Notes

A New Protecting Group for the Sulfhydryl Function of Cysteine[1-3]

Alayne L. Schroll*

Department of Chemistry, St. Michael's College, Winooski, Vermont 05404

George Barany

Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

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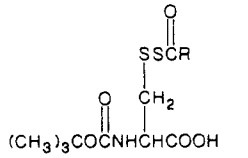
The availability of appropriate readily removable protecting groups for the sulfhydryl function of the amino acid cysteine[4,5] is a high priority of peptide synthesis. In some cases, a peptide is required with all cysteine residues as free thiols; in other cases, it is of interest to form one or more disulfide (cystine) bridges in a controlled fashion. The present paper reports the preparation, characterization, and application of a potentially useful derivative, N^{α}-(*tert*-butyloxycarbonyl)-*S*-[(*N'*-methyl-*N'*-phenylcarbamoyl)sulfenyl]-L-cysteine [Boc-Cys(Snm)-OH] (**1**). Properties of the related derivative[6] N^{α}-(*tert*-butyloxycarbonyl)-*S*-(carbomethoxysulfenyl)-L-cysteine [Boc-Cys(Scm)-OH] (**2**) have also been studied.



1: R = $N(CH_3)C_6H_5$ (Snm)

2: R = OCH_3 (Scm)

Scheme I

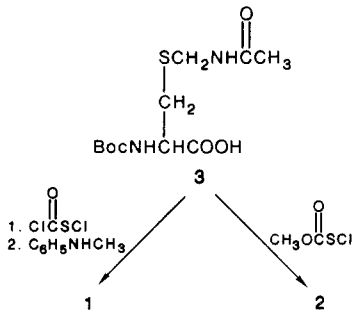


Table I. Results of Model Tripeptide Synthesis as a Function of Length of Neutralization and Coupling Solvent[a]

cysteine derivative	neutralization, min	coupling solvent	Gly:Val[b]
1	2 × 2	CH_2Cl_2	1.04
2	2 × 2	CH_2Cl_2	1.02
1	120	CH_2Cl_2	1.04
2	120	CH_2Cl_2	0.65
1	2 × 2	DMF	0.41
2	2 × 2	DMF	0.32

[a] See the Experimental Section. Neutralization was with DIEA/CH_2Cl_2 (1:19) and refers to the step after removal of Boc from incorporated derivatives **1** and **2**. Coupling solvent specified is for the subsequent coupling of Boc-Gly-OH. [b] As determined by amino acid analysis after acid hydrolysis of the peptide-resin.

The methodology for the synthesis of **1** was suggested by an earlier series of investigations in basic organosulfur chemistry.[7] The *S*-acetamidomethyl group of commercially available N^{α}-*t*-Boc-*S*-(acetamidomethyl)-L-cysteine [Boc-Cys(Acm)-OH] (**3**) was displaced with (chlorocarbonyl)sulfenyl chloride[7a] in chloroform, followed by treatment with excess *N*-methylaniline to provide a near quantitative yield of the desired compound as a solid (Scheme I, left). Similarly, the Scm derivative **2**, an oil, was best prepared by treating **3** directly with (methoxycarbonyl)sulfenyl chloride[7a] in *chloroform* (Scheme I, right).[8] Both **1** and **2** were readily converted in high yield to the corresponding dicyclohexylammonium (DCHA) salts, which were analytically pure crystalline compounds and surprisingly stable upon ambient storage.[9]

Derivatives **1** and **2** were evaluated for stepwise incorporation of cysteine as the central residue in the solid-phase synthesis of the model tripeptide **4**. This peptide was designed so that determinations (by amino acid

(1) A preliminary account of this work was presented at the 10th American Peptide Symposium, St. Louis, MO, May 23-28, 1987.

(2) Taken in part from the Ph.D. Thesis of A. L. Schroll, University of Minnesota, 1986.

