.[3,4]



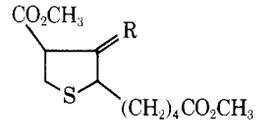

**1**

An examination of the structure of biotin (**1**) reveals the presence of three contiguous asymmetric centers, which requires a high degree of stereocontrol over synthetic intermediates. In addition, the three substituents on the tetrahydrothiophene ring are present in the thermodynamically least stable all-cis configuration.

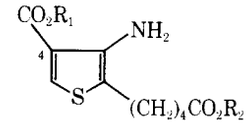
Recently, we have disclosed a solution to this problem which involved a novel oxidative rearrangement of an olefinic thiazolidine.[4] Earlier workers[5] have employed catalytic hydrogenation of a dihydrothiophene in this regard with varying degrees of success. Their efforts were often complicated by a lack of stereospecificity in the reduction step as well as other complications related to the chemistry of dihydrothiophenes. Therefore, it seemed reasonable that a synthesis of biotin based on readily available aromatic intermediates would offer several advantages. For example, the thiophene ring can be considered to be a protecting group for sulfur during the elaboration of the ring substituents. Furthermore, this protection may be dismantled by catalytic hydrogenation, conditions which in principle can simultaneously introduce the all-cis ring hydrogens of biotin in one operation. To date, no synthetically useful approach to biotin based on this concept has been reported,[6] reflecting the marked resistance of thiophenes to reduction.[7]

In this report we describe a highly stereospecific synthesis of biotin which incorporates an efficient reduction of the aromatic precursor **22** to the requisite oxidation level with concomitant introduction of the three cis hydrogens.

An easy entry into the appropriately substituted thiophenes begins with the readily available ketone **2**, prepared in large quantities from pimelic acid and methyl mercaptopropionate.[8] Treatment of **2** with hydroxylamine in pyridine at room temperature yielded the corresponding oxime **3** in quantitative yield. The two units of unsaturation present in the oxime functionality were induced to migrate into the thiophane ring system by simply dissolving the oxime **3** in ether saturated with hydrogen chloride for 24 h.[9] This rearrangement, which seems to require an electron-withdrawing group in position $\alpha$ to the oxime, afforded in 96% combined yield a mixture of the amino diester **4** and the corresponding amino acid **5**, in a ratio of 6:1. The water derived from the oxime dehydration presumably is the source of the by-product **5**, which is easily isolated by simple extraction.




**2**, R = O
**3**, R = NOH

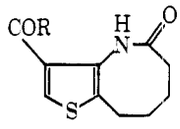



**4**, $R_1 = R_2 = CH_3$
**5**, $R_1 = CH_3$; $R_2 = H$
**6**, $R_1 = R_2 = H$; $\cdot HCl$

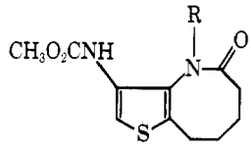
Our synthetic plan at this point required that a Curtius reaction be carried out on the aromatic carbomethoxy group attached to C(4). Treatment of the amine diester **4** with hydrazine failed to distinguish between the two esters. Although the amino acid **5** carried the requisite differentiated groups,

its preparation from the diester 4 could not be achieved in a practical manner. The problem of selecting between the redundant functionality present in 4 was efficiently solved by first hydrolyzing the crude mixture of 4 and 5 to the amino diacid 6, which was isolated as its hydrochloride salt in 95% overall yield based on the ketone 2. When a suspension of the amino diacid 6 in xylene was heated under reflux (Dean-Stark trap), a smooth cyclization to the eight-membered ring lactam 7 occurred. The product crystallized on cooling and was isolated in 87% yield. Similarly, the amino acid 5 underwent cyclization to the ester lactam 8; however, the diester 4 failed to react under these conditions.

Reaction of the ester 8 with hydrazine for 2 min at 25 °C yielded the carbohydrazide 9. Diazotization of 9 afforded the expected acyl azide 10, which underwent to Curtius rearrangement[10] upon heating in methanol under reflux. The product urethano lactam 11 was isolated in an overall yield of 95% based on the ester 8.

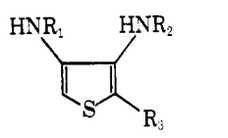


7, R = OH
8, R = $OCH_3$
9, R = $NHNH_2$
10, R = $N_3$



11, R = H
23, R = $CO_2Et$

We had secured in the urethane 11 a thiophene of general structure 12 which contained the required five-carbon side chain attached to C(2) and the necessary carbon–nitrogen bonds at both C(3) and C(4). The oxidation state of every atom in the substituents was now correct for conversion to biotin with the contrived exception of the thiophene nucleus. Reduction of the urethane 11 by catalytic hydrogenation led to the desired all-cis tetrahydrothiophene 13. The yield in this step was disappointingly low, and the intermediate 13 was not isolated from the product mixture but directly hydrolyzed with barium hydroxide to the diamino acid 14. Conversion to the target molecule was accomplished by exposure of 14 to phosgene, thereby yielding a sample of *dl*-biotin (1), isolated as its methyl ester 15, and shown to be identical in all respects with an authentic sample. This result served to establish the validity of our approach and encouraged further studies on the prime intermediate carboxy lactam 7.



