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Chem. Pharm. Bull. 26(1) 296—299 (1978)

UDC 547.466.1.04 : 547.541.5.04

Substituted Phenylsulfenyl Groups for Amino Protection during Peptide Synthesis

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(Received March 22, 1977)

Several substituted phenylsulfenyl chlorides were synthesized for the investigation of the properties of sulfenyl N-protecting groups and new sulfenyl (*o*-nitrophenylsulfenyl and 2,4-dinitrophenylsulfenyl) derivatives of some amino acids were obtained. The disubstituted phenylsulfenyl derivatives were far more stable than the monosubstituted ones in 80% acetic acid. Thus the sulfenyl derivatives of S-benzyl-L-cysteine was synthesized in pure state and used successfully to form cysteinyl peptides.

Keywords——substituted phenylsulfenyl chlorides; amino protection; peptide synthesis; Dnps amino acids; Nps amino acids

Since *o*-nitrophenylsulfenyl (Nps) group[2,3] can be selectively removed with mild acid treatment from their amino acid and peptide derivatives and the reagent, Nps chloride is easily synthesized, the Nps group has been used as an N-protecting group. However, Nps amino acids are relatively less stable, and according to our experiences, it is rather difficult to prepare them, especially N-Nps-S-benzyl-L-cysteine, in pure state.

In order to obtain more stable and useful sulfenyl N-protecting groups than the Nps groups, several substituted phenylsulfenyl chloride, such as 2,4,6-trinitrophenylsulfenyl (Tnps) chloride, 2,4-dinitrophenylsulfenyl (Dnps) chloride, 2,6-Dnps chloride, and *o*-nitro-*p*-methylphenylsulfenyl (Nmps) chloride, were synthesized by a method slightly modified the Kharasch and Langford's method.[4] The synthetic procedure is outlined in Chart 1.

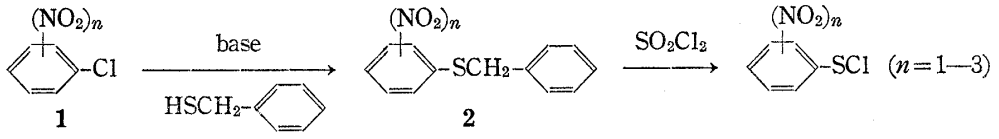

Chart 1

Although Kharasch and Langford had used pyridine as a base in the step **1**→**2** during the synthesis of 2,4-Dnps chloride, the reaction was now found to proceed smoothly in shorter time and the substance **2** ($n=2$) was obtained in better yields when a stronger base than pyridine, such as triethylamine or potassium hydroxide, was used. Accordingly we used potassium hydroxide as a base in all cases except that triethylamine was used in the preparation of 2,4,6-Tnps chloride.

Sulfenyl derivatives of amino acids were then prepared according to the procedure of Zervas, *et al.*[2,3] and Fontana, *et al.*[5] from sulfenyl chlorides and amino acids in a mixture of dioxane and water containing sodium hydroxide. Several new sulfenyl derivatives of nitro-L-arginine, O-tert-butyl-L-serine, alanine, and S-benzyl-L-cysteine were thus obtained and

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their characteristics are listed in Table I. However, no expected 2,6-Dnps and 2,4,6-Tnps derivatives of amino acids could be obtained by the reaction of the corresponding sulfenyl chlorides with L-valine, while unexpected 2,6-dinitrophenyl and 2,4,6-trinitrophenyl amino acids were obtained in this case respectively in low yield.

In order to examine stability of the sulfenyl protecting groups in acidic media, the rate of decomposition of sulfenyl amino acids was measured in 80% acetic acid at 23°. The "half-life period," the time required for the absorbance at absorption maximum of sulfenyl amino acids to reach the middle value of the initial and final values, are summarized in Table II. As expected, the results suggest that, in comparison with Nps group, 2,4-Dnps group containing an additional electron withdrawing group in the benzene ring gives more stable derivatives and Nmps group containing an electron releasing group gives more labile ones.

Noteworthy is the fact that N-2,4-Dnps-S-benzyl-L-cysteine was actually obtained in pure state and could be used for peptide synthesis, for example, the syntheses of N-2,4-Dnps-S-benzyl-L-cysteinyl-L-alanine benzyl ester, N-2,4-Dnps-S-benzyl-L-cysteinyl-L-leucine benzyl ester and N-2,4-Dnps-S-benzyl-L-cysteinylglycine ethyl ester, of which the corresponding Nps derivatives have so far been prepared with failure by the similar method. Practically no essential difference between 2,4-Dnps and Nps was observed in easiness of removal from amino groups by a 0.5 N solution of hydrogen chloride in dioxane.

