Chemistry of Aliphatic Disulfides. XI. The Synthesis of Unsymmetrical Bisdisulfides[1,2]

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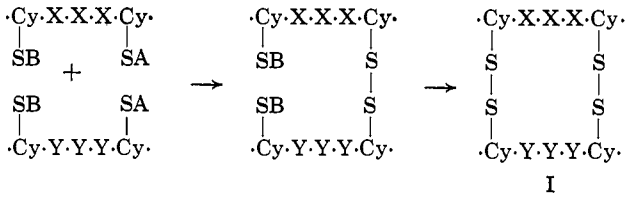
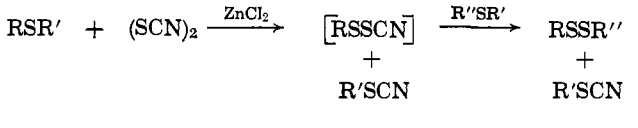
A general synthesis for unsymmetrical bisdisulfides from S-trityl disulfides is described. The application of this method to the synthesis of cystine-containing peptides is discussed.

Projected synthesis of molecules of type I involve several aspects that can be tested with model compounds. Among the problems that must be considered before polypeptides similar to I can be prepared are: (a) sulfur protective groups (A, B) that can be selectively removed, (b) methods for the direct conversion of suitably protected sulfur atoms to disulfides, and (c) a method of oxidation which will allow the stepwise formation of several disulfide bonds within the same molecule.

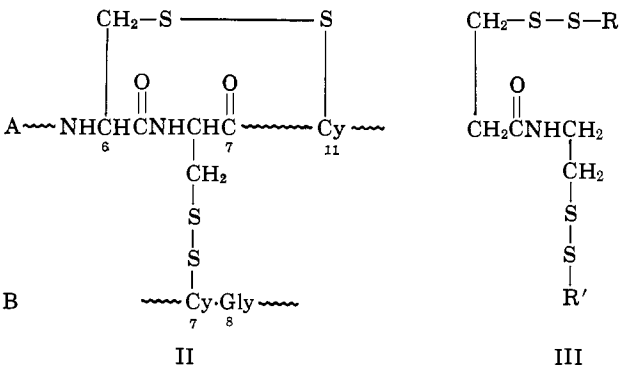


I

In an earlier report[5] on this subject several thio ethers, including S-trityl, S-benzhydryl, and S-tetrahydropyranyl derivatives, were converted to unsymmetrical disulfides by the action of thiocyanogen and zinc chloride. Recently, the formation of an unsymmetrical disulfide by the action of thiocyanogen–zinc chloride on two different S-trityl thio ethers was also observed.[6] These experiments suggested that the sulfenylthiocyanate method[5] of disulfide synthesis could be applied to the synthesis of a molecule of type I, provided that requirement c could be satisfied. The present report concerns an approach to this aspect of the problem and describes some results with model systems.



The choice of a suitable model was directed to some extent by the desire to approximate certain features of the hormone, insulin. The "loop" of insulin, comprising residues A_{6-11}, also contains the point at which one of the AB chain cross-links (A_7–B_7) occurs. Since any total synthesis of insulin would require the formation of two disulfide bonds (A_{6-11}, A_7–B_7) on adjacent cysteine residues (II) a model compound (III) approximating this relationship was desired. Presumably the successful synthesis of III would provide some indication as to the chances of constructing molecules of types I and II by stepwise formation of the disulfide bonds.

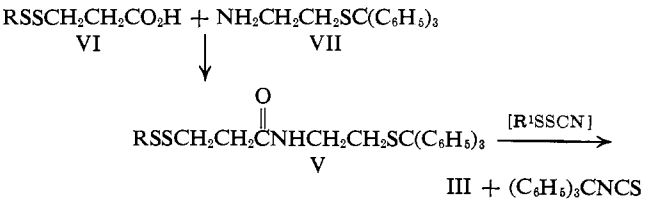


II III

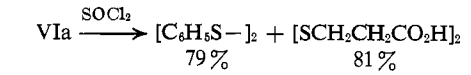
The bis-unsymmetrical disulfides (III) apparently represent an unknown class of compounds. The only related examples are the symmetrical bisdisulfides of type IV recently prepared by Field, *et al.*,[7] using the

$$CH_3CONHCH_2CH_2SSCH_2CH_2SSCH_2CH_2NHCOCH_3$$
IV

thiolsulfonate method. Since in the present work the stepwise introduction of the S–S bonds was desired, the synthetic route selected employed the reaction of a sulfenylthiocyanate with an unsymmetrical disulfide containing an S-trityl group (V). The disulfide V could in turn be prepared *via* the acid VI and 2-tritylthioethylamine (VII).