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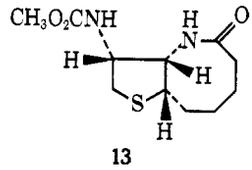

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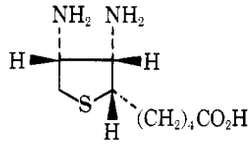

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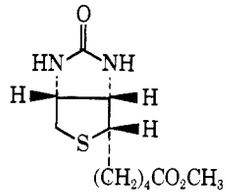
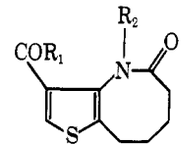
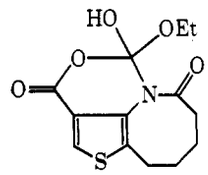


15

Treatment of 7 with ethyl chloroformate, the first step in the modified Curtius reaction,[11] did not yield the expected mixed anhydride 16. Instead, the carboxy imide 17, a product of acyl transfer to the relatively unreactive C(3) nitrogen, was generated. This result implicates the intramolecular acylation shown in structure 18. Addition of a second equivalent of ethyl chloroformate then presumably afforded the desired imido mixed anhydride 19 which was treated without isolation with sodium azide. Of the four potentially reactive carbonyls present in 19 only the one derived from the initial C(4) carboxyl group is affected. The resulting imido acyl azide 20 is thus available in virtually quantitative yield from the carboxy lactam 7. Heating 20 in methanol under reflux led primarily to the diurethane 21, a result of the expected Curtius rearrangement of the acyl azide [leading to the methyl urethane at C(4)] and a selective methanolysis of the imide function at the original lactam carbonyl [affording the ethyl urethane at C(3) and a methyl ester at the side chain terminus]. A small amount of by-product 11, arising from the alternate mode of imide methanolysis, was also obtained. Treatment of the mixture with aqueous sodium hydroxide yielded the desired acid 22 (easily separable from the unreactive 11 by simple extraction), which was isolated in 80% yield based on the acyl azide 20.



16, $R_1$ = $OCO_2Et$; $R_2$ = H
17, $R_1$ = OH; $R_2$ = $CO_2Et$
19, $R_1$ = $OCO_2Et$; $R_2$ = $CO_2Et$
20, $R_1$ = $N_3$; $R_2$ = $CO_2Et$



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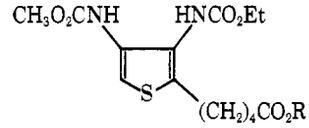
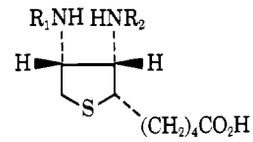


21, R = $CH_3$
22, R = H

A useful conversion of the by-product 11 to the main-line intermediate 21 was also achieved. Treatment of the urethane 11 in neat ethyl chloroformate under reflux quantitatively acylated the C(3) nitrogen and yielded the imido urethane 23. Methanolysis of 23 led directly to the desired diurethane 21. Fortunately, in spite of the presence of both a methyl and ethyl urethane in our intermediates, no traces of urethane exchange reactions in methanol were ever detected.

Catalytic hydrogenation of the now readily available thiophene acid 22 gave an excellent yield (>95%) of the corresponding all-cis tetrahydrothiophene acid 24.[12] The product was obtained as an oil, homogeneous in several TLC systems.



24, $R_1$ = $CO_2CH_3$; $R_2$ = $CO_2Et$
25, $R_1$ = $CO_2CH_3$; $R_2$ = H
26, $R_1$ = H; $R_2$ = $CO_2Et$

The all-cis structural assignment was easily confirmed by its conversion to *dl*-biotin (1) exclusively, without any detectable trace of the other biotin stereoisomers. This last transformation was achieved by simply treating the mixed diurethane 24 with aqueous barium hydroxide at reflux, conditions which served to cyclize the urethanes and generated the imidazolidone moiety. This led directly to *dl*-biotin (1), which separated in high yield upon acidification. The material so obtained was shown to be identical in all respects with an authentic sample.

This direct conversion to the cyclic urea portion of the biotin molecule implicates the intermediacy of the amino ure-

thanes **25** and/or **26**. We expected a difference in the rate of hydrolysis of a methyl vs. an ethyl urethane, especially with the latter group flanked by the side chain at C(2). Once generated, compounds such as **25** and **26** seem to undergo cyclization to biotin rather than suffer any further hydrolysis to the diamino acid **14**. This result obviates the need for a subsequent phosgene treatment of the hydrolysate, an undesirable feature of most published biotin syntheses.[13]

Thus, *dl*-biotin is available from the ketone **2** in an overall yield of 37% by a synthesis which features a number of novel steps: (1) the smooth closure of an eight-membered lactam ring (**6** → **7**); (2) the rearrangements of the various intermediates in the modified Curtius reaction (**7** → **21**); (3) a high-yield stereospecific hydrogenation of a trisubstituted thiophene (**22** → **24**); and (4) the ring closure of a diurethane to an imidazolidone derivative (**24** → **1**).

Finally, since the resolution of *dl*-biotin to the naturally occurring *d* enantiomer has been described,[14] these results constitute a total synthesis of *d*-biotin.

## Experimental Section

Melting points were determined on a Rinco Model M-50 melting point apparatus and are uncorrected. Ir spectra were obtained using a Beckman IR-9 spectrophotometer. A Cary 14 recording spectrophotometer was used for uv absorption spectra. NMR spectra were determined with Varian T-60 and HA-100 spectrometers using tetramethylsilane as the internal reference. Mass spectra were recorded on a CEC 21-110B mass spectrometer at 70 eV using a direct insertion probe. Thin layer chromatography was carried out using Merck F-254 silica gel plates.