(3) Abbreviations used are as follows: Acm, acetamidomethyl; Boc, *tert*-butyloxycarbonyl; Cys, cysteinyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DIEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; DTT, dithiothreitol; Gly, glycyl; HF, hydrogen fluoride; HOAc, acetic acid; Scm, *S*-carbomethoxysulfenyl; Snm, *S*-(*N'*-methyl-*N'*-phenylcarbamoyl)sulfenyl; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid; Val, valyl.

(4) Reviews: (a) Photaki, I. *Topics in Sulfur Chemistry*; Senning, A., Ed.; Georg Thieme: Stuttgart, 1976; Vol. 1, pp 115-133. (b) Barany, G.; Merrifield, R. B. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, pp 233-247. (c) Hiskey, R. G. In *The Peptides: Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1981; Vol. 3, pp 137-167. (d) Bodansky, M. *Principles of Peptide Synthesis*; Springer-Verlag: New York, 1984; pp 130-137.

(5) For some recent examples, see: Galakatos, N. G.; Kemp, D. S. *J. Org. Chem.* **1985**, *50*, 1302-1304 and references cited therein.

(6) Compound **2**, the free acid, is new. The corresponding Boc methyl ester has been previously reported: (a) Hiskey, R. G.; Muthukumaraswamy, N.; Vunnan, R. R. *J. Org. Chem.* **1975**, *40*, 950-953. (b) Other *S*-(alkoxycarbonyl)sulfenyl derivatives have been evaluated: Nokihara, K.; Berndt, H. *J. Org. Chem.* **1978**, *43*, 4893-4895. (c) Use of the Scm group in peptide synthesis, although not for stepwise incorporation, was pioneered by Kamber: Kamber, B. *Helv. Chim. Acta* **1973**, *56*, 1370-1381.

(7) (a) Barany, G.; Schroll, A. L.; Mott, A. W.; Halsrud, D. A. *J. Org. Chem.* **1983**, *48*, 4750-4761. (b) Barany, G. *Tetrahedron Lett.* **1983**, *24*, 5683-5686. (c) Barany, G.; Mott, A. W. *J. Org. Chem.* **1984**, *49*, 1043-1051. (d) Schroll, A. L.; Barany, G. *J. Org. Chem.* **1986**, *51*, 1866-1881 and references cited therein.

(8) When (methoxycarbonyl)sulfenyl chloride was used in neat methanol, substantial methyl ester formation was noted. This problem has been previously reported; see ref 6a.

(9) The DCHA salts of **1** and **2** were rechecked by ^1H NMR and melting point analyses after 4 months of ambient storage and were unchanged in both cases. In the case of **1**, there was no change even after 3 years. However, the DCHA salt of **2**, although still a solid, had a somewhat lower melting point.

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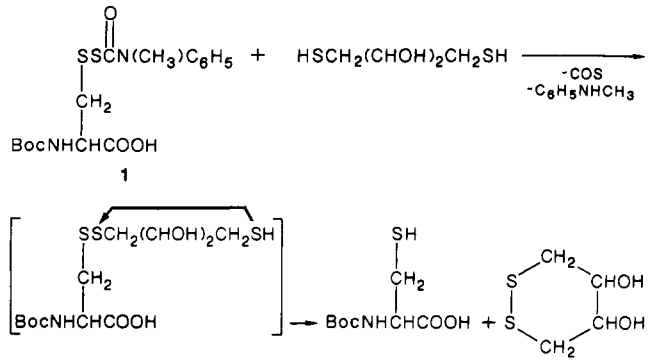
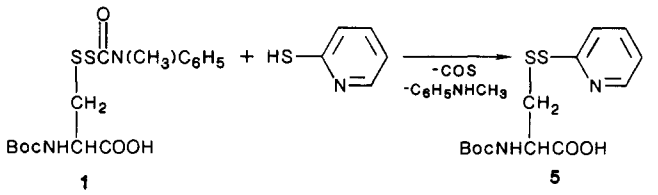
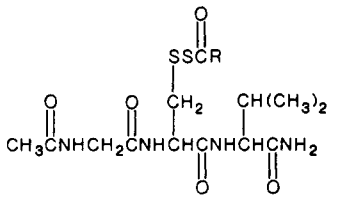
Scheme II



Scheme III



analysis)[10] of the Gly:Val ratio would indicate how well the coupling and deprotection of cysteine had proceeded.



