A Stable Reagent for the Preparation of *N*-*o*-Nitrophenylthio-amino-acids

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N-*o*-Nitrophenylthio-amino-acids were prepared in high yield by treatment of amino-acids with *o*-nitrophenylsulphenyl thiocyanate, a very stable compound, in the presence of silver nitrate. This reagent reacted selectively with lysine to give N^{α}-mono-*o*-nitrophenylthio- and $N^{\alpha}N^{\epsilon}$-bis-*o*-nitrophenylthio-lysine with virtually none of the N^{ϵ}-monosubstituted isomer. *o*-Nitrophenylsulphenyl *p*-nitrophenolate was used for the preparation of *N*-*o*-nitrophenylthio-amino-acids and for the direct preparation of *N*-*o*-nitrophenylthio-L-phenylalanine *p*-nitrophenyl ester in moderate yield.

THE use of the *o*-nitrophenylthio-group for *N*-protection during peptide synthesis has achieved considerable popularity. This group can be selectively removed under mild conditions;[1-4] it has recently been used as an activable protecting group in peptide bond formation [5] and as an *N*-protecting group removable *in situ* during peptide bond synthesis.[6,7]

The *N*-*o*-nitrophenylthio-derivatives of amino-acids are easily prepared in high yield by treatment with *o*-nitrophenylsulphenyl chloride,[8,9] but the reagent lacks stability. Use of material stored for more than two weeks gives lower yields.

A number of other potential reagents for the formation of *N*-*o*-nitrophenylthio-derivatives have been described.[10] Most sulphenyl compounds are even more unstable than *o*-nitrophenylsulphenyl chloride. However, *o*-nitrophenylsulphenyl thiocyanate (I), prepared in quantitative yield from *o*-nitrophenylsulphenyl chloride and potassium thiocyanate,[11] is very stable, highly resistant to hydrolysis, and can be stored indefinitely without special precautions. Lecher and Simon [11] have prepared *o*-nitrophenylsulphenamide in quantitative yield from *o*-nitrophenylsulphenyl thiocyanate and excess of ammonia. However, when an amino-acid is treated with an equimolar amount of this reagent (I) in the presence of sodium hydroxide, an equilibrium is rapidly reached with the formation of only about 40% of the *N*-protected amino-acid, as estimated visually on chromato-

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[1] A. Fontana, F. Marchiori, L. Moroder, and E. Scoffone, *Tetrahedron Letters*, 1966, 2985.
[2] W. Kessler and B. Iselin, *Helv. Chim. Acta*, 1966, **49**, 1330.
[3] K. Poduška and H. Maasen van den Brink-Zimmermannova, *Coll. Czech. Chem. Comm.*, 1968, **33**, 3769.
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[5] T. Nukaiyama, M. Ueki, H. Maruyama, and R. Matsueda, *J. Amer. Chem. Soc.*, 1968, **90**, 4490.
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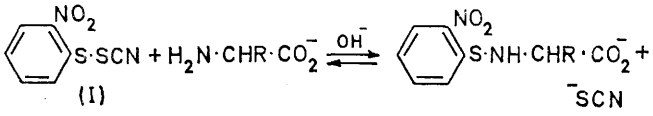
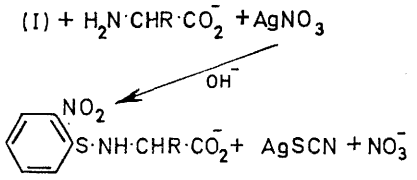
[11] H. Lecher and K. Simon, *Ber.*, 1921, **54**, 632.

grams. Apparently the thiocyanate ion produced is able to cleave the sulphenamide bond: [2]