**4-Carbomethoxy-2-[4,5-dihydrothiophen-3(2*H*)-one]valeric Acid Methyl Ester Oxime (3).** A solution of 151.4 g (0.553 mol) of 4-carbomethoxy-2-[4,5-dihydrothiophen-3(2*H*)-one]valeric acid methyl ester (**2**) in 470 ml of pyridine was treated with 42.2 g (0.608 mol) of hydroxylamine hydrochloride, and the reaction mixture was stirred at 25 °C for 24 h. Excess pyridine was removed on the rotary evaporator. The residue was taken up in 500 ml of dichloromethane and washed with 200 ml of 1 N hydrochloric acid. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness to yield 158.0 g (0.546 mol, 99%) of the oxime **3** as a pale yellow oil, suitable for use in the next step: ir ($CH_2Cl_2$) 3400, 3200 (oxime), 1740 $cm^{-1}$ (esters).

**3-Amino-4-carbomethoxy-2-thiophenevaleric Acid Methyl Ester (4) and 3-Amino-4-carbomethoxy-2-thiophenevaleric Acid (5).** Gaseous hydrogen chloride was bubbled into a round-bottom flask containing a solution of 110 g (0.381 mol) of the oxime **3** in 1500 ml of anhydrous ether previously cooled to 0 °C. After 1.0 h, the reaction flask was stoppered and stored at 25 °C for 24 h. The mixture was concentrated on a rotary evaporator, and the residue was taken up in 500 ml of water and made basic by the addition of 1000 ml of 10% sodium bicarboate solution. The mixture was then extracted three times with 500-ml portions of dichloromethane. The organic phases were dried over anhydrous sodium sulfate and evaporated to afford 90.0 g (0.316 mol, 83%) of the amino diester **4** as a pale yellow, crystalline solid, mp 50–52 °C. For analysis, a sample was recrystallized from ether and melted at 51–52 °C: ir (KBr) 3460, 3370 ($NH_2$), 1735, 1700 (esters), 1610 $cm^{-1}$; uv max ($CH_3OH$) 218 nm (sh) ($\epsilon$ 16 500), 248 (sh) (8500), 325 (2200); NMR ($CDCl_3$) $\delta$ 7.76 (s, 1 H, aromatic H), 4.32 (bs, 2 H, $NH_2$), 3.84 (s, 3 H, $OCH_3$), 3.66 (s, 3 H, $OCH_3$), 2.60 (t, 2 H, $ArCH_2$), 2.36 (t, 2 H, $CH_2$), 1.69 (m, 4 H, $CH_2CH_2$); mass spectrum *m/e* 271 ($M^+$), 170 (base), 138.

Anal. Calcd for $C_{12}H_{17}NO_4S$ (271.34): C, 53.12; H, 6.32; N, 5.16; S, 11.82. Found: C, 53.17; H, 6.35; N, 5.28; S, 11.80.

The aqueous phase was acidified with 6 N hydrochloric acid to pH 4 and extracted three times with 300-ml portions of dichloromethane. The organic extracts were dried over anhydrous sodium sulfate and evaporated to afford 13.0 g (0.048 mol, 13%) of the amino acid **5** as a white solid, mp 130–132 °C. An analytical sample was obtained by recrystallization from ethyl acetate and afforded colorless crystals: mp 131–132 °C; ir (KBr) 3450, 3360 ($NH_2$), 2700–2500 ($CO_2H$), 1720 (ester), 1705 $cm^{-1}$ (acid); uv max ($CH_3OH$) 203 nm ($\epsilon$ 1500), 220 (13 580), 249 (7900), 325 (2210); NMR ($Me_2SO$) $\delta$ 7.87 (s, 1 H, aromatic H), 3.77 (s, 3 H, $CO_2CH_3$), 2.60 (t, 2 H, $ArCH_2$), 2.25 (t, 2 H, $CH_2$), 1.54 (m, 4 H, $CH_2CH_2$); mass spectrum *m/e* 257 ($M^+$), 170 (base), 138.

Anal. Calcd for $C_{11}H_{15}NO_4S$ (257.31): C, 51.35, H, 5.88; N, 5.44; S, 12.46. Found: C, 51.47; H, 5.94; H, 5.60; S, 12.31.

**3-Amino-4-carboxy-2-thiophenevaleric Acid Hydrochloride (6).** A mixture of 18.64 g of the amino diester **4** and the amino acid **5** was prepared by the method outlined in the previous reaction. This material was dissolved in 400 ml of methanol and was treated with 185 ml (0.185 mol) of 1 N sodium hydroxide. The reaction mixture was refluxed for 1 hr, cooled, and concentrated. The residue was acidified to pH 1 with 50 ml of 6 N hydrochloric acid and evaporated to dryness leaving 23.0 g of the amino diacid **6** as its hydrochloride, admixed with the sodium chloride by-product. This mixture can be used directly in the next step. Further purification can be achieved by extraction of the residue with hot ethanol. The residue obtained by evaporation of the ethanol extract was recrystallized from methanol–ether to give **6** as a white solid: mp 186–187 °C dec; ir (KBr) 3000–2500 ($NH_3^+$), 1700–1660 $cm^{-1}$ (acids); uv max ($CH_3OH$) 240 nm ($\epsilon$ 6000), 320 (940); NMR ($CDCl_3$) $\delta$ 8.18 (s, 1 H, aromatic H), 8.00 (b, 4 H, $NH_2$ + 2 $CO_2H$), 2.89 (t 2 H, $ArCH_2$), 2.23 (t, 2 H, $CH_2$), 1.10 (m, 4 H, $CH_2CH_2$); mass spectrum *m/e* 243 ($M^+$), 224, 208, 197, 156 (base).