Since 5-phenyl-4,5-dithiapentanoic acid (VIa, R = C_6H_5) and VII could be obtained in quantity, several coupling methods were evaluated using this substrate. When VIa was warmed with a slight excess of thionyl



chloride in ether, complete disproportionation of the

(1) For part X of this series, see R. G. Hiskey, J. A. Kepler, and B. D. Thomas, *J. Org. Chem.*, **29**, 3684 (1964).

(2) Supported by Research Grants RG-7966 from the National Institute of General Medical Sciences and A-3416 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

(3) Abstracted in part from a dissertation by D. N. Harpp submitted in partial fulfillment of the requirements for the Ph.D. Degree to the University of North Carolina, Jan. 1965.

(4) Union Carbide Chemical Corporation Fellow, 1963–1964.

(5) R. G. Hiskey and W. P. Tucker, *J. Am. Chem. Soc.*, **84**, 4794 (1962).

(6) R. G. Hiskey and J. A. Kepler, *J. Org. Chem.*, **29**, 3678 (1964).

(7) L. Field, A. Ferretti, and T. C. Owen, *ibid.*, **29**, 2378 (1964).

Table I. Yields of Unsymmetrical Bisdisulfides

$$[R^1SSCN]^a + RS{-}SCH_2CH_2\overset{\overset{O}{\|}}{C}NHCH_2CH_2SC(C_6H_5)_3 \xrightarrow{NaOAc} RS{-}SCH_2CH_2\overset{\overset{O}{\|}}{C}NHCH_2CH_2S{-}SR^1 + (C_6H_5)_3CNCS$$

Compd.	Bisdisulfide R	Bisdisulfide R^1	Yield, %	M.p., °C.	Yield of trisdisulfide, %
IIIa	C_6H_5	$CH_2CH_2CO_2H$[b]	43	98.5–100.5	*c*
IIIb	C_6H_5	$CH_2C_6H_4$-*p*-NO_2	50	75–76	16
IIIc	C_6H_5	$CH_2\underset{\underset{NHCBZ}{|}}{C}HCO_2H$[d]	72	93–93.5	*c*
IIId	C_6H_5	$CH_2\underset{\underset{NHCBZ}{|}}{C}HCO_2CH_3$[e]	46	74.5–75.5	21
IIIf	2,4-$(NO_2)_2C_6H_3$	$CH_2C_6H_5$[f]	74	81.5–82.5	2
IIIg	C_6H_5	C_6H_4-*p*-CH_3[g]	71	66–67	*c*
IIIh	$C_6H_5CH_2$	$CH_2C_6H_4$-*p*-NO_2[h]	58	113–114	*c*

[a] Generated by the general procedure described in the preparation of IIIb. [b] *Anal.* Calcd. for $C_{14}H_{19}NO_3S_4$: C, 44.53; H, 5.07; N, 3.71; S, 33.97. Found: C, 44.31; H, 5.11; N, 3.81; S, 34.00; recrystallized from methylene chloride–hexane. [c] Not detected. [d] *Anal.* Calcd. for $C_{22}H_{26}N_2O_5S_4$: C, 50.17; H, 4.98; N, 5.32; S, 24.35. Found: C, 50.28; H, 4.81; N, 5.23; S, 24.75, 23.65; $[\alpha]^{24}D$ −20.3 (*c* 1.0, dioxane); recrystallized from ethyl acetate–hexane. [e] *Anal.* Calcd. for $C_{23}H_{28}N_2O_5S_4$: C, 51.09; H, 5.22; N, 5.18; S, 23.72. Found: C, 50.75; H, 5.36; N, 5.15; S, 23.58; $[\alpha]^{24}D$ −54.8 (*c* 2.0, C_2H_5OH); recrystallized from methanol–hexane. [f] *Anal.* Calcd. for $C_{18}H_{19}N_3O_5S_4$: C, 44.52; H, 3.94; N, 8.65; S, 26.41. Found: C, 44.88; H, 4.14; N, 8.74; S, 26.05; recrystallized from ethanol–hexane. [g] *Anal.* Calcd. for $C_{17}H_{21}NOS_4$: C, 54.65; H, 5.35; N, 3.54; S, 32.42. Found: C, 54.87; H, 5.62; N, 3.71; S, 32.30; recrystallized from hexane–ethanol. [h] *Anal.* Calcd. for $C_{19}H_{22}N_3O_3S_4$: C, 50.19; H, 4.88; N, 6.16; S, 28.21. Found: C, 50.18; H, 4.93; N, 6.32; S, 28.10; recrystallized from ethanol.