4 Ac-Gly-Cys(Snm, Scm)-Val-NH_2

A: R = $N(CH_3)C_6H_5$

B: R = OCH_3

Results of a variety of experiments are presented in Table I. An optimized protocol (see the Experimental Section) featuring DCC-mediated couplings in CH_2Cl_2 and normal neutralizations with DIEA/CH_2Cl_2 gave the desired Gly:Val ratio of 1.0 for both derivatives 1 and 2 (Table I, lines 1 and 2).

Some exaggerated conditions were also explored. When neutralization was carried out for 2 h with derivative 1 (Table I, line 3), the Gly:Val ratio was 1.0; the same conditions with derivative 2 (Table I, line 4) provided a Gly:Val ratio of 0.65, suggesting that a terminating side reaction had taken place for derivative 2 but not for derivative 1. It is possible, although specific evidence is not on hand, that this side reaction involves an S → N acyl migration.[11]

A similar terminating side reaction also appears to take place when coupling of glycine is carried out in DMF. Derivative 1 provided a Gly:Val ratio of 0.41 (Table I, line 5), while the ratio for 2 was 0.32 (Table I, line 6). As earlier, a side reaction was found to be more severe for 2 by comparison to 1.

An interesting caveat concerning the use of Boc-Cys(Scm)-OH or Boc-Cys(Snm)-OH during peptide synthesis relates to results of a qualitative ninhydrin test[12] applied to N-deblocked 1 or 2. These tests provided a negative or only slightly positive result when in fact a strong positive result is expected. The reason for this anomaly is not understood.

Both the Snm and Scm groups were stable to strong acids (e.g. anhydrous hydrogen fluoride, trifluoromethanesulfonic acid) used to cleave the peptides from the resin. This orthogonality[4b,13] feature should be of considerable significance because relatively few acid-stable cysteine protecting groups are known.[4,14]

As was anticipated from earlier work on carbamoyl disulfides,[15] the new Snm protecting group was shown to be quantitatively removed under mild thiolytic conditions. It can be rapidly cleaved by dithiothreitol (DTT) in the presence of *N*-methylmorpholine to provide free cysteine and the cyclic disulfide (Scheme II). In addition, the Snm group is readily converted to the 2-pyridyl disulfide 5 when treated with 2-mercaptopyridine (Scheme III). Derivatives such as 5, generated in other ways, have been shown to be useful intermediates[16] for directed disulfide bond formation.

In conclusion, the new derivative 1, Boc-Cys(Snm)-OH, appears to have promise for the stepwise incorporation of cysteine residues for solid-phase peptide synthesis in standard protocols[4b,c,d,17,18] involving temporary protection with Boc and final cleavage with hydrogen fluoride or trifluoromethanesulfonic acid. The related Scm derivative 2 can also be used for stepwise incorporation, but is somewhat inferior to 1 for several reasons. It is noteworthy that whereas 1 is highly acid stable, it can be readily reduced to the free thiol or it can be converted into derivative 5 suitable for directed disulfide bond formation. Future work is planned to test these concepts on larger peptide targets.

Experimental Section

General Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. ^1H NMR spectra were observed in $CDCl_3$ with a Varian HFT80/CFT20 spectrometer or a Nicolet NT-300 instrument. N^{α}-*t*-Boc-*S*-(acetamidomethyl)-L-cysteine [Boc-Cys(Acm)-OH] (3) was obtained from Chemical Dynamics Corp. Benzhydrylamine-functionalized polystyrene resin (0.24 mmol/g as determined by picric acid titration[19]) was obtained from Lab Systems, Inc. Dichloromethane, for peptide synthesis, was dried over anhydrous potassium carbonate and distilled from it immediately before use. DMF, for peptide synthesis, was stored over 4-Å molecular sieves and Amberlyst-15 (H form), and nitrogen was bubbled through

(10) Both glycine and valine are stable amino acids that are fully recovered upon acid hydrolysis, in contrast to protected cysteine, which is only partially recovered as cystine.