**3-Amino-4-carboxy-2-thiophenevaleric Acid ζ-Lactam (7).** A suspension of 29.0 g (0.104 mol) of the amino diacid hydrochloride **6** in 3.8 l. of xylene was heated under reflux for 2.0 days, using a Dean-Stark trap to remove water. The solution was filtered to remove polymeric material and the filtrate was allowed to cool. The product carboxy lactam **7** separated, and was filtered and washed with ether. The yield of pure material was 19.8 g (0.088 mol, 85%). A sample was recrystallized from xylene–ethanol (trace)–petroleum ether to afford white crystals: mp 216–217 °C; ir (KBr) 3280 (N–H), 2700–2500 ($CO_2H$), 1680 (aromatic acid), 1630 (amide), 1260 $cm^{-1}$; uv max ($CH_3OH$) 213 nm ($\epsilon$ 22 100), 275 (infl) (1810); NMR ($Me_2SO$) $\delta$ 12.50 (b, 1 H, $CO_2H$), 8.82 (bs, 1 H, NH), 8.06 (s, 1 H, aromatic H), 2.69 (t, 2 H, $ArCH_2$), 2.05 (t, 2 H, $CH_2$), 1.67 (m, 4 H, $CH_2CH_2$); mass spectrum *m/e* 225 ($M^+$), 169 (base), 138.

Anal. Calcd for $C_{10}H_{11}NO_3S$ (225.27): C, 53.32; H, 4.92; N, 6.22; S, 14.23. Found: C, 53.64; H, 5.00; N, 6.41; S, 14.07.

**3-Amino-4-carbomethoxy-2-thiophenevaleric Acid Lactam (8).** A suspension of 15.0 g (0.0554 mol) of the amino acid **5** in 1500 ml of xylene was heated to reflux and maintained at that temperature for 1 week employing a Dean-Stark trap to remove water. The solvent was removed on the rotary evaporator using a high vacuum pump. The residue was taken up in 100 ml of dichloromethane and washed with 30 ml of 10% sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate and evaporated to afford 13.5 g (0.0534 mol, 96%) of crude ester lactam **8**. Recrystallization from ethyl acetate yielded 11.8 g (0.0467 mol, 84%) of the product as a white solid: mp 167–168 °C; ir (KBr) 3230 (NH), 1703 (ester), 1675 (lactam), 1250, 745 $cm^{-1}$; uv max ($CH_3OH$) 213 nm ($\epsilon$ 2300), 245 (infl) (7000), 275 (sh) (1880); NMR ($CDCl_3$) $\delta$ 8.02 (bs, 1 H, NH), 7.84 (s, 1 H, aromatic H), 3.82 (s, 3 H, $OCH_3$), 2.78 (t, 2 H, $ArCH_2$), 2.25 (t, 2 H, $CH_2$), 1.83 (m, 4 H, $CH_2CH_2$); mass spectrum *m/e* 239 ($M^+$), 211, 208, 196, 183 (base).

Anal. Calcd for $C_{11}H_{13}NO_3S$ (239.29): C, 55.21; H, 5.48; N, 5.85; S, 13.40. Found: C, 55.25; H, 5.49; N, 5.89; S, 13.39.

**3-Amino-4-carbazoyl-2-thiophenevaleric Acid Lactam (9).** A sample of 15.0 g (0.0628 mol) of the ester lactam **8** was dissolved at 25 °C in 25 ml of 95% hydrazine. After 2 min, the product began to crystallize. The reaction was allowed to proceed for 0.5 h and then the mixture was evaporated to dryness. The residue was filtered and washed with cold ethanol to afford 15.0 g (0.0628 mol, 100%) of the carbohydrazide **9**, mp 191–192 °C. An analytical sample was prepared by recrystallization from ethanol: ir (KBr) 3390–3050 ($NHNH_2$), 1640 (amide, lactam), 1250, 990 $cm^{-1}$; uv max ($CH_3OH$) 213 nm ($\epsilon$ 25 100), 260 (infl) (4300); NMR ($Me_2SO$) $\delta$ 9.37 (bs, 1 H, NH), 8.85 (bs, 1 H, NH), 7.77 (s, 1 H, aromatic H), 4.44 (b, 2 H, $NH_2$). 2.70 (t, 2 H, aromatic $CH_2$), 2.00 (t, 2 H, $CH_2$), 1.75 (m, 4 H, $CH_2CH_2$); mass spectrum *m/e* 239 ($M^+$), 208 (base).

Anal. Calcd for $C_{10}H_{13}N_3O_2S$ (239.30): C, 50.19; H, 5.48; N, 17.56; S, 13.40. Found: C, 50.25; H, 5.39; N, 17.71; S, 13.47.