unsymmetrical disulfide occurred. Although "disulfide interchange" is well known in basic or strongly acidic media,[8] interchange under the present conditions was surprising. When either 5-(2,4-dinitrophenyl)-4,5-dithiapentanoic acid (VIb, R = 2,4-$(NO_2)_2C_6H_3$) or 6,6-dimethyl-4,5-dithiaheptanoic acid (VIc, R = $(CH_3)_3C$) was treated with thionyl chloride under the same conditions, no interchange occurred and only starting material was recovered. Under slightly modified conditions VIb afforded a crude acid chloride which provided a complex mixture when treated with VII. Thus, a coupling reaction involving an acid chloride was abandoned. Likewise the attempted coupling of VIa and VII *via* the mixed anhydride method provided a crude product which exhibited amide absorption in the infrared but could not be crystallized.

The use of N,N′-dicyclohexylcarbodiimide (DCC) however afforded the desired amide; N-(2-tritylthioethyl)-5-phenyl-4,5-dithiapentanamide (Va, R = C_6H_5) was obtained in 93% yield from VIa and VII; N-(2-tritylthioethyl)-5-(2,4-dinitrophenyl)-4,5-dithiapentanamide (Vb, R = 2,4-$(NO_2)_2C_6H_3$) and N-(2-tritylthioethyl)-6-phenyl-4,5-dithiahexanamide (Vc, R = $C_6H_5CH_2$) were obtained in respective yields of 58 and 77%.

The problem of removal of the S-trityl group and subsequent formation of the second disulfide bond was then considered. When Va was treated with thiocyanogen and zinc chloride, followed by addition of 3-mercaptopropionic acid, the desired bisdisulfide, N-(6-carboxy-3,4-dithiahexyl)-5-phenyl-4,5-dithiapentanamide (IIIa, R = C_6H_5; R^1 = $CH_2CH_2CO_2H$) was obtained in only 10% yield. A similar yield of N-(4-nitrophenyl)-3,4-dithiapentyl-5-phenyl-4,5-dithiapentanamide (IIIb, R = C_6H_5; R^1 = *p*-$NO_2C_6H_4CH_2$) resulted from Va and *p*-nitrobenzyl mercaptan. The use of boron trifluoride as a catalyst also gave poor yields (20–30%) of IIIa,b.

(8) R. E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **80**, 1666 (1958).

A more detailed investigation of the thiocyanogen–zinc chloride reaction with Va indicated that the combination of the three materials produced an insoluble, amorphous, zinc-containing precipitate. The S-trityl group was removed in this process but the lowered amide carbonyl stretching frequency (1610 $cm.^{-1}$) suggested that a zinc-containing complex had been produced. This substance is uncharacterized at present but may be similar to the complexes prepared by Busch, *et al.*,[9] from various transition metals and 2-aminoethanethiol.

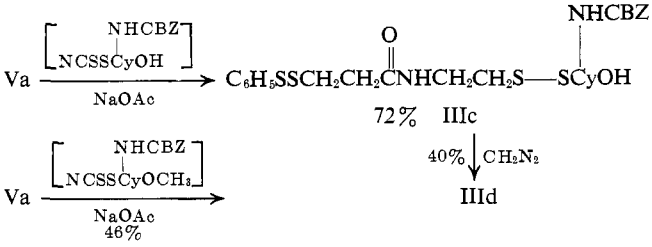
A more effective system for detritylation of V proved to be a thiocyanogen–sodium acetate combination. A summary of the results obtained from the reaction of various sulfenylthiocyanates (generated from the mercaptans with thiocyanogen) with several analogs of V is given in Table I. In each experiment the S-trityl group was almost quantitatively removed and about 90% yields of trityl isothiocyanate (or triphenylcarbinol) were obtained in each case. In several experiments symmetrical trisdisulfides (VIII) were isolated; these compounds were independently synthesized from the appropriate derivative of V by the action of 0.5 equiv. of thiocyanogen and boron trifluoride.