(11) (a) Zervas, L.; Photaki, I.; Cosmatos, A.; Borovas, D. *J. Am. Chem. Soc.* **1965**, *87*, 4922–4933. (b) Guttmann, S. *Helv. Chim. Acta* **1966**, *49*, 83–96. (c) Hiskey, R. G.; Mizoguchi, T.; Inui, T. *J. Org. Chem.* **1966**, *31*, 1192–1195. (d) Hammerstrom, K.; Lunkenheimer, W.; Zahn, H. *Nakromol. Chem.* **1970**, *133*, 41–51.

(12) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598. The ninhydrin test is usually an accurate way to monitor the absence or presence of free amino groups.

(13) Barany, G.; Merrifield, R. B. *J. Am. Chem. Soc.* **1977**, *99*, 7363–7365.

(14) For a recent discussion of this problem, see: Nutt, R. F.; Brady, S. F.; Lyle, T. A.; Ciccarone, T. M.; Paleveda, W. J.; Colton, C. D.; Veber, D. F.; Winquist, R. J. In *Protides of Biological Fluids*; Peeters, H., Ed.; Pergammon: Oxford, 1986; Vol. 34, pp 55–58.

(15) (a) Barany, G.; Merrifield, R. B. *J. Am. Chem. Soc.* **1980**, *102*, 3084–3095. (b) Barany, G. *Anal. Biochem.* **1980**, *109*, 114–122. (c) Barany, G. *Int. J. Pept. Protein Res.* **1982**, *19*, 321–324.

(16) King, T. P.; Li, Y.; Kochoumian, L. *Biochemistry* **1978**, *17*, 1499–1506. These workers prepared 2-mercaptopyridyl derivatives of peptide/protein-A and reacted these with peptide/protein-B containing a free thiol to form the A–B disulfide while 2-mercaptopyridine was released.

(17) (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154. (b) Stewart, J. M.; Young, J. D. In *Solid Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Co.: Rockford, IL, 1984; p 105. (c) Barany, G.; Kneib-Cordonier, N. K.; Mullen, D. G. *Int. J. Pept. Protein Res.* **1987**, *30*, 705–739.

(18) It should be stressed that the exact details of the deprotection/coupling protocol are important (see Table I). Some widely used protocols are based on DMF as solvent. These procedures would result in partial termination of peptide chains containing Cys(Snm) or Cys(Scm).

(19) Gisin, B. F. *Anal. Chim. Acta* **1972**, *58*, 248–249.

for 2 days prior to use. Peptide-resins were hydrolyzed under nitrogen for 20 h at 130 °C in 12 N HCl/HOAc (1:1) containing a crystal of phenol. Other materials and general methods for manual solid-phase peptide synthesis as practiced in this laboratory were as summarized in previous publications.[20]

N^{α}-t-Boc-S-[(N'-methyl-N'-phenylcarbamoyl)-sulfenyl]-L-cysteine [Boc-Cys(Snm)-OH] (1). N^{α}-t-Boc-S-(acetamidomethyl)-L-cysteine (3) (840 mg, 2.9 mmol) was stirred for 15 min with chloroform (14 mL) to form a hazy solution, which was chilled to 5 °C. (Chlorocarbonyl)sulfenyl chloride[7a] (0.24 mL, 2.9 mmol) in chloroform (2.9 mL) was added dropwise over 5 min. After a further 5 min of stirring, the relatively clear reaction mixture was filtered through glass wool directly into a solution of N-methylaniline in chloroform (14 mL, 2.0 M, 28 mmol) at 5 °C. The reaction mixture after 5 min was washed with 1 N aqueous hydrochloric acid (2 × 30 mL) and water (30 mL), dried ($MgSO_4$), and concentrated in vacuo to provide a brittle expanded mass, which was broken into a gold powder (1.1 g, 98%). Recrystallization from methylene chloride/petroleum ether yielded an amorphous yellow solid, mp 141–146 °C (59% recovery). A second recrystallization yielded an off-white powder: mp 145–148 °C (2 crops, 76% total recovery); one spot by TLC ($CHCl_3$/HOAc, 19:1), R_f 0.48; ^{1}H NMR δ 7.45 (m, 3 H), 7.32 (m, 2 H), 4.36 (m, α-H), 3.42 (s, 3 H), 3.26 (dd, J = 4.2, 14.4 Hz, one β-H), 2.78 (dd, J = 9.0, 14.4 Hz, other β-H), 1.47 (s, 9 H). Anal. Calcd for $C_{16}H_{23}N_2O_{5.5}S_2$, MW 395.49 (hemihydrate of title compound): C, 48.59; H, 5.86; N, 7.08; S, 16.21. Found: C, 48.63; H, 5.84; N, 7.04; S, 16.24.

