The Synthesis of *N*-Acyl-α-mercaptoalanine Derivatives[1]

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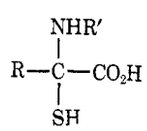
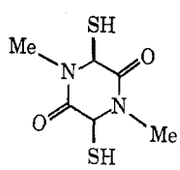
N-Acyl-α-mercapto-DL-alanine derivatives have been prepared by the reaction of hydrogen sulfide with the corresponding *N*-acyl-α-halo-DL-alanines. In a similar manner, reaction of *N*-acyl-α-haloalanine derivatives with thiolacetic acid or benzhydryl mercaptan gave the corresponding α-acetylthiol and α-benzhydrylmercaptoalanines, which upon removal of the *S*-acetyl and *S*-benzhydryl groups yielded α-mercaptoamino acid derivatives. Reaction of *N*-acyl-2,3-dihaloalanines with hydrogen sulfide or thiolacetic acid effected displacement of the α-halo group to yield 3-halo-2-mercapto- and 3-halo-2-acetylthioalanine derivatives, respectively. Deprotection of *N*-benzyloxycarbonyl-α-mercaptoalanine gave α-mercapto-DL-alanine hydrobromide, which proved to be quite unstable.

α-Mercapto-α-amino acids (**1**) are of interest in relationship to certain antibiotics, *i.e.*, gliotoxin,[2a] sporidesmin,[2b] aranotin,[2c] chaetocin,[2d] and the quinomycins,[3] which contain amino acid moieties possessing a sulfur function in the α position. The synthesis of 3,6-dimercaptopiperazine-2,5-dione derivatives **3**[4] and **4**[5] has been reported; these α-mercapto-α-amino acid derivatives are known to possess antiviral activity.[4a] Recently, Pojer and Rae reported[6] the preparation of *N*-benzoyl-2-mercapto-DL-alanine (**2**). We wish to



1, R′ = H
2, R = methyl; R′ = benzoyl



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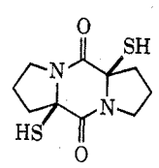

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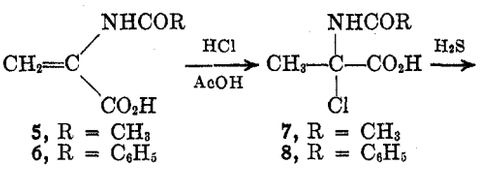
report in this paper a convenient synthesis of *N*-acyl-α-mercaptoalanine derivatives.

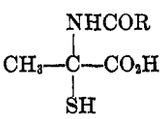
Treatment of the α-chloroalanine **7**, formed *in situ* by the addition[7] of hydrogen chloride to 2-acetamidoacrylic acid (**5**), with hydrogen sulfide in acetic acid gave *N*-acetyl-2-mercapto-DL-alanine (**9**) as a crystalline, readily isolable material. The nmr spectrum of **9** in dimethyl sulfoxide-d_6 consisted of three singlets, in a relative intensity of 3:4:1, at δ 1.68 due to the β protons, 1.84 due to the acetyl group superimposed upon the mercapto proton, and 7.50 assignable to the amide proton. Addition of deuterium oxide effected hydrogen–deuterium exchange of the mercapto proton, as evidenced by equal relative intensities, following exchange, of the peaks at δ 1.68 and 1.84.

In a similar manner, 2-benzamidoacrylic acid (**6**) was converted to the mercaptoalanine **10**; the physical and spectral properties of **10** corresponded in all respects with data reported[6] for this compound.



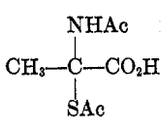
5, R = CH_3
6, R = C_6H_5

7, R = CH_3
8, R = C_6H_5



9, R = CH_3
10, R = C_6H_5

The α-mercaptoalanine **9** also was prepared by treatment of the α-chloro compound **7** with thiolacetic acid to give the α-acetylthioalanine **11**. Removal of the *S*-acetyl group in **11** by methanolysis[8] gave **9**.