In conclusion, more stable and useful protecting groups than Nps group could not be found in the substituted phenylsulfenyl groups except 2,4-Dnps group, though the modified method of the synthesis of phenylsulfenyl chloride described above will become valuable hereafter.

Table I. Sulfenyl Derivatives of Some Amino Acids

Derivative	mp (°C)	$[\alpha]_D$ (c, 2)[a]	Formula	Analysis (%) Calcd. (Found) C	H	N
Nps-L-Arg(NO_2)–OH DCHA salt	149—151	+10.0	$C_{24}H_{37}N_7O_6S$	52.05 (52.33)	7.01 (7.19)	17.66 (17.89)
Nps-L-Ser(tBu)–OH DCHA salt	159—161	−16.5	$C_{25}H_{41}N_3O_5S$	60.58 (60.62)	8.34 (8.40)	8.48 (8.30)
2,4-Dnps-L-Arg(NO_2)–OH DCHA salt	124—125	−7.9	$C_{23}H_{39}N_7O_6S$	49.39 (49.06)	6.39 (6.84)	16.80 (16.56)
2,4-Dnps-L-Ser(*t*Bu)–OH DCHA salt	127—128	+40.0	$C_{24}H_{40}N_4O_7S$	55.54 (54.80)	7.46 (7.29)	10.36 (9.55)
2,4-Dnps-L-Ala–OH	117—118	+35.8	$C_{21}H_{32}N_4O_6S$	53.79 (53.76)	6.90 (6.69)	11.96 (11.27)
2,4-Dnps-L-Cys(Bzl)–OH	138—139	−96.4	$C_{28}H_{36}N_4O_6S_2$	56.11 (56.89)	6.49 (6.44)	9.03 (9.09)
Nmps-L-Ala–OH DCHA salt	153—155	−50.7	$C_{22}H_{35}N_3O_4S$	60.37 (60.41)	8.08 (8.07)	9.60 (9.28)

Arg(NO_2)=nitroarginine, DCHA=dicyclohexylamine, *t*Bu=*tert*-butyl, Bzl=benzyl.
a) in MeOH.

Table II. "Half-life Period" of Sulfenyl Amino Acids in 80% Acetic Acid at 23°

Amino acid	Nps-derivative (hr)	2,4-Dnps-derivative (hr)	Nmps-derivative (hr)
Ala	4.5	29.8	1.7
Arg(NO_2)	5.3	77.0	
Ser(*t*Bu)	4.0	50.3	
Cys(Bzl)	2.0	58.0	

Experimental

All melting points are uncorrected. The mass spectra were taken on a Hitachi RMU-6M mass spectrometer operating at 70 eV with a direct inlet system.

Preparation of 2,4-Dnps Chloride——a) 2,4-Dinitrophenyl Benzyl Sulfide (**3**): A solution of KOH (0.6 g) in MeOH (2 ml) was added dropwise to an ice-cold solution containing 2,4-dinitrochlorobenzene (2.02 g, 10 mmol), MeOH (4 ml) and benzylmercaptan (1.24 g 10 mmol). After the reaction mixture had been allowed to stand for 1 hr at 0°, the crystals separated out were collected by filtration, washed with water and ice-cold MeOH, and dried at 60—80° to afford **3**; wt 2.61 g (90%); mp 128—129°; lit[4]: mp 128—129°.

b) 2,4-Dnps Chloride: The sulfenyl chloride was prepared from **3** and sulfuryl chloride according to the procedure of Kharasch and Langford.[4] The yield was 80%; mp 95—96°; lit[4]: mp 95—96°.

Preparation of 2,6-Dnps Chloride——a) 2,6-Dinitrophenyl Benzyl Sulfide (**4**): **4** was prepared from 2,6-dinitrochlorobenzene and benzylmercaptan in the same manner as with **3**. The yield was 80%; mp 95.5—97.5°; MS *m/e* 290 (M^+); *Anal.* Calcd. for $C_{13}H_{10}N_2O_4S$: C, 53.80; H, 3.47; N, 9.67. Found: C, 53.77; H, 3.50; N, 9.58.

b) 2,6-Dnps Chloride: Sulfurylchloride (2.4 g, 18 mmol) was added to **4** (2.32 g, 8 mmol) at room temperature. The reaction mixture was allowed to stand for 10 min and concentrated to an oil at 40° under aspirator vacuum. To the residual oil, dry petroleum ether (5 ml) was added with vigorous handswirling. The crystals separated out were collected by filtration and washed well with dry petroleum ether; wt 1.6 g (85%); mp 50° (dec.); MS *m/e*: 234 (M^+). This sulfenyl chloride is extremely unstable when allowed to stand in contact with the atmosphere.