The dicyclohexylammonium (DCHA) salt[9] of the title compound was prepared by dissolving the best recrystallized material (170 mg, 0.43 mmol) in ethyl acetate (5 mL), chilling to 5 °C, and adding dropwise a solution of dicyclohexylamine in ethyl acetate (0.17 mL, 2.5 M, 0.43 mmol). Within a few hours at 4 °C, a white solid (210 mg, 86%) formed: mp 160–168 °C; ^{1}H NMR δ 7.38 (m, 3 H), 7.28 (m, 2 H), 4.20 (m, 1 H), 3.40 (m, 1 H), 3.35 (s, 3 H), 3.23 (m, 1 H), 2.98 (br t, 2 H), 2.0 (br d, 4 H), 1.8 (br s, 4 H), 1.42 (s, 9 H) superimposed over 1.4 (m, 4 H), 1.2 (m, 8 H). Anal. Calcd for $C_{28}H_{45}N_3O_5S_2$, MW 567.80: C, 59.23; H, 7.99; N, 7.40; S, 11.29. Found: C, 59.15; H, 7.93; N, 7.21; S, 11.21.

N^{α}-t-Boc-S-(carbomethoxysulfenyl)-L-cysteine [Boc-Cys(Scm)-OH] (2). A hazy solution of N^{α}-t-Boc-S-(acetamidomethyl)-L-cysteine (3) (350 mg, 1.2 mmol) in chloroform (6 mL) was treated at 5 °C with (methoxycarbonyl)sulfenyl chloride[7a] (0.12 mL, 1.3 mmol). The resultant clear yellow solution was concentrated after 10 min on a rotary evaporator (bath 25 °C, pressure ~10 mm) to remove any excess sulfenyl chloride; the remaining oil was redissolved in chloroform (10 mL), washed with 1 N aqueous hydrochloric acid (2 × 10 mL) and water (10 mL), dried ($MgSO_4$), and concentrated to yield a viscous, pale yellow oil (360 mg, 96%): ~90% purity by TLC ($CHCl_3$/HOAc, 19:1), R_f 0.52 (major), R_f 0.20 (minor); ^{1}H NMR δ 4.59 (m, α-H), 3.92 (s, 3 H), 3.36 (dd, J = 5.5, 14.4 Hz, 1 H), 3.27 (dd, J = 4.8, 14.4 Hz, 1 H), 1.46 (s, 9 H).

Derivative 2 was best handled as its dicyclohexylammonium (DCHA) salt. The crude product (320 mg, 1.03 mmol) was dissolved in minimal ethyl acetate (~2 mL), chilled to 5 °C, and treated with a solution of dicyclohexylamine in ethyl acetate (0.40 mL, 2.5 M, 1.0 mmol). Excess solvent was removed by a gentle stream of nitrogen, and a precipitate formed immediately. This first crop was rinsed with ethyl acetate and thoroughly dried to yield 340 mg (69%) of a white solid: mp 140–145 °C; ^{1}H NMR δ 4.26 (m, 1 H), 3.86 (s, 3 H), 3.4 (m, 2 H), 3.0 (br t, 2 H), 2.0 (br d, 4 H), 1.8 (br s, 4 H), 1.44 (s, 9 H) superimposed over 1.4 (m, 4 H), 1.2 (m, 8 H). A second crop (100 mg, 20% more for overall yield of 89%), mp 133–138 °C, was isolated from ethyl acetate rinses kept overnight at 4 °C. The DCHA salt thus obtained did not contain the impurity reported above for the crude free acid. Anal. Calcd for $C_{22}H_{40}N_2O_6S_2$, MW 492.69: C, 53.63; H, 8.18; N, 5.69; S, 13.01. Found: C, 53.64; H, 8.17; N, 5.76; S, 12.88.