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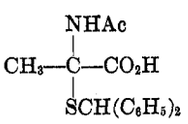

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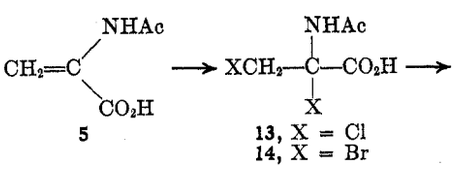
Likewise, the α-benzhydrylmercapto derivative **12**, prepared by reaction of benzhydryl mercaptan with **7**, yielded **9** upon cleavage[8] of the benzhydryl moiety with trifluoroacetic acid.

Addition[9] of chlorine to **5** gave *N*-acetyl-2,3-dichloro-DL-alanine (**13**). Treatment of **13** with hydrogen sulfide yielded the 3-chloro-2-mercaptoalanine **15**. Similarly, the dibromo compound **14** yielded the 2-acetylthio-3-bromoalanine **16** upon treatment with



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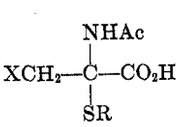
13, X = Cl
14, X = Br



15, R = H; X = Cl
16, R = acetyl; X = Br

(1) Presented in part at the 27th Annual Northwest Regional Meeting of the American Chemical Society, Corvallis, Ore., June 1972.

(2) (a) M. R. Bell, J. R. Johnson, B. Wyldi, and R. B. Woodward, *J. Amer. Chem. Soc.*, **80**, 1001 (1958). (b) J. Fridrichson and A. McL. Mathieson, *Tetrahedron Lett.*, 1265 (1962); R. Hodges, J. W. Ranaldson, A. Taylor, and E. P. White, *Chem. Ind. (London)*, 42 (1963). (c) R. Nagarajan, N. Neuss, and M. M. Marsh, *J. Amer. Chem. Soc.*, **90**, 6518 (1968); D. B. Cosulich, N. R. Nelson, and J. H. van den Herde, *ibid.*, **90**, 6519 (1968). (d) D. Hauser, H. P. Weber, and H. P. Sigg, *Helv. Chim. Acta*, **53**, 1061 (1970).

(3) W. Keller-Schierlein, M. Lj. Mihailovic, and V. Prelog, *Helv. Chim. Acta*, **42**, 305 (1959); H. Ōtsuka and J. Shōji, *Tetrahedron*, **23**, 1535 (1967).

(4) (a) P. W. Trown, *Biochem. Biophys. Res. Commun.*, **33**, 402 (1968); (b) H. Poisel and U. Schmidt, *Chem. Ber.*, **104**, 1714 (1971).

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(6) P. M. Pojer and I. D. Rae, *Tetrahedron Lett.*, 3077 (1971).

(7) A. L. Love and R. K. Olsen, *J. Org. Chem.*, **37**, 3431 (1972).

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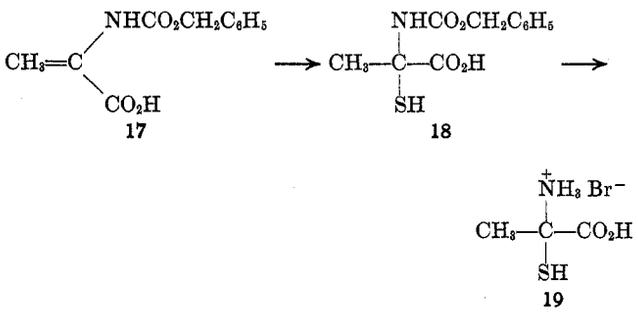
(9) O. V. Kil'disheva, L. P. Rasteikene, and I. L. Kunyants, *Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 231 (1955); *Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk*, 260 (1955).

excess thiolacetic acid. These reactions, which lead only to displacement of the α-halo group, are in contrast to the reported[10] displacement of both the 2- and 3-halo groups by alkyl mercaptans.

An interesting phenomenon was observed in the nmr spectra of the 2,3-disubstituted alanine derivatives **13** and **15**. The β-methylene protons appeared as an AB pattern when the spectra were recorded in trifluoroacetic acid; however, in dimethyl sulfoxide-d_6 or deuterium oxide as solvent, the β-methylene protons were observed to occur as a singlet. The acetylthiolalanine **16**, however, maintained the expected AB pattern in both trifluoroacetic acid and dimethyl sulfoxide.