Preparation of 2,4,6-Tnps Chloride——a) 2,4,6-Trinitrophenyl Benzyl Sulfide (**5**): Triethylamine (1.5 ml, 10 mmol) was added dropwise to an ice-cold solution containing 2,4,6-trinitrochlorobenzene (1.5 g, 6 mmol) and benzylmercaptan (0.7 ml, 6 mmol). The crystals separated out were collected by filtration and washed with water and MeOH; mp 112—114°; MS *m/e*: 335 (M^+); *Anal.* Calcd. for $C_{13}H_9N_2O_6S$: C, 46.57; H, 2.71; N, 12.54. Found: C, 46.49; H, 2.74; N, 12.49.

b) 2,4,6-Tnps Chloride: Sulfurylchloride (0.6 ml, 7 mmol) was added to **5** (0.3 g, 1 mmol) at room temperature. The reaction mixture was concentrated to an oil at 60° under aspirator vacuum. Dry petroleum ether (10 ml) was added to the residual oil with vigorous handswirling. After the mixture had been allowed to stand for 1 hr at room temperature, the crystals separated out were collected by filtration and washed with petroleum ether; wt 0.22 g (80%); mp 90° (dec.); MS *m/e*: 279 (M^+). This sulfenyl chloride is also unstable.

Preparation of Nps Chloride——a) *o*-Nitrophenyl Benzyl Sulfide (**6**): A solution of KOH (0.6 g, 11 mmol) in MeOH (2 ml) was added dropwise to a solution containing *o*-nitrochlorobenzene (1.57 g, 10 mmol), MeOH (4 ml) and benzylmercaptan (1.24 g, 10 mmol). After the reaction mixture had been allowed to stand for 3 hr at 0°, the crystals separated out were collected by filtration and washed with MeOH; wt 1.96 g (80%); mp 80—83°; lit[6]: mp 82—83°.

b) Nps Chloride: Sulfurylchloride (1.19 g, 8.8 mmol) was added to a suspension of **6** (1.96 g, 8 mmol) in ethylene chloride (4 ml) at room temperature. The reaction mixture was allowed to stand for 10 min and concentrated to an oil at 80° under aspirator vacuum. To the residual oil, dry petroleum ether (5 ml) was added with vigorous handswirling. The crystals separated out were collected by filtration, washed with petroleum ether and dried at 60—80°; wt 1.1 g (70%); mp 73—74°; lit[7]: mp 75°.

Preparation of Nmps Chloride——a) *o*-Nitro-*p*-methylphenyl Benzyl Sulfide (**7**): A solution of KOH (2.2 g, 40 mmol) in MeOH (20 ml) was added dropwise to a solution containing 4-chloro-3-nitrotoluene (3.4 g, 20 mmol) and benzylmercaptan (2.4 ml, 20 mmol). After the reaction mixture had been allowed to stand overnight, the crystals separated out were collected by filtration and washed with MeOH; wt 2.9 g (57%); mp 99—100°; MS *m/e*: 259 (M^+); *Anal.* Calcd. for $C_{14}H_{13}NO_2S$: C, 64.86; H, 5.05; N, 5.40. Found: C, 64.85; H, 5.05; N, 5.54.

b) Nmps Chloride: Sulfurylchloride (3.2 ml, 4 mmol) was added to a solution of **7** (0.78 g, 3 mmol) in methylene chloride (20 ml) at room temperature and the reaction mixture was treated in the same manner as with Nps chloride; wt 0.52 g (85%); mp 89—90°; MS *m/e*: 203 (M^+).

Preparation of 2,4-Dnps-L-Cys(Bzl)-L-Ala-OBzl (8)——**8** was prepared from 2,4-Dnps-L-Cys(Bzl)-OH and L-Ala-OBzl by the DCC[8] method. The yield was 70%; mp 142—144°; $[\alpha]_D$ +53.7 (*c*, 0.7 in ethyl acetate *Anal.* Calcd. for $C_{26}H_{26}N_4O_7S_2$: C, 54.72; H, 4.60; N, 9.82. Found: C, 54.63; H, 4.72; N, 9.59.