**3-Amino-4-azidocarbonyl-2-thiophenevaleric Acid Lactam (10).** To a solution of 12.24 g (0.052 mol) of the carbohydrazide **9** in 100 ml of 1 N hydrochloric acid was added dropwise at 0 °C 4.4 g (0.062 mol) of sodium nitrite in 30 ml of water (previously cooled to 0 °C) over a 15-min period. The heterogeneous mixture was stirred for 0.5 h, and then extracted four times with 50-ml portions of chloroform. The extracts were dried over anhydrous sodium sulfate and evaporated to afford 13.0 g (0.052 mol, 100%) of the acyl azide **9** as a colorless oil, suitable for use directly in the next step: ir ($CH_2Cl_2$) 2100 $cm^{-1}$ ($CON_3$).

**3-Amino-4-carbomethoxyamino-2-thiophenevaleric Acid Lactam (11).** A solution of 13.0 g (.052 mol) of the acyl azide **10** in 500 ml of dry methanol was heated to 50 °C. After 15 min at that tem-

perature, the solution was brought up to reflux; the rate of heating was determined by the amount of vigorous gas evolution. The solution was then refluxed for 6.0 h. The solution was cooled and evaporated to afford 12.5 g (0.0491 mol, 95%) of the urethane **11** as a white, crystalline solid, mp 208–210 °C. For analysis, a sample was recrystallized from methanol to yield colorless crystals: mp 209–210 °C; ir (KBr) 3210 (NH), 1705, 1695 (urethane), 1647 (lactam), 1570, 1070 $cm^{-1}$; uv max ($CH_3OH$) 220 nm ($\epsilon$ 22 300), 250 (infl) (5980); NMR ($Me_2SO$) $\delta$ 8.82 (bs, 1 H, NH), 8.60 (bs, 1 H, NH), 7.03 (s, 1 H, aromatic), 3.63 (s, 3 H, $OCH_3$), 2.60 (t, 2 H, aromatic $CH_2$), 1.9 (t, 2 H, $CH_2$), 1.7 (m, 4 H, $CH_2CH_2$); mass spectrum *m/e* 254 ($M^+$), 226, 225, 211 (base), 198.

Anal. Calcd for $C_{11}H_{14}N_2O_3S$ (254.31): C, 51.95; H, 5.55; N, 11.02; S, 12.61. Found: C, 51.74; H, 5.52; N, 10.92; S, 12.88.

**Reduction of the Urethane 11.** A solution of 1.0 g (0.004 mol) of the urethane **11** in 200 ml of glacial acetic acid was placed in a steel autoclave. After addition of 1.0 g of 10% Pd/C catalyst the reaction mixture was hydrogenated at 100 °C and 1800 psi for 10.0 h. The autoclave was cooled and vented, and the catalyst was filtered and washed with 100 ml of acetic acid. The solvent was removed, and the residue containing the tetrahydrothiophene **13** was taken up in 50 ml of water to which 10.0 g of $Ba(OH)_2 \cdot 8H_2O$ has been added.

The reaction mixture was refluxed for 20.0 h and cooled. Carbon dioxide was bubbled in until the pH dropped to 4. The precipitated barium carbonate was filtered and washed with 20 ml of water. The filtrate was acidified with 1 N sulfuric acid and the precipitated barium sulfate was filtered. The filtrate was then evaporated to dryness, and the residue was taken up in 120 ml of 10% by weight sodium carbonate and cooled to 0 °C. Gaseous phosgene was bubbled in for 5 min until the medium was acidic to Congo red. After 2.0 h an impurity was filtered off and the filtrate was evaporated to dryness. The residue, containing *dl*-biotin, was suspended in 70 ml of dry methanol and treated with 1 drop of sulfuric acid. The mixture was refluxed for 1 h, cooled, and filtered. The filtrate was evaporated, and the residue was partitioned between 50 ml of chloroform and 20 ml of water. The aqueous phase was extracted three times with 20-ml portions of chloroform. The organic phases were combined, dried over anhydrous sodium sulfate, and evaporated to give 0.300 g (0.00116 mol, 29%) of crude *dl*-biotin methyl ester. The mixture was taken up in 3 ml of dichloromethane and plated on three thick layer silica plates. Elution was with 10% by volume methanol–chloroform solution. After two elutions, a sample of pure *dl*-biotin methyl ester, mp 131–132 °C, mmp 131–132 °C, was obtained by removal of the band at $R_f$ 0.26 and recrystallization from ethyl acetate.

**4-Azidocarbonyl-3-carbethoxyamino-2-thiophenevaleric Acid Lactam (20).** A solution of 2.25 g (0.010 mol) of the carboxy lactam **7** in 40 ml of acetone to which 2 ml of water had been added was cooled in an ice bath for 15 min. At this point, 4.6 ml (0.033 mol) of triethylamine in 40 ml of acetone was added, followed immediately by the dropwise addition of 3.3 ml (0.033 mol) of ethyl chloroformate in 4.5 ml of acetone over a 10-min period. The reaction mixture was stirred at 0 °C for 1 h and then treated dropwise with a solution of 2.13 g (0.33 mol) of sodium azide in 10 ml of water over a 5-min period. The reaction mixture was further stirred at 0 °C for 2 h and then partitioned between 100 ml of dichloromethane and 75 ml of ice water. The aqueous phase was extracted three times with 30-ml portions of dichloromethane. The organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to leave 3.20 g (0.009 mol, 100%) of the acyl azide **20** as a crystalline solid, which was used directly in the next step, ir ($CH_2Cl_2$) $cm^{-1}$ ($CON_3$).