Efforts to prepare *N*-benzyloxycarbonyl-2-mercapto-DL-alanine (**18**) and to effect subsequent deprotection of **18** to yield 2-mercapto-DL-alanine hydrobromide (**19**) proved to be unsatisfactory. Unstable oils were obtained in each case; however, nmr spectra provided evidence for the predominant presence of **18** and **19** in the product mixtures. Thus, the nmr spectrum of



17 **18** **19**

an impure sample of **19**, obtained by deprotection of **18** with hydrogen bromide in acetic acid, showed the β protons as a singlet at δ 1.58, the mercapto proton as a singlet at 2.38, and the ammonium protons as a triplet at 7.40 (J = 51 Hz). Attempts to purify **19** lead only to the isolation of ammonium bromide. Treatment of a solution of **19** in pyridine with acetic anhydride lead to a multicomponent mixture in which none of the possible acetylated products **9** or **11** were detected.

The studies reported herein establish that reaction of appropriate sulfur nucleophiles with α-haloalanines affords a convenient method for the preparation of α-mercapto-α-amino acid derivatives. Further studies on the chemistry of this novel class of amino acids are underway.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR-20A spectrophotometer. Nmr data were obtained with a Varian A-60 nmr spectrometer at 60 MHz. Mass spectra were measured on a Hitachi Perkin-Elmer RMU-6E mass spectrometer. Tlc data were measured on Brinkmann precoated silica gel plates in the following solvents: solvent A, chloroform:methanol:acetic acid (10:5:1); solvent B, chloroform:methanol:acetic acid (85:10:5); solvent C, chloroform:methanol:acetic acid (7:2:1). The nmr spectral data recorded in trifluoroacetic acid (TFA) were measured relative to an external TMS standard. Evaporation *in vacuo* was carried out with a Buchler rotary evaporator. Elemental analyses were performed by M-H-W Laboratories, Garden City, Mich.

(10) O. V. Kil'disheva, M. G. Lin'kova, V. M. Savina, and I. L. Kunyants, *Izv. Akad. Nauk. SSSR; Otdel. Khim. Nauk*, 1348 (1958).

***N*-Acetyl-2-mercapto-DL-alanine (9).**—To a solution of 2-acetamidoacrylic acid[9] (**5**) (0.30 g, 2.33 mmol) in 3 ml of trifluoroacetic acid and 25 ml of glacial acetic acid was added 0.70 ml (2.57 mmol) of 4 *N* hydrogen chloride in dioxane. After 5 min, hydrogen sulfide gas was passed into the solution for 10 min, following which the reaction mixture was stirred overnight at room temperature. The solvent was evaporated *in vacuo;* the solid obtained was recrystallized from ethyl acetate to give 0.24 g (63%) of **9**: mp 154–156°; a sodium nitroprusside test[11] on **9** was positive; tlc R_{fA} 0.46; ir (KBr) 3320, 2650, 1705, 1605, 1520, 1430, 1360, 1295, 1250, 1165, 1125, 1100, 1015, 975, 935, 860, 780, 720, 690, 640, 570, 500, and 365 cm^{-1}; nmr (DMSO-d_6) δ 1.68 (s, 3 H, β-methyl), 1.84 (s, 4 H, acetyl, SH); nmr (DMSO-d_6 + D_2O) 1.72 (s, 3 H), 1.90 (s, 3 H); nmr (TFA) 1.47 (s, 3 H), 1.80 (s, 4 H), 7.50 (s, 1 H); mass spectrum *m/e* (rel intensity) 163 (3.5), 130 (22), 129 (16), 88 (36), 87 (26), 59 (75), 58 (28), 45 (27), 44 (62), 43 (97), 42 (100), 41 (52), 36 (13), 34 (100), 33 (50).

Anal. Calcd for $C_5H_9NO_3S$ (163.2): C, 36.8; H, 5.53; N, 8.59; S, 19.5. Found: C, 36.6; H, 5.80; N, 8.76; S, 19.6.