$$V \xrightarrow[BF_3]{0.5\ \text{equiv. of}\ (SCN)_2} [RSSCH_2CH_2\overset{\overset{O}{\|}}{C}NHCH_2CH_2S{-}]_2$$

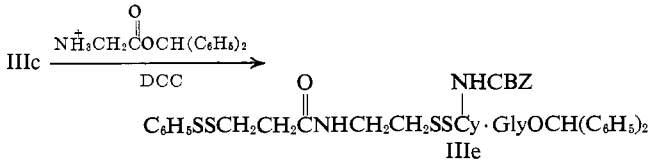
VIII

The preparation of N-(6-carboxy-6-carbobenzoxyamino-3,4-dithiahexyl)-5-phenyl-4,5-dithiapentanamide (IIIc, R = C_6H_5; R^1 = $CH_2C(NHCBZ)HCO_2H$) was of particular interest since the adaptability of a cysteine residue to this process could be determined. Further, the presence of a free carboxyl group in the molecule would allow coupling reactions at that point to be studied. The acid IIIc was converted to the methyl ester IIId with diazomethane; IIId was also prepared

(9) D. H. Busch, D. C. Jicha, M. C. Thompson, J. W. Wrathall, and E. Blinn, *ibid.*, **86**, 3651 (1964); M. C. Thompson and D. H. Busch, *ibid.*, **86**, 3651 (1964).

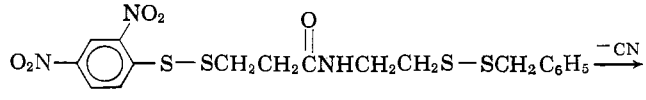


directly from Va using the sulfenylthiocyanate generated from N-carbobenzoxy-L-cysteine methyl ester. The carboxyl group of IIIc was coupled with glycine benzhydryl ester to provide the bisdisulfide, IIIe, in

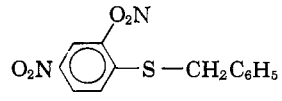


68% yield. The formation of IIIe carries the analogy of the bisdisulfide models somewhat closer to the insulin segment (II) in that residues B_7 and B_8 have been incorporated. The ease of removal of benzhydryl esters[10] permits deblocking of the carboxyl group and presumably further extension of the peptide chain.

A preliminary study of the nucleophilic cleavage of the bisdisulfides suggests a complicated reaction pathway. Treatment of IIIf (R = 2,4-$(NO_2)_2C_6H_3$; R^1 = $CH_2C_6H_5$) with sodium cyanide in acetonitrile provided a 45% yield of benzyl 2,4-dinitrophenyl sulfide (IX); at least four other products were indicated by thin layer chromatography. Additional experi-



IIIf



IX

ments on the nucleophilic and electrophilic cleavage of the bisdisulfides as well as the application of the synthetic procedure to the preparation of unsymmetrical cystine peptides will be reported separately.

Experimental[11]

6-Phenyl-4,5-dithiahexanoic Acid (*VId*, R = $C_6H_5CH_2$) was prepared by the sulfenylthiocyanate method.[12] The product was recrystallized from hexane, m.p. 59–60.5°, 48%.

Anal. Calcd. for $C_{10}H_{12}O_2S_2$: C, 52.61; H, 5.30; S, 28.09. Found: C, 52.92; H, 5.46; S, 27.91.

5-Phenyl-3,4-dithiapentanoic acid (*VIa*, R = C_6H_5) was prepared by the sulfenylthiocyanate method[12] in 74–86% yield. The product was recrystallized from a hexane–ether mixture, m.p. 57–58°.

Anal. Calcd. for $C_9H_{10}O_2S_2$: C, 50.44; H, 4.70; S, 29.92. Found: C, 50.20; H, 4.71; S, 30.10.

5-(2,4-Dinitrophenyl)-4,5-dithiapentanoic acid (*VIb*, R = 2,4-$(NO_2)_2C_6H_3$) was prepared in 95% yield by the method of Parker and Kharasch,[13] m.p. 121–124°. The analytical sample, recrystallized from acetic acid–water, melted at 126–128°.[14]

Anal. Calcd. for $C_9H_8N_2O_6S_2$: C, 35.59; H, 2.65; N, 9.18; S, 21.02. Found: C, 35.51; H, 2.51; N, 9.39; S, 20.96.