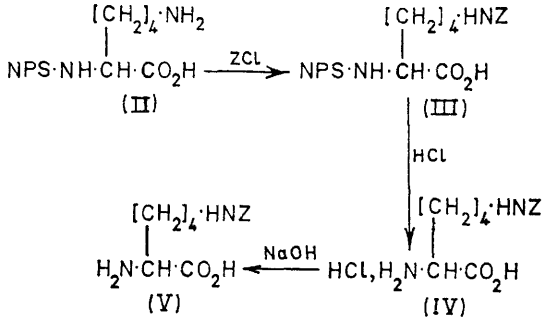
We attempted to drive the reaction to completion by removal of the thiocyanate ion with a heavy metal. Almost quantitative transformation, as shown by chromatograms, was achieved in the presence of mercuric chloride (see Table), but the derivatives were difficult to isolate, probably because of unfavourable solubility properties shown by certain types of complexes that mercury is able to give with thiocyanate ions. However the use of silver nitrate in aqueous solution at pH 8—9 proved satisfactory (see Table).



The *N*-*o*-nitrophenylthio-amino-acids, isolated as their dicyclohexylammonium salts, were usually sufficiently pure to render recrystallisation unnecessary.

N-*o*-Nitrophenylthio-L-proline, obtained in moderate yield by the sulphenyl chloride method,[8] could not be isolated under our experimental conditions. The reaction apparently proceeded normally after addition of the reagent (I) and *ca.* 0·5 equiv. of silver nitrate, but further addition of silver nitrate resulted in the precipitation of silver oxide, showing that no more thiocyanate ion was available.

With lysine the *o*-nitrophenylsulphenyl thiocyanate (I) reacted selectively to give N^{α}-mono-*o*-nitrophenylthiolysine and $N^{\alpha}N^{\varepsilon}$-bis-*o*-nitrophenylthiolysine in about equal amounts; only a trace of a compound believed to be N^{ε}-mono-*o*-nitrophenylthiolysine was detected on chromatograms. The silver nitrate present was not responsible for the observed selectivity and served only to bind the thiocyanate ions. In fact, chromatography showed that the relative proportions of the derivatives formed in the absence of silver nitrate were not significantly altered on addition of the silver salt. After removal of silver thiocyanate the $N^{\alpha}N^{\varepsilon}$-bis-*o*-nitrophenylthiolysine crystallised out in 21% yield at low pH; the N^{α}-mono-*o*-nitrophenylthio-derivative, almost free of the *N*-monosubstituted isomer, separated out at pH 6·2 in 29% yield. The crude mono-N^{α}-*o*-nitrophenylthiolysine (II), after benzyloxycarbonylation and treatment with dicyclohexylamine, gave a compound identical with an authentic sample of N^{ε}-benzyloxycarbonyl-N^{α}-*o*-nitrophenylthio-L-lysine dicyclohexylammonium salt.[8] After removal of the N^{α}-*o*-nitrophenylthio-group [with hydrochloric acid (1 equiv.)] from the $N^{\alpha}N^{\varepsilon}$-disubstituted derivative (III), N^{ε}-benzyloxycarbonyl-L-lysine hydrochloride (IV) was obtained. Adjustment to pH 6·2 of an aqueous solution of the hydrochloride (IV) caused the N^{ε}-benzyloxycarbonyl-L-lysine (V) to precipitate.



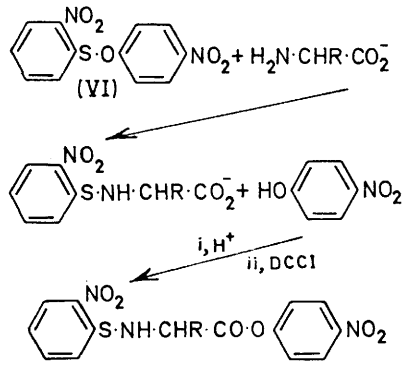
o-Nitrophenylsulphenyl *p*-nitrophenolate (VI), prepared from sodium *p*-nitrophenolate and *o*-nitrophenylsulphenyl chloride, can also be used to prepare *N*-*o*-nitrophenylthio-amino-acids in good yields (see Table).