**3-Carbethoxyamino-4-carbomethoxyamino-2-thiophenevaleric Acid Methyl Ester (21).** A solution of 3.20 g (0.099 mol) of the acyl azide **20** in 75 ml of methanol was heated slowly to reflux. The reaction was allowed to proceed for 6 h at this temperature. The methanol was then removed, leaving 3.12 g (0.087 mol, 87%) of the diurethane **21**. Recrystallization from diethyl ether afforded 2.88 g (80%) of pure **21** as a white solid: mp 60–61 °C; ir (KBr) 3330 (NH), 1740 (ester), 1720 (urethanes), 1560, 1260 $cm^{-1}$; ir (KBr) 3330 (NH), 1740 (ester), 1720 (urethanes), 1560, 1260 $cm^{-1}$; uv max ($CH_3OH$) 206 nm ($\epsilon$ 24 980), 258 (infl) (5200); NMR ($CDCl_3$) $\delta$ 7.22 (bs, 1 H, NH), 7.14 (s, 1 H, aromatic), 6.41 (bs, 1 H, NH), 4.20 (q, 2 H, $CH_2O$), 3.73 (s, 3 H, urethane), 3.63 (s, 3 H, $OCH_3$), 2.67 (t, 2 H, aromatic $CH_2$), 2.32 (t, 2 H, $CH_2$), 1.65 (m, 4 H, $CH_2CH_2$), 1.26 (t, 3 H, $CH_3$); mass spectrum *m/e* 358 ($M^+$), 326, 312, 298, 280.

Anal. Calcd for $C_{15}H_{22}N_2O_6S$ (358.41): C, 50.27; H, 6.19; N, 7.82; S, 8.95. Found: C, 50.34; H, 6.33; N, 7.95; S, 8.95.

The mother liquors from the recrystallization were chromatographed over silica (eluent chloroform–methanol, 98:2) to yield 0.210 g (8%) of the by-product **11**, identical in all respects with the sample prepared from the carbohydrazide **9**.

**Conversion of the Urethane 11 to the Diurethane 21.** A suspension of 1.0 g (0.00393 mol) of the urethane **11** in 15 ml of ethyl chloroformate was heated under reflux for 2.0 h. The reaction mixture was cooled and evaporated to dryness. The residue, consisting primarily of the imide **23**, was taken up in 25 ml of anhydrous methanol and heated under reflux for 3–5 h. The reaction mixture was cooled and evaporated to dryness to give 1.4 g (0.00390 mol, 100%) of the diurethane **21**, identical in all respects with the sample prepared from the carboxy lactam **7**.

**3-Carbethoxyamino-4-carbomethoxy-2-thiophenevaleric Acid (22).** A solution of 0.136 g (0.000380 mol) of the diurethane **21** in 5 ml of methanol was treated with 0.55 ml of 1 N sodium hydroxide. The reaction mixture was heated under reflux for 3.0 h, cooled, and evaporated. The residue was partitioned between 30 ml of dichloromethane and 30 ml of water. The aqueous phase was acidified with 2 ml of 1 N hydrochloric acid and extracted three times with 30-ml portions of dichloromethane. The organic phases were pooled, dried over anhydrous sodium sulfate, and evaporated to yield 0.129 g (000376 mol, 99%) of the acid **22** as a white solid. An analytical sample, mp 159–160 °C, was prepared by recrystallization from methanol: ir (KBr) 3340 (NH), 3150–2850 (acid), 1720 (urethanes), 1700 (acid), 1550, 1241 $cm^{-1}$; uv max ($CH_3OH$) 206 nm ($\epsilon$ 21 500), 250 (infl) (5300); NMR ($Me_2SO$) $\delta$ 9.05 (b, 1 H, OH), 8.9 (b, 2 H, NH), 7.05 (s, 1 H, aromatic H), 4.04 (q, 2 H, $OCH_2$), 3.14 (s, 3 H, $OCH_3$), 2.58 (t, 2 H, $ArCH_2$), 2.18 (t, 2 H, $CH_2$), 1.54 (m, 4 H, $CH_2CH_2$), 1.30 3 H, $CH_3$); mass spectrum *m/e* 344 ($M^+$), 330, 312, 298 (base), 280.

Anal. Calcd for $C_{14}H_{20}N_2O_6S$ (344.39): C, 48.83; H, 5.85; N, 8.13; S, 9.31. Found: C, 48.61; H, 5.94; N, 8.26; S, 9.00.

***all-cis*-3-Carbethoxyamino-4-carbomethoxyamino-2-tetrahydrothiophenevaleric Acid (24).** A solution of 0.344 g (0.001 mol) of the acid **22** in 200 ml of glacial acetic acid was hydrogenated at 1800 psi in a steel autoclave at 50 °C for 10 h using 0.344 g of 10% Pd/C catalyst. The autoclave was cooled and vented, and the catalyst was filtered and washed with 100 ml of glacial acetic acid. The solvent was removed under vacuum to afford 0.328 g (0.00095 mol, 95%) of the tetrahydrothiophene acid **24** as a colorless oil, homogeneous in several TLC systems: ir ($CH_2Cl_2$) 3280 (NH), 2850–2500 (acid), 1740 (urethanes), 1720 (acid), 1520, 915 $cm^{-1}$; NMR ($CDCl_3$) $\delta$ 5.5 (bs, 2 H, NH), 5.5 (b, 1 H, OH), 4.30 (q, 2 H, $CH_2O$), 4.30 (m, 2 H, CHN), 3.7 (s, 3 H, $OCH_3$), 3.21 (m, 2 H, $CH_2$), 2.40 (t, 2 H, $CH_2S$), 2.21 (t, 2 H, $CH_2$), 1.51 (m, 6 H, $CH_2CH_2CH_2$), 1.2 (t, 3 H, $CH_3$); mass spectrum *m/e* 331, 273, 259, 247 (base), 184.