Liberation of Derivatives 1 and 2 from the DCHA Salts. The DCHA salts of 1 and 2 were dissolved in CH_2Cl_2 to prepare ~0.1 M solutions. Dowex 50X80-400 ion-exchange resin (1 g for each millimole of DCHA salt; 2 equiv per ion-exchange sites) was added, and the suspensions were vigorously agitated on a Vortex mixer for 1 min. The neutralization mixture was filtered through glass wool, and the resin was washed with a small volume of CH_2Cl_2. The combined soluble extracts were suitable for direct use in coupling reactions.

Ac-Gly-Cys(Snm,Scm)-Val-NH_2 (4A and 4B) (Optimal Procedure). Benzhydrylamine-functionalized polystyrene resin (1.00 g, 0.24 mmol) was placed in a solid-phase reaction vessel (1.5 × 8 cm) and washed (all wash volumes = 15 mL) with CH_2Cl_2 (4 × 1 min), TFA/CH_2Cl_2 (1:3) (2 × 1 min, then 1 × 20 min), CH_2Cl_2 (5 × 1 min) DIEA/CH_2Cl_2 (1:19) (1 × 1 min, then 1 × 10 min), and CH_2Cl_2 (5 × 1 min). Next, Boc-Val-OH (139 mg, 0.62 mmol) in CH_2Cl_2 (4 mL) was added and agitated for 5 min, followed without filtering by DCC (128 mg, 0.62 mmol) in CH_2Cl_2 (1 mL). After being shaken for 18 h at 25 °C, the resultant Boc-Val-resin was washed with CH_2Cl_2 (4 × 1 min) and gave a negative ninhydrin test.[12] Portions (50–200 mg) of this Boc-Val-resin were used in several subsequent syntheses of **4A** and **4B**. Incorporation of the cysteine derivative (1 or 2) was achieved according to the following deprotection/coupling cycle: Boc removal was carried out with TFA/CH_2Cl_2 (1:3) (2 × 1 min, then 1 × 20 min), followed by CH_2Cl_2 washes (4 × 1 min), neutralization with DIEA/CH_2Cl_2 (1:19) (1 × 1 min, then 1 × 10 min), and CH_2Cl_2 washes (4 × 1 min). Solid derivative 1 was used directly for the coupling step, whereas suitable 2 was obtained by neutralizing the DCHA salt just prior to use. The appropriate cysteine derivative (2.5–3.0 equiv) was dissolved in a minimal amount of CH_2Cl_2 and added to the deprotected Val-resin, which was briefly shaken (1 min) prior to adding DCC (2.5–3.0 equiv from a 0.5 M stock solution) to effect coupling (120 min). There followed washes with CH_2Cl_2 (4 × 1 min) and a ninhydrin test[12] (in the few cases when the ninhydrin test was positive, the coupling step was repeated). Next, Boc removal and washes were carried out as previously stated, whereas neutralization with DIEA/CH_2Cl_2 was varied (Table I, lines 1–4). Then, Boc-Gly-OH was coupled according to the standard protocol, except that in two cases the coupling was carried out in DMF rather than CH_2Cl_2 (see Table I, lines 5 and 6). Finally, the Boc group was removed and DCC-mediated acetylation with acetic acid was carried out according to the standard protocol.