N-*o*-Nitrophenylthio-amino-acids

Amino-acid	Method [a]	Yield (%)	M.p.	$[\alpha]_D^{22}$	Reported [8,9] M.p.	Reported [8,9] $[\alpha]_D$
L-Phenylalanine	A	41 [b]	127—131° [b]	−46·5° [c,d]	134—135°	−47·6°
	C	87 [b]	128—135 [b]			
L-Alanine [e]	A	48 [b]	173—175 [b]	−56·9 [b,f]		
	B	78 [e]	175—178 [e]	−57·5 [e,f]	176—178	−56·5
	C	71 [d]	173—178 [b]			
L-Leucine [e]	A	53 [b]	180—182 [b]	−72·8 [b,g]		
	B	85 [e]	182—184 [e]	−72·8 [e,g]	182—183	−76·1
	C	60 [b]	180—184 [b]			
L-Isoleucine [e]	B	89 [e]	186—187 [e]	−54·2 [e,f]	188—189	−53·8
β-Benzyl L-aspartate [e]	B	85	162—164 [e]	−38·0 [e,h]	165—166	−38·0
L-Proline [e]	B	0				
Glycine [e]	B	88 [e]	190—191 [e]	(Analysis [e,i])	190—191	
L-Serine [e]	B	75 [e]	171—173 [e]	−91·5 [e,j]	171—173	−89·0
L-Phenylalanine *p*-nitrophenolate	C	44 [b]	105—113 [b]	−113·5 [b,k]	116—118	−115·5

[a] A: *o*-nitrophenylsulphenyl thiocyanate with mercuric chloride; B: *o*-nitrophenylsulphenyl thiocyanate with silver nitrate; C: *o*-nitrophenylsulphenyl *p*-nitrophenolate. [b] After recrystallisation. [c] Crude product. [d] *c* 4·0 in $Me_2N{\cdot}CHO$. [e] Dicyclohexylammonium salt. [f] *c* 2·0 in MeOH. [g] *c* 0·7 in $Me_2N{\cdot}CHO$. [h] *c* 4·0 in $CHCl_3$. [i] Found: C, 58·1; H, 7·6; N, 10·3; S, 7·6. Calc. for $C_{20}H_{31}N_3O_4S$: C, 58·65; H, 7·6; N, 10·3; S, 7·8%. [j] *c* 1·0 in $Me_2N{\cdot}CHO$. [k] *c* 3·0 in $Me_2N{\cdot}CHO$.

We tried to use this reagent (VI) for the direct preparation of *p*-nitrophenyl esters of *N*-*o*-nitrophenylthio-amino-acids by analogy with the reactions described by Wolman, Ladkany, and Frankel.[12] However, only



DCCI = *NN′*-dicyclohexylcarbodi-imide

in the case of phenylalanine were we able to isolate the *N*-*o*-nitrophenylthio-*p*-nitrophenyl ester, in moderate yield.

EXPERIMENTAL

Thin-layer chromatograms were run on silica gel G in butan-1-ol–[acetic acid–pyridine (pH 5·3)]–water (3 : 1 : 1) (R_{FA}) or butan-1-ol–acetic acid–water (3 : 1 : 1) (R_{FB}). Spots were revealed with ninhydrin or with the reagent of Reindel and Hoppe.[13] Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

o-*Nitrophenylsulphenyl* p-*Nitrophenolate* (VI).—Sodium *p*-nitrophenolate (17 g.) and *o*-nitrophenylsulphenyl chloride (5 g.) were suspended in benzene (150 ml.) and shaken in the dark for 48 hr. at room temperature under a calcium chloride guard-tube. Excess of sodium *p*-nitrophenolate (13·3 g.) was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The yellow crystalline residue was washed with ethanol, and was precipitated from acetone by addition of water. Two recrystallisations from benzene–ethanol gave the *ester* (4·4 g., 57%), m.p. 90—93° (decomp.) (Found: C, 48·9; H, 3·0; N, 9·6; S, 11·0. $C_{12}H_8N_2O_5S$ requires C, 49·3; H, 2·8; N, 9·6; S, 11·0%).