2-Tritylthioethylamine (*VII*) was obtained in 69% yield from trityl mercaptan and 2-bromoethylamine hydrobromide, m.p. 94–96.5° (lit.[15] m.p. 90–93°).

Attempted Preparation of 5-Phenyl-4,5-dithiapentanoyl Chloride. A solution containing 2.14 g. (0.01 mole) of VIa and 1.40 g. (0.018 mole) of freshly distilled thionyl chloride in 8 ml. of anhydrous ether was concentrated on a steam bath. The residue was dried over phosphorus pentoxide and washed with 20 ml. of pentane. The filtrate was evaporated to dryness. The residue, 1.02 g., was recrystallized from ethanol and yielded 0.86 g. (78.9%) of diphenyl disulfide, m.p. 57–58°. A mixture melting point with an authentic sample was not depressed. The pentane-insoluble solid (1.00 g.) was recrystallized from an ethyl acetate–benzene mixture to yield 0.84 g. (80.8%) of 7-carboxy-4,5-dithiaheptanoic acid, m.p. 149–152°, identical in all respects with an authentic sample. A similar experiment using sodium bicarbonate solution, rather than pentane, provided similar results.

Preparation of N-(2-Tritylthioethyl)-5-phenyl-4,5-dithiapentanamide (*Va*). A solution containing 31.95 g. (0.10 mole) of VII and 22.69 g. (0.11 mole) of DCC in 130 ml. of methylene chloride was cooled to 10° and treated with a cold solution containing 22.5 g. (0.105 mole) of VIa in 90 ml. of methylene chloride. The addition required 5 min. The mixture was stirred at 10° for 2 hr. and at 25° for 3 hr. The suspension was then treated with 1 ml. of acetic acid and filtered, and the filtrate was washed with successive 100-ml. portions of 1 *N* hydrochloric acid, 5% sodium bicarbonate, and distilled water. The organic layer was concentrated *in vacuo*, taken up in ethyl acetate, and decolorized with charcoal. Addition of hexane caused crystallization; the amide Va was obtained as 47.7 g. (92.5%) of white solid, m.p. 98–100°. One recrystallization from hexane–ethyl acetate gave white needles: m.p. 100–101°; ν_{Max}^{KBr} 3401, 1642, and 1546 cm.$^{-1}$; τ 2.42 (20 H, m), 7.00 (4 H, crude triplet), 7.59 (4 H, crude triplet), and 4.24 (1 H,m). In several preparations the yields averaged 85%.

Anal. Calcd. for $C_{30}H_{29}NOS_3$: C, 69.86; H, 5.67; N, 2.72; S, 18.65. Found: C, 69.93; H, 5.74; N, 2.79; S, 18.72.

Preparation of N-(2-Tritylthioethyl)-5-(2,4-dinitrophenyl)-4,5-dithiapentanamide (*Vb*). The coupling of 3.195 g. (10 mmoles) of VII and 3.195 g. (10.5 mmoles) of VIb with 2.269 g. (11.0 mmoles) of DCC, using the

(10) J. B. Adams, unpublished observation.

(11) Melting points are uncorrected. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill., and Triangle Chemical Laboratory, Carrboro, N. C. Thin layer chromatograms were conducted on microscope slides and large plates. Organic acids were eluted on silica gel G, using benzene–dioxane–acetic acid (90:25:4). Optical rotations were determined with a Rudolph polarimeter Model 80, equipped with a Model 200 photoelectric attachment.

(12) R. G. Hiskey, F. I. Carroll, R. M. Babb, J. O. Bledsoe, R. T. Puckett, and B. W. Roberts, *J. Org. Chem.*, **26**, 1512 (1961).

(13) A. J. Parker and N. Kharasch, *J. Am. Chem. Soc.*, 82, 3071 (1960).

(14) This substance was initially prepared by Mr. J. E. Reece.

(15) F. I. Carroll, H. M. Dickson, and M. E. Wall, *J. Org. Chem.*, 30, 33 (1965).

conditions previously described, provided 4.70 g. of yellow solid contaminated with two impurities (t.l.c.). Chromatography of 2.50 g. of the solid on a 100–200 mesh Florisil column, using an elution gradient from benzene to 17% ethyl acetate–benzene, provided 1.86 g. (50%) of pure amide Vb: 138.5–140°; ν_{Max}^{KBr} 1656, 1548, 1513, and 1337 cm.$^{-1}$.