Preparation of 2,4-Dnps-L-Cys(Bzl)-Gly-OEt (9)——**9** was prepared from 2,4-Dnps-L-Cys(Bzl)-OH and Gly-OEt by the DCC[8] method. The yield was 63%; mp 165—166°; $[\alpha]_D$ −68.4 (*c*, 1.2 in ethyl acetate); *Anal.* Calcd. for $C_{20}H_{22}N_4O_7S_2$: C, 48.57; H, 4.45; N, 11.39. Found: C, 48.48; H, 4.45; N, 11.39.

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Preparation of 2,4-Dnps-L-Cys(Bzl)-L-Leu-OBzl (10)——10 was prepared from 2,4-Dnps-L-Cys(Bzl)-OH and L-Leu-OBzl by the DCC[8] method. The yield was 62%; mp 140—141°; $[\alpha]_D$ −155.6 (*c*, 0.3 in ethyl acetate); *Anal.* Calcd. for $C_{29}H_{30}N_4O_7S_2$: C, 57.04; H, 4.95; N, 9.17. Found: C, 57.11; H, 5.04; N, 9.15.

Preparation of Sulfenyl Amino Acids[2,3] (Table I)——The amino acid (0.02 mol) was dissolved in a mixture of 2 N NaOH (10 ml) and dioxane (25 ml). During a period of 30 min, sulfenyl chloride (0.022 mol) was added in 10 equal portions as 2 N NaOH (12 ml) was added dropwise, with vigorous shaking. After 1 hr, the solution was diluted with water (300 ml), filtered and acidified at 0° with 1 N sulfuric acid. The product was filtered off, washed with water and dried under high vacuum over P_2O_5. For recrystallization the crude product was dissolved in ethyl acetate or ether and precipitated with petroleum ether.

The sulfenyl amino acids can also be purified as dicyclohexylammonium salt. For this purpose, after acidification with sulfuric acid, the product was redissolved in ethyl acetate. The solution was repeatedly washed with water and then dried over sodium sulfate; upon addition of dicyclohexylamine (4 ml) the corresponding salt separated out, in most cases, in the form of needles.

Measurement of the "Half-life Period" of the Sulfenyl Amino Acids in Acidic Media——A solution of sulfenyl amino acids (0.1 mmol) in 80% acetic acid was kept at 23°. After aliquots (20 μl) of the solution had been diluted with MeOH (10 ml) at regular time intervals, the absorbance of Nps-, 2,4-Dnps- and Nmps-amino acids were measured respectively at 380, 335 and 388 nm. From the time course of a decrease in absorbance, the time required for the absorbance at absorption maximum) of sulfenyl amino acids to reach the middle value ((Di+Df)/2) of the initial (Di) and final (Df) values was determined.

Removal of the 2,4-Dnps Group——To a 0.5 N solution of hydrogen chloride in dioxane (40 ml), 2,4-Dnps-L-Ala–Gly–OBzl (1 g, 2.5 mmol) was added. After the reaction mixture had been stirred for 20 min at room temperature, the solvent was removed *in vacuo*. Dry dioxane was added to the residual oil with handswirling and then ethyl acetate was added to the resulting mixture. After the mixture had been allowed to stand for 4 hr in refrigerator, the crystals separated out were collected by filtration and washed first with ethyl acetate and then with ether. The yield was 0.25 g (84%), mp 176—179°. The product gave single Ninhydrin-positive spot having *Rf* of 0.42 on Silica Gel thin-layers with the solvents of methylene chloride /methanol 5: 1.

[Chem. Pharm. Bull. 26(1) 299—302 (1978)]

UDC 547.571.04 : 546.74-39.04

Oxidation with Nickel Peroxide. XI.[1] Oxidation of Aromatic Aldehydes to Carboxylic Acids in Aqueous Alkaline Solution

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(Received May 24, 1977)

Nickel Peroxide (Ni-PO) was shown to be useful oxidizing agent for a preparation of substituted benzoic acids from the corresponding benzaldehydes in aqueous alkaline solution. Some kinds of aromatic carboxylic acids were also effectively obtained from the corresponding aldehydes in a similar way. The mechanism of these oxidations were discussed.

Keywords——nickel peroxide oxidation; aromatic aldehyde; aromatic carboxylic acid; alkaline medium; mechanism

In our previous paper,[3] it was shown that allylic and benzylic alcohols were oxidized by Nickel Peroxide (Ni-PO) in organic solvents to give the corresponding carbonyl compounds. While oxidation of alcohols in aqueous alkaline solution gave carboxylic acids.

In the case of oxidation in aqueous alkaline solution, it seemed that the reaction proceeded *via* the formation of aldehyde as an intermediate follow by further oxidation to give carboxylic acid.

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