N-o-*Nitrophenylthio-amino-acids*.—(a) *By use of* o-*nitrophenylsulphenyl thiocyanate* (I) *and silver nitrate*. N-Sodium hydroxide was added to the amino-acid (0·001 mole) dissolved in water (2 ml.) to bring the pH to 8—9. In the case of the less soluble amino-acids, up to 1 equiv. of base was added. During 15 min. *o*-nitrophenylsulphenyl thiocyanate[11] (I) (0·001 mole) was added in portions while N-silver nitrate (0·5 ml., 0·5 equiv.) was added dropwise, with stirring and while the pH was maintained at 8—9 with N-sodium hydroxide. After the addition of the thiocyanate was completed, more N-silver nitrate (0·5 ml.) was added dropwise during 15 min. at pH 8—9. The silver thiocyanate precipitate was then filtered off and washed with water. The combined filtrates were cooled to 0° and acidified with N-sulphuric acid. The *o*-nitrophenylthio-amino-acid, which usually crystallised out, was extracted with ethyl acetate or ether. The extract was washed with water and dried (Na_2SO_4). Addition of dicyclohexylamine (0·22 ml.) precipitated the corresponding salt of the *N*-protected amino-acid in high purity, in 75—90% yield.

(b) *By use of* o-*nitrophenylsulphenyl thiocyanate* (I) *and mercuric chloride*. Procedure (a) was followed, except that water–dioxan (1 : 1) or water was used as solvent, and that solid mercuric chloride instead of aqueous silver nitrate was added to the reaction mixture. In the case of *o*-nitrophenylthio-L-phenylalanine, which was prepared as the free carboxylic acid, the ether extract of the *N*-protected phenylalanine was evaporated to dryness and the solid residue was dissolved in hot methanol. Upon cooling, *N*-*o*-nitrophenylthio-L-phenylalanine crystallised out (78%), m.p. 124—130°, $[\alpha]_D^{22}$ 45·1° (*c* 4·0 in Me_2N·CHO). Two recrystallisations from methanol gave material (41%), m.p. 127—131°, $[\alpha]_D^{22}$ −46·5° (lit.,[8] m.p. 134—135°, $[\alpha]_D$ −47·6°).

(c) *By use of* o-*nitrophenylsulphenyl* p-*nitrophenolate* (VI). The amino-acid (0·003 mole) was dissolved in a mixture of N-sodium hydroxide (3 ml.) and tetrahydrofuran (10 ml.). *o*-Nitrophenylsulphenyl *p*-nitrophenolate (VI) (0·003 mole) was added in one portion and the mixture was stirred for 5 min. at room temperature. After acidification to pH 7 with N-sulphuric acid the tetrahydrofuran was evaporated off under reduced pressure and, after being cooled to 0°, the aqueous mixture was further acidified with 0·2N-sulphuric acid. The dicyclohexylammonium salts of the *N*-protected amino-acids including that of *N*-*o*-nitrophenylsulphenyl-L-phenylalanine were then obtained as described in (a) and (b). One recrystallisation (see the Table) gave sufficiently pure products.

N-o-*Nitrophenylthio*-L-*phenylalanine* p-*Nitrophenyl Ester*.—L-Phenylalanine was treated with *o*-nitrophenylsulphenyl *p*-nitrophenolate (VI) as in (c). After acidification of the mixture with 0·2N-sulphuric acid, the *N*-protected L-phenylalanine and the *p*-nitrophenol formed were extracted with ethyl acetate, and the extracts were washed with water, dried (Na_2SO_4), and filtered. *NN′*-Dicyclohexylcarbodi-imide (1 equiv.) was added to the cooled solution and the mixture was kept for 12 hr. at 0°. The *NN′*-dicyclohexylurea formed was filtered off and the filtrate was evaporated to dryness under reduced pressure. The oily residue was dissolved in hot methanol. The cooled solution deposited the crude *p*-nitrophenyl ester (65%). Two recrystallisations from methanol gave material (44%), m.p. 105—113°, $[\alpha]_D^{24}$ −113·5° (*c* 3·0 in Me_2N·CHO) (Found: C, 56·9; H, 3·7; N, 9·5; S, 7·3. Calc. for $C_{21}H_{17}N_3O_6S$: C, 57·4; H, 3·9; N, 9·6; S, 7·3%) (lit.,[8] m.p. 116—118°, $[\alpha]_D$ −115·5°).