***N*-Benzoyl-2-mercapto-DL-alanine (10).**—Following the same procedure as described above for **9**, 2-benzamidoacrylic acid[9] (**6**) (0.40 g, 2.1 mmol) was converted to **10** (0.25 g, 53%): mp 146–148° from chloroform (lit.[6] mp 146–147°): tlc R_{fB} 0.20; nmr δ 1.78 (s, 3 H, β-methyl), 3.80 (br s, 1 H, SH), 7.50–8.20 (m, 6 H, phenyl plus amide), 9.20 (s, 1 H, carboxyl proton).

***N*-Acetyl-2-acetylthio-DL-alanine (11).**—2-Acetamidoacrylic acid[9] (**5**) (1.20 g, 9.32 mmol) was dissolved in 10 ml of trifluoroacetic acid and an additional 100 ml of glacial acetic acid was added. To this solution was added 3.0 ml (11 mmol) of 4 *N* hydrogen chloride in dioxane. After approximately 5 min, an excess of thiolacetic acid was added and the reaction mixture was stirred for 2 hr. The solvent was removed *in vacuo* and the solid obtained was triturated with diethyl ether and collected by filtration (1.42 g, 74%), mp 145–146°. Recrystallization from ethyl acetate yielded 1.0 g (53%) of **11**: mp 150–151°; tlc R_{fA} 0.55; ir (KBr) 3330 and 3280 (NH and OH), 2650 (acid dimer), 1715 (carboxyl), 1675 cm^{-1} (amide); nmr (trifluoroacetic acid) δ 1.60 (s, 3 H, β-methyl), 1.80 (s, 3 H, acetyl), 1.95 (s, 3 H, acetyl), 7.94 (s, 1 H, amide).

Anal. Calcd for $C_7H_{11}NO_4S$ (205.3): C, 41.0; H, 5.36; N, 6.83. Found: C, 41.3; H, 5.15; N, 6.63.

***N*-Acetyl-2-benzhydrylmercapto-DL-alanine (12).**—2-Acetamidoacrylic acid[9] (**5**) (1.20 g, 9.32 mmol) was treated as above with hydrogen chloride, following which an excess of benzhydryl mercaptan[12] was added to the reaction mixture. After the solution was stirred for 1.5 hr at room temperature, the solvent was evaporated *in vacuo* to yield, after trituration with diethyl ether, 2.80 g (92%) of a white solid. Recrystallization of this material from 95% ethanol gave 2.10 g (70%) of **12**: mp 166–167°; tlc R_{fA} 0.74; nmr (DMSO-d_6) δ 1.55 (s, 3 H, β-methyl), 1.65 (s, 3 H, acetyl), 5.27 (s, 1 H, benzhydryl proton), 7.27 (br s, 10 H, aromatic), 8.17 (s, 1 H, amide proton).

Anal. Calcd for $C_{18}H_{19}NO_3S$ (329.4): C, 65.7; H, 5.77; N, 4.25. Found: C, 66.1; H, 5.98; N, 3.98.

Preparation of *N*-Acetyl-2-mercapto-DL-alanine (9) from 11.—**11** (0.3 g, 1.5 mmol) was allowed to stir for 4 hr at room temperature in 5 ml of a solution prepared by the addition of 5 ml of concentrated hydrochloric acid in 15 ml of water and 15 ml of methanol. Evaporation of the solvent *in vacuo* gave a white solid; recrystallization of this material from acetone–ligroin (bp 60–90°) yielded 0.10 g (41%) of **9**, mp 154–156°. This material was indistinguishable (tlc, ir, mixture melting point) from a sample of **9** as prepared above.

Preparation of *N*-Acetyl-2-mercapto-DL-alanine (9) from 12.—A solution of **12** (0.33 g, 1.0 mmol) in 12 ml of a 2.5% solution of phenol in trifluoroacetic acid was allowed to stir for 18 hr at room temperature. The solvent was removed *in vacuo* to obtain an oil. After trituration of the oil with diethyl ether, 0.10 g (60%) of **9** was collected by filtration, mp 154–156°. This material was indistinguishable (ir, mixture melting point) from a sample of **9** as prepared above using hydrogen sulfide.