***dl*-Biotin (1).** A suspension of 150 mg (0.43 mmol) of the all-cis tetrahydrothiophene acid **24** in 7 ml of water was treated with 1.0 g (5.2 mmol) of barium hydroxide monohydrate. The mixture was heated under reflux for 1 h, cooled, and filtered to remove inorganics. The solids were washed with water, and the filtrate was acidified with 1 N hydrochloric acid and concentrated. Pure *dl*-biotin crystallized from the solution upon cooling and was obtained as 50.0 mg (48%) of a white solid, mp 232–233 °C, mmp 232–233 °C. From the mother liquors, a second crop of 28.1 mg (27%) was isolated to give a total yield of 75% of pure material identical in all respects with an authentic sample of *dl*-biotin (1): ir (KBr) 3250, 3125 (NH), 2700–2500 (acid), 1705 (urea), 1695 $cm^{-1}$ (acid); NMR ($Me_2SO$) $\delta$ 6.56 (bs, 1 H, NH), 6.44 (bs, 1 H, NH), 4.28 (m, 2 H, NCHCHN), 3.15 (b, 1 H, CHS), 2.72 (m, 2 H, $CH_2S$), 2.22 (t, 2 H, $CH_2$), 1.45 (bm, 6 H, $CH_2CH_2CH_2$); mass spectrum *m/e* 244 ($M^+$), 184, 112, 97 (base), 85.

Anal. Calcd for $C_{10}H_{16}N_2O_3S$ (244.29): C, 49.16; H, 6.60; N, 11.47; S, 13.12. Found: C, 49.22; H, 6.62; N, 11.34; S, 13.20.

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**Registry No.**—**1**, 22377-59-9; **1** Me ester, 60562-11-0; **2**, 59851-05-7; **3**, 59851-06-8; **4**, 59851-07-9; **5**, 59851-08-0; **6** HCl, 59851-10-4; **7**, 59851-11-5; **8**, 59851-12-6; **9**, 59851-13-7; **10**, 59851-14-8; **11**, 59851-15-9; **20**, 59851-19-3; **21**, 59851-20-6; **22**, 59851-21-7; **24**, 60512-75-6; hydroxylamine hydrochloride, 5470-11-1; ethyl chloroformate, 541-41-3.

## References and Notes

(1) (a) For preliminary communication see P. N. Confalone, G. Pizzolato, and M. R. Uskoković, *Helv. Chim. Acta*, **59**, 1005 (1976); (b) M. A. Boas, *Biochem. J.*, **21**, 712 (1927).
(2) R. Blair and C. Whitehead, *Feedstuffs*, **48**, 30 (1976); D. B. McCormick, *Nutr. Rev.*, **33**, 97 (1975); J. O. Anderson and R. E. Warnick, *Poult. Sci.*, **49**, 569 (1970); J. C. Bauerfeind, *Feedstuffs*, **41**, 32 (1969); J. Labuda, *Polnohospodarstvo*, **12**, 707 (1966).
(3) S. Bory, M. J. Luche, B. Moreau, S. Lavielle, and A. Marquet, *Tetrahedron*

Lett., 827 (1975); H. Kuzuhara, H. Ohrui, and S. Emoto, ibid, 1185 (1975); H. Ohrui and S. Emoto, ibid., 2765 (1975); S. I. Zav'yalov et al., Bull. Acad. Sci. USSR, Div. Chem. Sci., 24, 1533 (1975).
(4) P. N. Confalone, G. Pizzolato, E. G. Baggiolini, D. Lollar, and M. R. Uskoković, J. Am. Chem Soc., 97, 5936 (1975).
(5) S. A. Harris, D. E. Wolf, R. Mozingo, and K. Folkers, Science, 97, 447 (1943); S. A. Harris et al., J. Am. Chem. Soc., 66, 1756 (1944); S. A. Harris et al., ibid., 67, 2096 (1945). Catalytic hydrogenation of an exocyclic double bond at C(2) is used in the commercial synthesis of biotin by M. W. Goldberg and L. H. Sternbach, U.S. Patents 2 489 232, 2 489 235, and 2 489 238 (1949). For a related reductive approach using a 2,5-dihydrothiophene see A. Grussner, J. P. Bourquin, and O. Schnider, Helv. Chim. Acta, 28, 510, 517 (1945); B. R. Baker et al., J. Org. Chem., 12 167, 186, 322 (1949).
(6) Notable attempts have been reported by S. Nishimura and E. Imoto, Meeting of the Society of Organic Synthetic Chemistry, Tokyo, Oct. 1959; L. C. Cheney, U.S. Patent 2 502 422; Ya. L. Gol'dfarb et al., Zh. Obshch. Khim., 31, 1244 (1961); Zh. Org. Khim., 1, 1507 (1965); Dokl. Akad. Nauk SSSR, 162, 120 (1965).
(7) C. M. Cawley and C. C. Hall, J. Soc. Chem. Ind., London, 62, 116 (1943); R. Mozingo et al., J. Am. Chem. Soc., 67, 2092 (1945); E. B. Maxted and H. C. Evans, J. Chem. Soc., 603 (1937); H. D. Hartough, "The Chemistry of Heterocyclic Compounds", Vol. III, Interscience, New York, N.Y., 1952, pp 159, 167–169.
(8) G. B. Brown, M. D. Armstrong, A. W. Moyer, W. P. Anslow, Jr., B. R. Baker, M. V. Querry, S. Bernstein, and S. R. Safir, J. Org. Chem., 12, 160 (1947).
(9) L. C. Cheney and J. R. Piening, J. Am. Chem. Soc., 67, 729 (1945).
(10) P. A. S. Smith, Org. React., 3, 000 (1945).
(11) A. J. H. Klunder and B. Zwanenburg, Tetrahedron, 28, 4131 (1972).
(12) This hydrogenation is remarkably substrate specific. For example, the corresponding ester 21 does not hydrogenate at all under these conditions. Presumably, the acid function can enter the coordination sphere of the palladium catalyst. Once this occurs, the coiled side chain of 22 can bring the aromatic substrate in close proximity to the catalytic surface and thus favor the reduction step.
(13) In fact, only S. I. Zav'yalov et al., Bull. Acad. Sci. USSR, Div. Chem. Sci., 24, 1533 (1975), can claim the only other synthesis of biotin whose imidazolidone carbonyl did not originate from phosgene.
(14) D. E. Wolf, R. Mozingo, S. A. Harris, R. C. Anderson, and K. Folkers, J. Am. Chem. Soc., 67, 2100 (1945).