Anal. Calcd. for $C_{30}H_{27}N_3O_5S_3$: C, 59.48; H, 4.49; N, 6.94; S, 15.88. Found: C, 59.42; H, 4.48; N, 7.15; S, 15.96.

Preparation of N-(2-Tritylthioethyl)-6-phenyl-4,5-dithiahexanamide (Vc). Following the general procedure previously described 4.47 g. (0.014 mole) of VII and 3.36 g. (0.014 mole) of VId were coupled using 3.18 g. (0.0154 mole) of DCC. The amide Vc was obtained as 6.71 g. (77%) of white solid. Recrystallization from a methylene chloride–hexane mixture provided plates: m.p. 110–112°; ν_{Max}^{KBr} 3367, 1647, and 1570 cm.$^{-1}$.

Anal. Calcd. for $C_{31}H_{31}NOS_3$: C, 70.28; H, 5.90; N, 2.64; S, 18.16. Found: C, 70.00; H, 6.08; N, 2.91; S, 18.09.

Reaction of Va with Thiocyanogen in the Presence of Zinc Chloride. A solution containing 0.005 mole of thiocyanogen in 30 ml. of ethyl acetate was treated with 0.90 g. (0.065 mole) of anhydrous zinc chloride and 2.579 g. (0.005 mole) of Va. A precipitate formed almost immediately and the solution was filtered after 0.25 hr. The insoluble material was dried *in vacuo;* the dried solid was insoluble in common organic solvents and water but turned to a paste in acetonitrile. The substance gave a positive test for zinc ion using the ferrocyanide reagent and exhibited infrared absorption bands at 3370, 2170, 2090, 1610 (broad), and 1560 cm.$^{-1}$.

Anal. Found: C, 33.20; H, 4.50; N, 5.72; S, 26.13; residue, 6.91.

Further experiments established that three reagents (thiocyanogen, zinc chloride, and Va) were necessary for formation of the solid. Recrystallization of the solid from an N,N-dimethylformamide–ether mixture provided a new material, insoluble in all solvents tested: m.p. 117.5–120.5°; ν_{Max}^{KBr} 3415, 1652, and 1542 cm.$^{-1}$.

Anal. Found: C, 36.84; H, 5.53; N, 8.49; S, 34.85; residue, 0.24.

Preparation of N-(3-Thiapropyl)-5-phenyl-4,5-dithiapentanamide Disulfide (VIIIa, R = C_6H_5). A cold thiocyanogen solution (1.74 mmoles) in ethyl acetate (10 ml.) was pipetted from the lead thiocyanate and transferred to a cold solution containing 1.289 g. (2.5 mmoles) of Va in 10 ml. of ethyl acetate. The solution was treated with 5 ml. of a 10^{-7} *M* solution of boron trifluoride etherate in ethyl acetate and stirred for 30 min. at 0° and 2.5 hr. at room temperature. The precipitated solid, 0.31 g., was collected and the filtrate was washed with water, dried, and concentrated *in vacuo.* The residue was extracted with petroleum ether (b.p. 30–60°) for 24 hr. in a Soxhlet; evaporation of the petroleum ether provided 97.4% of trityl isothiocyanate.

The residue remaining in the thimble (0.060 g.) was combined with the original precipitate and the combined solid was dissolved in ethyl acetate. Addition of *n*-hexane provided 0.304 g. (44.3%) of the sulfide VIIIa: m.p. 110.5–111.5°; ν_{Max}^{KBr} 3436, 1643, and 1536 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{28}N_2O_2S_6$: C, 48.50; H, 5.18; N, 5.14; S, 35.31. Found: C, 48.46; H, 5.08; N, 5.14; S, 35.07.

Preparation of N-(3-Thiapropyl-6-phenyl-4,5-dithiahexanamide Disulfide (VIIIb, R = $C_6H_5CH_2$). Compound VIIIb was prepared from Vc by the procedure used for VIIIa. The yield was 29.8%, m.p. 103–106°, from hexane–ethanol.

Anal. Calcd. for $C_{24}H_{32}N_2O_2S_6$: C, 50.31; H, 5.63; N, 4.89; S, 33.58. Found: C, 50.01; H, 5.64; N, 5.17; S, 33.16.