***N*-Acetyl-3-chloro-2-mercapto-DL-alanine (15).**—A solution of 0.50 g (3.8 mmol) of 2-acetamidoacrylic acid[9] in 10 ml of trifluoroacetic acid was stirred while a saturated solution of chlorine in carbon tetrachloride was added slowly until a faint green color

(11) K. Shinohra and M. Kilpatrick, *J. Biol. Chem.*, **105**, 241 (1934).

(12) M. M. Klenk, C. M. Suter, and S. Archer, *J. Amer. Chem. Soc.*, **70**, 3846 (1948).

remained. The dichloroalanine **13** thus obtained showed the following nmr spectral data: nmr (TFA) δ 1.79 (s, 3 H, acetyl), 3.73 and 4.41 (AB doublets, J = 12 Hz, 2 H, CH_2); nmr (DMSO-d_6) 1.90 (s, 3 H, acetyl), 3.93 (s, 2 H, CH_2). After the solution was stirred for 10 min, hydrgen sulfide was bubbled in rapidly for 10 min. After an additional 10 min, the solvent was removed *in vacuo*. Trituration four times with small portions of diethyl ether gave 0.65 g (85%) of a white solid: mp 113–114° dec; nmr (TFA) δ 2.36 (s, 4 H, acetyl, SH), 4.36 and 4.74 (AB doublet, J = 12 Hz, 2 H, CH_2), and 7.80 (broad s, 1 H, NH); nmr (DMSO-d_6) δ 1.87 (s, 4 H, acetyl, SH), 3.89 (s, 2 H, CH_2).

Anal. Calcd for $C_5H_8ClNO_3S$ (197.7): C, 30.39; H, 4.07; N, 7.09. Found: C, 30.39; H, 4.07; N, 7.04.

Similar results, as evidenced by nmr spectral data, were obtained when the dibromo compound **14** was treated with hydrogen sulfide in trifluoroacetic acid. However, the product was an unstable oil and attempts to effect purification were unsuccessful.

***N*-Acetyl-2-acetylthio-3-bromo-DL-alanine (16).**—2-Acetamidoacrylic acid (**5**) (0.30 g, 2.33 mmol) was suspended in 9 ml of glacial acetic acid. A solution of bromine in acetic acid was added until the bromine color was no longer discharged. An excess of thiolacetic acid was added and the reaction mixture was stirred at room temperature for 1.5 hr. The solvent was evaporated *in vacuo* and the solid residue was triturated with diethyl ether to yield 0.47 g (70%) of crystalline material. Recrystallization from ethyl acetate gave material melting at 127–129° dec: tlc R_{fA} 0.62; nmr (trifluoroacetic acid) δ 1.80 (s, 3 H, acetyl), 1.95 (s, 3 H, acetyl), 3.43 and 4.27 (AB doublet, J = 11 Hz, CH_2, 2 H), 7.70 (s, 1 H, amide proton).

Anal. Calcd for $C_7H_{10}BrNO_4S$ (284.2): C, 29.6; H, 3.53; N, 4.94. Found: C, 29.5; H, 3.71; N, 4.91.

***N*-Benzyloxycarbonyl-2-mercapto-DL-alanine (18).**—2-(Benzyloxycarbonylamino)acrylic acid (17)[9] (500 mg, 2.26 mmol) was stirred in 3 ml of trifluoroacetic acid while hydrogen chloride gas was passed in for 10 min. After the solution was stirred for an additional 10 min, hydrogen sulfide was introduced into the reaction mixture for 15 min. The solvent was removed at reduced pressure followed by a vacuum of <1 mm. Ether was added slowly to the resulting oil until no more solid precipitated (~20 ml). Filtration and removal of solvent gave an oil with a structure apparently that of an impure sample of the thiol **18**: nmr ($CDCl_3$) δ 1.86 (s, 3 H, CH_3), 2.35 (s, 0.8 H, SH), 5.10 (s, 2.5 H, CH_2), and 7.60 (s, 8.5 H, phenyl). The instability of the product precluded further purification; however, tlc showed a major component (R_{fC} 0.60) with only minor impurities.