Reaction of o-*Nitrophenylsulphenyl Thiocyanate* (I) *with Lysine in the Presence of Silver Nitrate*.—(*a*) L-Lysine monohydrochloride (0·02 mole) was dissolved in water (40 ml.) and 2N-nitric acid was added to bring the pH to 1·5. A solution of silver nitrate (0·02 mole) in water (10 ml.) was added with stirring and the silver chloride formed was filtered off and washed with 0·1N-nitric acid (15 ml.). The pH of the combined filtrates was adjusted to 9·0 with N-sodium hydroxide and *o*-nitrophenylsulphenyl thiocyanate (I) (0·02 mole) was added in one portion.

[12] Y. Wolman, D. Ladkany, and M. Frankel, *J. Chem. Soc.* (*C*), 1967, 689.
[13] F. Reindel and W. Hoppe, *Naturwiss.*, 1953, **40**, 221.

The mixture was stirred at 20° while the pH was maintained at 8·8—9·0 with N-sodium hydroxide. After 5 min. a solution of silver nitrate (0·02 mole) in water (20 ml.) was added dropwise at pH 8·8—9·0 during 40 min. Chromatography then revealed one ninhydrin-positive spot (R_{FA} 0·03, R_{FB} 0·05; lysine), two yellow ninhydrin-positive spots of approximately equal intensity [R_{FA} 0·74, R_{FB} 0·89 ($N^{\alpha}N^{\varepsilon}$-bis-*o*-nitrophenylthiolysine) and R_{FA} 0·35, R_{FB} 0·33 (N^{α}-mono-*o*-nitrophenylthiolysine)], and a very weak yellow ninhydrin-positive spot (R_{FA} 0·45, R_{FB} 0·39) which we believe to be N^{ε}-mono-*o*-nitrophenylthiolysine on the grounds of the similar chromatographic behaviour of the corresponding *N*-benzyloxycarbonyl-lysine derivatives. The pH of the mixture was then adjusted to 7 * with N-sulphuric acid and the precipitated silver thiocyanate was centrifuged off and washed twice with water (centrifuged off each time). The solid silver thiocyanate still contained the major part of the $N^{\alpha}N^{\varepsilon}$-bis-*o*-nitrophenylthio-L-lysine. The combined mother liquor and washings were acidified to pH 2·5 with 5N-sulphuric acid and the precipitated $N^{\alpha}N^{\varepsilon}$-bis-*o*-nitrophenylthio-L-lysine was filtered off. The filtrate was worked up [see (*c*)].

(*b*) $N^{\alpha}N^{\varepsilon}$-*bis*-O-*nitrophenylsulphenyl*-L-*lysine dicyclohexylammonium salt*. The solid $N^{\alpha}N^{\varepsilon}$-disubstituted derivative and the centrifuged silver thiocyanate obtained in (*a*) were dispersed in cold 0·1N-sulphuric acid and extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure after addition of dicyclohexylamine (0·01 mole). The residue was triturated with ether and the crystalline $N^{\alpha}N^{\varepsilon}$-*bis*-O-*nitrophenylthio*-L-*lysine dicyclohexylammonium salt* was filtered off (2·65 g., 0·0042 mole, 21%), m.p. 154—159°. Recrystallisation from ethanol gave material (2·1 g.), m.p. 156—158°, R_{FA} 0·74, R_{FB} 0·89, $[\alpha]_D^{24}$ −19·2° (*c* 1·0 in $Me_2N{\cdot}CHO$) (Found: C, 56·6; H, 6·8; N, 11·1; S, 10·0. $C_{30}H_{43}N_5O_6S_2$ requires C, 56·8; H, 6·8; N, 11·0; S, 10·1%).