Preparation of N-[3-Thiapropyl-5-(2,4-dinitrophenyl)]-4,5-dithiapentanamide Disulfide (VIIIc, R = 2,4-$(NO_2)_2$-C_6H_3). Compound VIIIc was prepared from Vb as described above. The yield was 31.8%, m.p. 145–146°, from ethyl acetate–hexane.

Anal. Calcd. for $C_{22}H_{24}N_6O_{10}S_6$: C, 36.45; H, 3.34; N, 11.59; S, 26.54. Found: C, 36.90; H, 3.39; N, 11.52; S, 26.70.

Preparation of N-[5-(4-Nitrophenyl)-3,4-dithiapentyl]-5-phenyl-4,5-dithiapentanamide (IIIb). General Procedure for the Preparation of Unsymmetrical Bisdisulfides. A thiocyanogen solution was prepared from 2.25 g. (0.007 mole) of lead thiocyanate, suspended in 15 ml. of methylene chloride, and 0.815 g. of bromine, in 10 ml. of methylene chloride. The stirred solution was cooled to 0° and treated with a solution containing 0.846 g. (0.005 mole) of *p*-nitrobenzyl mercaptan in 20 ml. of methylene chloride. The addition required 1.5 hr. The suspension was stirred for 15 min. and 0.41 g. (0.005 mole) of anhydrous sodium acetate was added in one portion. The reaction mixture was then treated with a solution of 2.579 g. (0.005 mole) of Va in 20 ml. of methylene chloride; the addition required 15 min. The reaction mixture was stirred at 0° for 45 min. and at room temperature for 1 hr. and filtered. The filtrate was washed with three 30-ml. portions of 0.3% sodium bicarbonate solution and 30 ml. of water, dried, and concentrated to a semisolid. The residue (3.55 g.) was dissolved in a petroleum ether–benzene mixture (1:9) and adsorbed on a 3 × 60 cm. column of Florisil (250 g., 100–200 mesh). The chromatogram was developed as follows: fractions 2 and 3, 250-ml. fractions of benzene; 6–8, 200-ml. fractions of benzene–ethyl acetate (18:1); 9–12, 200-ml. fractions of benzene–ethyl acetate (9:1); 13–17, 200-ml. fractions of benzene–ethyl acetate (3:1); 18 and 19, 300-ml. fractions of benzene–ethyl acetate (1:1); and 25–28, 200-ml. fractions of ethyl acetate–methanol (1:1).

Fractions 2 and 3 gave 1.215 g. (93.4%) of triphenylcarbinol,[16] m.p. 158–160°. A mixture melting point with an authentic sample was not depressed. Fractions 6–8 gave 0.155 g. of an uncharacterized semisolid. Fractions 9–12 afforded 1.290 g. of an oil which solidified on standing. Recrystallization from aqueous ethanol gave 1.09 g. (49.6%) of pale yellow solid (IIIb); m.p. 75–76° (darkening on exposure to light); ν_{Max}^{KBr} 3472, 1661, 1538, 1517, and 1342 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{20}N_2O_3S_4$: C, 49.06; H, 4.58;

(16) In another experiment the semisolid residue was extracted with petroleum ether (b.p. 30–60°) and quantitative yields of trityl isothiocyanate were obtained from the extract. Subsequently, the quantitative conversion of the isothiocyanate to the carbinol, during chromatography on Florisil, was demonstrated with authentic samples. Chromatography of similar reaction mixtures on silicic acid columns afforded only trityl isothiocyanate.

N, 6.36; S, 29.11. Found: C, 49.33; H, 4.69; N, 6.46; S, 29.38.

Fractions 13–17 gave 0.12 g. of an uncharacterized oily solid. Fractions 18 and 19 provided 0.12 g. (16.2%) of the trisulfide VIIIa, m.p. 108–111°. A mixture melting point with an authentic sample was not depressed.

Preparation of Methyl N-Carbobenzoxy-L-cysteinate. A solution of 2.78 g. (0.01 mole) of N-carbobenzoxy-L-cysteine[17] in 15 ml. of dry methanol was treated with 5 drops of concentrated sulfuric acid and allowed to stand at room temperature for 20 hr. The solution was concentrated *in vacuo* and the residue was extracted with ether. The extract was washed with 3% sodium bicarbonate solution and water. The dried extract was concentrated *in vacuo* to provide 2.52 g. (86%) of ester as a clear, viscous oil. The substance exhibited a single spot on the thin layer chromatogram.