The desired peptides (**4A**, **4B**) were released from the resins by either TFMSA or HF cleavage. In the first case, peptide-resin (20–60 mg) was placed in a solid-phase reaction vessel, washed with CH_2Cl_2 (2 × 1 min) and TFA/CH_2Cl_2 (1:1) (2 × 1 min), and treated with TFA/TFMSA/CH_2Cl_2 (10:1:10) (24 h). The cleavage mixture was collected, combined with washes with TFA/CH_2Cl_2 (1:1) (4 × 1 min), and concentrated. The residue was redissolved in a small volume of 50% aqueous HOAc and treated with Dowex 1×8-400 ion-exchange resin (Ac form) (~200 mg). Resin was removed by filtration, and the filtrate was evaporated to dryness and taken up in CD_3CN. Alternatively, peptide-resin (50–80 mg) was treated with anhydrous HF/anisole (15:1) for 1 h at 0 °C. Workup proceeded in the standard way.[17b]

Irrespective of how the tripeptides were cleaved, they showed ^{1}H NMR spectra entirely consistent with the proposed structures including fully intact cysteine protection. **4A**: δ 7.25–7.50 (m, 5 H), 4.58 (m, Cys α-H), 4.15 (dd, J = 5.4, 8.7 Hz, Val α-H), 3.99 (d, J = 5.2 Hz, Gly α-H), 3.34 (s, 3 H), 3.21 (dd, J = 5.0, 14.0 Hz, one of Cys β-H), 2.86 (dd, J = 6.6, 14.0 Hz, other Cys β-H), 1.70 (m, Val β-H), 0.92 (d, J = 6.6 Hz, 3 Val γ-H), 0.89 (d, J = 6.2 Hz, 3 Val γ-H); note N-terminal acetyl group obscured by CD_3CN solvent peaks. **4B**: δ 4.55 (m, 1 H), 4.11 (m, 1 H), 3.88 (s, 3 H), 3.74 (d, J = 5.6 Hz, 2 H), 3.19 (m, 2 H), 1.70 (m, 1 H), 0.91 (d, J = 5.0 Hz, 3 H), 0.88 (d, J = 5.0 Hz, 3 H).

Protecting Group Removal. A. In $CDCl_3$, derivative 1 (0.05 M) was treated with dithiothreitol (0.05 M) and N-methylmorpholine (0.10 M). As monitored by ^{1}H NMR, the $t_{1/2}$ for Snm removal was approximately 16 min. B. In $CDCl_3$, derivative 1 (0.025 M) was treated with 2-mercaptopyridine (0.05 M, 2 equiv). ^{1}H NMR showed that 5 formed with $t_{1/2}$ ~7 min. Additional 2-mercaptopyridine (up to 10 equiv) did not change this outcome, as no 2,2'-dithiodipyridine was detected.

Acknowledgments. We thank Robert Hammer for his help in extending certain aspects of this work and the National Institutes of Health (Grant GM 28934) and the

(20) Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1987**, *30*, 177–205 and references cited therein.

Graduate School of the University of Minnesota for financial support.

Registry No. 1, 117310-05-1; 1·DCHA, 117310-06-2; 2, 45234-12-6; 2·DCHA, 40044-22-2; 3, 19746-37-3; 4A, 117310-03-9; 4B, 117310-04-0; 5, 117310-07-3; ClC(O)SCl, 2757-23-5; MeNHPh, 100-61-8; MeOC(O)SCl, 26555-40-8; BOC-Val-OH, 13734-41-3; H-Cys-OH, 52-90-4; 2-mercaptopyridine, 2637-34-5.

Pressure Effect on the Product Distribution in Competing Reactions: Formation of a Bis Diels–Alder Adduct via an Aromatizable Intermediate

S. Srivastava,[1a] A. P. Marchand,*[,1b] V. Vidyasagar,[1b] J. L. Flippen-Anderson,[1c] R. Gilardi,[1c] C. George,[1c] Z. Zachwieja,[1d] and W. J. le Noble*[,1a]

Department of Chemistry, State University of New York, Stony Brook, New York 11794, Department of Chemistry, North Texas State University, Denton, Texas 76203, Naval Research Laboratory, Laboratory for the Structure of Matter, Code 6030, Washington, D.C. 20375-5000, and Department of Organic Chemistry, School of Medicine, 30-048 Krakow, Poland