(*c*) N^{α}-*Mono*-O-*nitrophenylsulphenyl*-L-*lysine* (II). The pH of the filtrate from (*a*), free of the disubstituted lysine, was adjusted to 6·2 with N-sodium hydroxide, and the solution was evaporated at 45° under reduced pressure to 30 ml. and left for 2 hr. in a refrigerator. The precipitate was filtered off and washed with a little cold water. The crude derivative (1·73 g., 29%) (R_{FA} 0·35, R_{FB} 0·33) was contaminated by a trace of a faster-moving component (R_{FA} 0·45, R_{FB} 0·39, yellow and ninhydrin-positive) and by an unknown amount of silver thiocyanate. The crude material was dissolved in hot pyridine (10 ml.) in the presence of a little water; after cooling and addition of more pyridine, the pure *derivative* (II) crystallised out, was filtered off, and was washed with pyridine and ether; yield 1·49 g. (25%), m.p. 186—188°, $[\alpha]_D^{23}$ −19·2° (*c* 2·0 in 0·1N-KOH) (Found: C, 47·6; H, 5·7; N, 14·0; S, 10·5. $C_{12}H_{17}N_3O_4S$ requires C, 48·1; H, 5·7; N, 14·0; S, 10·7%).

N^{ε}-*Benzyloxycarbonyl*-N^{α}-O-*nitrophenylthio*-L-*lysine* (III) *Dicyclohexylammonium Salt*.—The crude N^{α}-mono-*o*-nitrophenylthio-L-lysine (II) (0·002 mole) was suspended in water (20 ml.) and N-sodium hydroxide was added until a clear solution was obtained. Benzyloxycarbonyl chloride (0·0027 mole) was added in five portions during 30 min. to the cooled (0°) and vigorously stirred liquor while the pH was maintained at 10·2—10·6 with N-sodium hydroxide. The mixture was then stirred at 20° for 1 hr., washed with ethyl acetate, acidified to pH 3 with N-sulphuric acid, and extracted with ethyl acetate. The extract was washed with water and dried (Na_2SO_4). On addition of dicyclohexylamine (0·002 mole) the derivative crystallised out (0·80 g., 65%), m.p. 185—187°, $[\alpha]_D^{25}$ −29·9° (*c* 0·70 in $Me_2N{\cdot}CHO$) (lit.,[8] m.p. 184—187°, $[\alpha]_D$ −29·1°).

N^{ε}-*Benzyloxycarbonyl*-L-*lysine* (V).—(*a*) The dicyclohexylammonium salt of (III) (0·001 mole) was suspended in ether and shaken with 0·2N-sulphuric acid until the material dissolved. The ethereal phase was washed thoroughly with water, dried (Na_2SO_4), and evaporated to dryness. The solid lysine derivative, so liberated from its dicyclohexylammonium salt, was dissolved in acetone (3 ml.). After addition of 5N-hydrochloric acid (0·22 ml.) the mixture was left at 20° for 1 hr. On addition of more acetone and ether the *hydrochloride of* (V) crystallised out, and was filtered off and washed with ether; yield 0·285 g. (90%), m.p. 189°, $[\alpha]_D^{23}$ +13·1° (*c* 1·6 in 1 equiv. of HCl), R_{FA} 0·43, R_{FB} 0·33. Authentic N^{ε}-benzyloxycarbonyl-L-lysine[14] gives the same R_F values; N^{α}-benzyloxycarbonyl-L-lysine[15] gives R_{FA} 0·35, R_{FB} 0·25.[16]

(*b*) The hydrochloride of (V) obtained in (*a*) was dissolved in 0·1N-hydrochloric acid (20 ml.) and the pH was adjusted to 6·2 with N-sodium hydroxide. The *product* (V) separated out and, after being left overnight in the refrigerator, was filtered off and washed with cold water; yield 82%, m.p. 245—253° $[\alpha]_D^{22}$ +14·1° (*c* 1·0 in 2 equiv. of HCl) (lit.,[17] m.p. 255°, $[\alpha]_D$ +14·0°).

[0/433 *Received, March 20th,* 1970]

* During the reaction the silver thiocyanate separated out as a very fine precipitate; some of it even appeared to be kept in solution probably through co-ordination with the free amino-groups of lysine. At about pH 7—7·5 the silver thiocyanate precipitate was centrifuged off more easily.

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