Preparation of IIId by Esterification of IIIc. Treatment of an ether suspension of 0.150 g. (0.29 mmole) of IIIc with excess diazomethane solution provided 0.06 g. (39.6%) of IIId, m.p. 67–69°. One recrystallization from methanol–hexane raised the melting point to 71–74°. A mixture melting point with the material obtained from the thiocyanogen reaction melted at 71–74°.

Preparation of IIIe. A solution of 0.091 g. (0.44 mmole) of DCC, 0.165 g. (0.40 mmole) of glycine benzhydryl ester *p*-toluenesulfonic acid salt,[18] and 0.041 g. (0.40 mmole) of triethylamine in 8 ml. of methylene chloride was treated with a solution containing 0.211 g. (0.4 mmole) of IIIc in 7 ml. of methylene chloride. The mixture was stirred in the cold for 1 hr. and at room temperature overnight. The filtrate was washed with successive 30-ml. portions of 1% sodium bicarbonate solution and 0.5 *N* hydrochloric acid. The solution was dried and concentrated *in vacuo* to yield 0.3 g. of white solid. Recrystallization from an ethanol–hexane mixture provided 0.205 g. (68.3%) of IIIe, m.p. 87–88°. The analytical sample was prepared by filtering a solution of IIIe through a short Florisil column followed by recrystallization of the resulting solid from an ethanol–hexane mixture: m.p. 87.5–88.5°, $[\alpha]^{24}D$ −17.8° (*c* 1.0, dioxane); ν_{Max}^{KBr} 3414, 1748, 1718, and 1664 cm.$^{-1}$.

Anal. Calcd. for $C_{37}H_{39}N_3O_6S_4$: C, 59.25; H, 5.24; N, 5.60; S, 17.10. Found: C, 59.11; H, 5.54; N, 5.39; S, 17.12.

Cyanide Ion Cleavage of IIIf. Treatment of a solution of 0.486 g. (0.001 mole) of IIIf in 40 ml. of acetonitrile with 0.060 g. (0.001 mole) of sodium cyanide provided a black solution. Thin layer chromatography (benzene–ethyl acetate, 1:1) of the reaction mixture after 3 hr. showed the presence of at least four compounds. After 11 hr. the reaction mixture was worked up as previously described.[19] The only pure component of the reaction mixture which could be isolated by chromatography on Florisil was identified as 2,4-dinitrophenyl benzyl sulfide, 0.130 g. (44.7%), m.p. 124–126°. A mixture melting point with an authentic sample was 127–129°. The infrared spectrum of the substance was identical with that of the authentic sulfide and different from the infrared spectrum of 2,4-dinitrophenyl benzyl disulfide.

Treatment of Va with Sodium Acetate. Anhydrous sodium acetate, 0.082 g. (0.001 mole), was added to a solution of 0.516 g. (0.001 mole) of Va in 10 ml. of methylene chloride. The mixture was stirred at 5° for 1 hr. and at room temperature for 1 hr. Work-up in the usual manner provided 0.470 g. (91.2%) of Va, m.p. 99.5–101°. A mixture melting point with authentic Va was not depressed. A similar experiment using sodium thiocyanate provided 0.470 g. (91.2%) of recovered Va.

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Sulfur-Containing Polypeptides. I. Use of the N-Benzhydryloxycarbonyl Group and the Benzhydryl Ester[1,2]

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The N-benzhydryloxycarbonyl (BhOC) group has been utilized in the synthesis of several peptides. The group is introduced via the stable, solid reagent, benzhydryl azidoformate. The group is removed by mild acid hydrolysis and is stable to many conditions encountered in peptide synthesis. The benzhydryl ester has been found useful in conjunction with the BhOC group.

Introduction

Since the introduction of the carbobenzoxy fragment as an amino-protecting group in peptide synthesis a number of analogous groups have been utilized.[4] While several reagents have found limited application, none surpasses the general applicability of carbobenzoxy chloride. There are, however, circumstances in which the N-carbobenzoxy group is

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(3) Shell Chemical Corporation Fellow, 1963–1964.

(4) Several excellent reviews on amino-protecting groups have appeared, including: (a) R. A. Boissonnas in "Advances in Organic Chemistry: Methods and Results," Vol. 3, R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., Interscience Publishers, Inc., New York, N. Y. 1963, p. 183; (b) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1961, p. 885.