2-Mercapto-DL-alanine Hydrobromide (19).—A solution of *N*-benzyloxycarbonyl-2-mercaptoalanine (**18**) was prepared as above but in acetic acid. To this solution was added 6 ml of a saturated solution of hydrogen bromide in acetic acid. Within 5 min, gas evolution began. After the solution was stirred for 1 hr, the mixture was filtered and the solvent was reduced *in vacuo* to 1/3 its original volume. The slow addition of 40 ml of diethyl ether, while cooling the mixture, gave a small amount of white solid (NH_4Br) which was filtered off. Removal of the solvent from the filtrate gave a viscous and somewhat unstable oil: nmr (DMSO-d_6) δ 1.58 (s, CH_3), 2.38 (s, SH), and 7.40 (t, J = 51 Hz, NH_3^+). Further attempts at purification of **19** led to loss of ammonium bromide.

An impure sample of freshly prepared **19** in pyridine cooled to 0° was treated with acetic anhydride and allowed to stand in a refrigerator for 2 days. Following work-up of the reaction mixture, no evidence for the presence of **9** or **11** was detected by tlc or nmr. The crude product obtained consisted of several components as shown by tlc.

Registry No.—**9**, 36871-62-2; **11**, 36871-63-3; **12**, 36871-64-4; **15**, 36871-65-5; **16**, 36871-66-6; **18**, 36871-67-7.

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Reaction of Hexafluoroacetone with Certain Simple Peptides and Related Compounds

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Hexafluoroacetone in dimethyl sulfoxide reacts with simple *N*-glycyl peptides and glycine esters to form fluorinated derivatives which contain an oxazolidine ring. When the N-terminal residue of the peptide is α-methylalanyl, the product is a polyfluorinated imidazolidinyl peptide.

For the last four years, our research group has been concerned with the interaction of aldehydes and ketones with amino acids and simple peptides and their derivatives. Thus far, we have studied a fairly general reaction between carbonyl compounds (acetone, cyclohexanone, cyclopentanone, or isobutyraldehyde) and dipeptides[1] which has afforded novel imidazolidine ring systems. In general, polyhalogenated ketones react quite differently from other carbonyl compounds with amino acids, peptides, and their derivatives. Hexachloroacetone and *sym*-trichlorotrifluoroacetone afforded *N*-trichloroacetyl[2] and *N*-trifluoroacetyl[3] derivatives, respectively. Hexafluoroacetone condenses with amino acids to yield 2,2-bistrifluoromethyl-5-oxazolidones (**1**).[4] All of the foregoing reactions of polyhalogenated ketones were run with dimethyl sulfoxide as the solvent. The interaction of hexafluoroacetone with certain low-molecular-weight peptides in dimethyl sulfoxide is the subject of the present paper.

Hexachloroacetone and *sym*-trichlorotrifluoroacetone both suffered facile cleavage during reactions with simple peptides. In both cases, the trichloromethyl-to-carbonyl carbon bond was ruptured and chloroform was the by-product. Under identical reaction conditions, the cleavage of a carbon–carbon bond of hexafluoroacetone was never observed. Instead, one or two molecules of hexafluoroacetone condensed with the peptides studied in this work and produced relatively nonpolar and volatile peptide derivatives.

When glycylglycine was treated with hexafluoroacetone in dimethyl sulfoxide at −28 to +25°, a crystalline product was obtained, the solubility of which indicated that it was less polar than the parent dipeptide. Elemental analysis indicated that two hexafluoroacetone molecules condensed with one molecule of glycylglycine with the loss of a molecule of water.

(1) C. A. Panetta and M. Pesh-Imam, *J. Org. Chem.*, **37**, 302 (1972).
(2) C. A. Panetta and T. G. Casanova, *ibid.*, **35**, 2423 (1970).
(3) C. A. Panetta and T. G. Casanova, *ibid.*, **35**, 4275 (1970).
(4) F. Weygand, K. Burger, and K. Engelhardt, *Chem. Ber.*, **99**, 1461 (1966).