# Reaction of O-Methyl-N,N'-diisopropylisourea with Amino Acids and Amines

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The reaction of O-methyl-N,N'-diisopropylisourea with amino acids and amines, as their hydrochlorides, has been examined, and exhaustive methylation has been found to be the reaction pattern. Thus conversion of L-proline, L-N-methylproline, L-4-hydroxyproline, and DL-2-aminobutyric acid to the corresponding betaines was effected in good yield. Also, the hydrochlorides of benzylamine and codeine gave the quaternary derivatives, benzyltrimethylammonium chloride and codeine methochloride. Clean conversion of morphine to codeine could be realized without N-methylation; however, reaction of this aminophenol was solvent sensitive and in methanol and acetonitrile α-codeimethine was formed as a minor side product. In each case, the more nucleophilic site in the molecule was selectively alkylated.

The utility of O,N,N'-trialkylisoureas in the conversion of carboxylic acids to esters,[1,2] phenols to arylalkyl ethers,[3,4] thiophenols to arylalkyl sulfides,[5,6] and thiols to dialkyl sulfides,[7] and in the alkylation of β-diketones[6] and thymidine and uridine,[8] has been demonstrated. Much less is known about the reactivity of these reagents toward compounds containing more than a single nucleophilic site, and such reactions have received only cursory attention.[5-7]

We now report our findings on the reaction of O-methyl-N,N'-diisopropylisourea (1) with amino acids, amine hydrochlorides, and an aminophenol. These reactions and results appear to be applicable to a variety of other amines and O,N,N'-trialkylisoureas.

**Conversion of Amino Acids to Betaines.** When the amino acids, L-proline (2), L-N-methylproline (4), L-4-hydroxyproline (5), and DL-2-aminobutyric acid (7), were allowed to react with excess isourea 1 in methanol at room temperature for several days, the corresponding betaines 3, 6, and 8 were isolated in moderate to high yield (Table I). In no case was esterification observed, and no etherification of the hydroxyl group of 5 was detected.

The rationalization for betaine formation lies in the relative nucleophilicities of the amino and carboxyl functionalities present in the reaction. Following protonation of 1, the amino acid species is present as the carboxylate anion. N-Methylation leading to betaine formation is attributed to the amino group being a more powerful nucleophile than the carboxylate anion in the ensuing SN2 reaction.

An interesting solvent dependence was observed in the reaction of L-proline (2) with isourea 1. Whereas betaine formation was the sole process occurring at room temperature in methanol or water, both formation of betaine 3 and L-proline methyl ester were detected in *tert*-butyl alcohol or methoxyacetonitrile under reflux. O-Alkylation leading to methyl ester formation can be attributed to the decreased solvation, and, hence, greater availability as a nucleophile, of the more electronegative carboxylate oxygen in *tert*-butyl alcohol and methoxyacetonitrile relative to the more polar and protic methanol and water.

Use of isourea 1 to prepare betaines directly from amino acids affords a mild and effective alternative to the more classical procedures of treating an amino acid with silver oxide and methyl iodide,[9-11] an alkali metal hydroxide and a methylating agent,[9,12,13] or diazomethane[9,14,15] that have been employed to prepare some of these, as well as other betaines. Further, optical activity measurements show the recrystallized product obtained by the isourea method to be optically pure.

**Exhaustive Methylation of Amine Hydrochlorides.** The mechanism for alkylation with O,N,N'-trialkylisoureas requires a proton source and a sufficiently powerful nucleophile.[1,2,16] In view of the ease of betaine formation from amino acids, it was postulated that an amine hydrohalide could serve as both proton source and nucleophile and react with excess O-methyl-N,N'-diisopropylisourea (1) to yield the corresponding quaternary ammonium halide. In agreement with the prediction, treatment of benzylamine hydrochloride (9) and codeine hydrochloride (11) with excess isourea 1 in methanol at room temperature for several days resulted in the formation of benzyltrimethylammonium chloride (10) and codeine methochloride (12), respectively. The low isolated yields of 10 and 12, 17 and 25%, were attributed to incomplete