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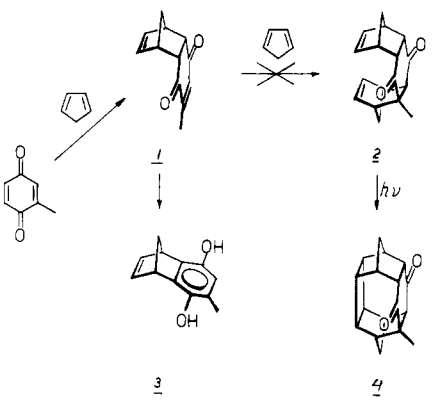
The exceptionally large accelerating effect of pressure on the Diels–Alder reaction has long been known,[2] and this phenomenon has been exploited both for mechanistic[3] and for synthetic[4] purposes. While the availability of very extensive listings of activation volumes[5] makes the choice of further such applications of pressure a simple matter indeed, it is often less obvious what the effect of compression will be in the case of competing reactions, and we begin to address that concern here: any additional tools in the manipulation of the relative rates of desired conversions and side reactions are of obvious value to the synthetic chemists.

In the construction of many polycyclic cage compounds such as **4**, a 2-fold Diels–Alder reaction can be an indispensable tool; however, side reactions may intervene after the first step. In the sequence shown in Scheme I, for example, aromatization of **1** to give **3** may interfere with the formation of **2**. This side reaction has been shown to be promoted by either acidic or basic impurities.[6] It can be avoided by the use of well-purified **1**, however, even then, no **2** is formed.

We reasoned that the second-stage cycloadditions would be accelerated more by compression than by the proton transfers required for enolization, *and* that the relative rates of these two reactions under ambient conditions should be sufficiently similar that the former might, if needs be, overtake the latter at high pressure.

Scheme I

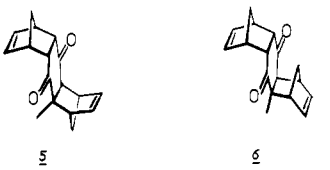


Results and Discussion

The experimental results confirm this assessment. Compression of a solution of methyl-*p*-benzoquinone and excess cyclopentadiene in toluene at 75–80 °C to 700–800 MPa leads to an excellent yield of bisadducts. The conversion is low (20%) because the competing diene dimerization is also accelerated; we therefore use a procedure of several cycles of compression, pressure release, and diene replenishment. After the conversion has reached about 60%, the reaction is stopped as the growing dimer concentration begins to pose purification problems.

HPLC analysis showed the presence of two products in a 4:1 ratio with very similar R_f values; ^{13}C NMR spectra of this mixture confirmed that both were stereoisomeric bisadducts, but the configurations could not immediately be assigned. In the hope of obtaining solid derivatives, we attempted to prepare semicarbazone derivatives, but no reaction occurred under the usual conditions.[7] Once again, the application of high pressure was expected to be helpful as the activation volumes for the related acyl transfer reactions are large and negative, particularly for hindered reactions;[5] indeed, both isomers did form semicarbazones at 800 MPa. The crystals obtained were not suitable for X-ray diffraction purposes, however.

The search for separation procedures was eventually rewarded with substantial quantities of both isomers. Irradiation experiments failed to produce any evidence for the formation of cage ketone **4**, leading us to doubt that isomer **2** was formed at all. Indeed, the configurations of the major and minor products were proved to be *endo-anti-endo* (**5**) and *endo-anti-exo* (**6**), respectively. The steric factor is evidently decisive in directing the second molecule to the convex face of **1**. The proofs proceeded as follows.



Reduction of the major and minor isomers by means of sodium borohydride–cerium(III) chloride[8,9] gave a hydroxy ketone in each case in which the hydroxyl groups originated from the less hindered carbonyl groups. Oxidation

(1) (a) At Stony Brook (S.S. present address: E. R. Squibb and Sons, P.O. Box 191, New Brunswick, NJ 08903-0191). (b) At Denton. (c) At Washington. (d) At Krakow.

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