Synthesis of Cystine-peptide by a New Disulphide Bond-forming Reaction using the Silyl Chloride–Sulphoxide System

Kenichi Akaji, Tadashi Tatsumi, Makoto Yoshida, Tooru Kimura, Yoichi Fujiwara and Yoshiaki Kiso*

Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan

Methyltrichlorosilane or tetrachlorosilane in trifluoroacetic acid, in the presence of diphenylsulphoxide, is found to cleave various *S*-protecting groups of cysteine to form cystine directly by the reduction–oxidation reaction; this new disulphide bond forming reaction is successfully applied to the syntheses of oxytocin and human brain natriuretic peptide.

The disulphide bond forming reaction is a key step in the synthesis of cystine-containing peptides.[1] Usually, air oxidation or iodine oxidation[2] has been employed for this reaction. However, the former is time-consuming under highly diluted conditions and the latter needs particularly controlled conditions. We now report that methyltrichlorosilane or tetrachlorosilane in trifluoroacetic acid (TFA), in the presence of sulphoxide, can cleave various *S*-protecting groups of cysteine to form cystine directly.

It has been reported that a sulphoxide can be reduced with trimethylsilylchloride in the presence of benzenethiol to form the corresponding sulphide and diphenyl disulphide in nearly quantitative yield.[3] We treated Boc-Cys(Acm)-OH,[4]† with tetrachlorosilane or methyltrichlorosilane in TFA in the presence of diphenylsulphoxide at 4 °C for 30 min and the reaction mixture was separated into water- and ether-soluble fractions. From the aqueous fraction, cystine rather than cysteine was recovered nearly quantitatively on an amino acid analyser. The mass spectrum of the ether-soluble fraction showed the presence of a compound of M 186, which corresponds to diphenylsulphide. Thus, the silylchloride–sulphoxide system was considered to be applicable for the disulphide bond forming reaction and its usefulness in the synthesis of cystine-containing peptides was examined.

Each *S*-protected Cys-derivative in TFA was treated with methyltrichlorosilane (20 mol equiv.) in the presence of diphenylsulphoxide (5 mol equiv.) at 4 °C and the recovery of cystine and each parent Cys-derivative were examined quantitatively on an amino acid analyser (Table 1). All *S*-protecting groups examined (Acm, Tacm,[5] Bam,[6] Bu^t[7] and MBzl[8]) were cleaved to form cystine as a sole product except for Cys(4-MeBzl),[9] which was cleaved incompletely. Cys(4-MeBzl) (26%) remained after 30 min, but no other byproduct was detected. Tetrachlorosilane, in the presence of diphenylsulphoxide, could convert each *S*-protected Cys-derivative to cystine as in the case of methyltrichlorosilane. However, trimethylsilyl chloride converted Cys(Acm) to cystine in only 6% yield even after 30 min at 25 °C and tetramethylsilane showed no cystine formation. Met, Tyr, His and Trp(For) were recovered unchanged after the above methyltrichlorosilane treatment, but unmasked Trp gave unidentified products (recovery of Trp, 36%). The N^{in}-For group of Trp was known to be removable by short treatment with NaOH after disulphide bond formation.[10] From these experimental data, the disulphide bond formation was assumed to proceed through an initial *O*-silylation of diphenylsulphoxide and subsequent electrophilic attack of the resulting sulphonium ion on the sulphur atom of *S*-protected cysteine (the reduction–oxidation reaction) (Fig. 1).

Table 1 Cleavage of various *S*-protecting groups of cysteine and formation of cystine by methyltrichlorosilane–diphenylsulphoxide at 4 °C

	10 min		30 min	
	Starting compound (%)	Cystine (%)	Starting compound (%)	Cystine (%)
Cys(Acm)	15	79	0	93
Cys(Tacm)	9	82	0	88
Cys(Bam)	4	98	0	100
Cys(Bu^t)	0	99		
Cys(MBzl)	0	95		
Cys(4-MeBzl)	51	49	26	76

† Abbreviations used: Boc = t-butyloxycarbonyl, Fmoc = fluoren-9-ylmethoxycarbonyl, Acm = acetamidomethyl, Tacm = trimethylacetamidomethyl, Bam = benzamidomethyl, MBzl = 4-methoxybenzyl, MeBzl = 4-methylbenzyl, For = formyl.

Oxytocin was obtained as a sole product when H-Cys(R)-Tyr-Ile-Gln-Asn-Cys(R)-Pro-Leu-Gly-NH_2 (11.6 μmol; R = Acm, Tacm or Bu^t) in TFA (3.5 ml) was treated with methyltrichlorosilane (100 equiv.) in the presence of diphenylsulphoxide (10 equiv.) at 25 °C. The reaction mixture was examined by analytical HPLC and the reaction completed within 10 min. No intermolecular disulphide bond formation was observed using HPLC. The product was isolated by fast protein liquid chromatography (FPLC, Pharmacia) on a column packed with YMC gel ODS-AQ 120A S-50 using a gradient of 60% aq. acetonitrile (0–100%) in 0.1% aq. TFA (56, 69 and 64% yield from *S*-Acm, *S*-Tacm, and *S*-Bu^t oxytocin, respectively). The purified oxytocin‡ possessed the same elution time based on analytical HPLC as that of an authentic sample (Peptide Institute Inc., Osaka).

In order to demonstrate the usefulness of the silylchloride–sulphoxide system for practical synthesis of cystine-containing peptides, we synthesized human brain natriuretic peptide

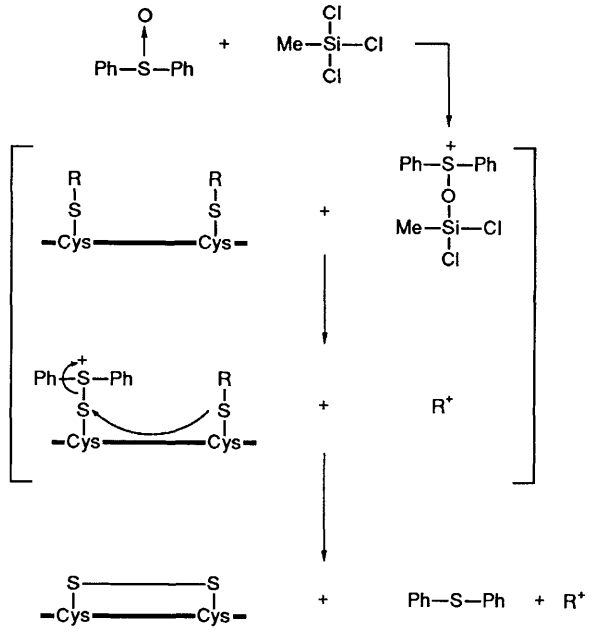

Fig. 1 Proposed pathway for the synthesis of cystine–peptide using silylchloride–sulphoxide system

‡ Amino acid ratios in HCl (6 mol dm^{-3}) hydrosylate; Asp 1.01, Glu 1.04, Gly 1.00, Cys 0.73, Ile 1.02, Leu 0.98, Tyr 0.82, Pro 0.95; HPLC [YMC AM 302, 4.6 × 150 mm, MeCN (10–60%, 30 min) in aq. 0.1% TFA, 0.7 ml min^{-1}], retention time 15.26 min.

(hBNP),[11] consisting of 32 amino acid residues, including two Met and a His, and containing one disulphide bridge. The *S*-protected hBNP [H-Ser-Pro-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys(R)-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys(R)-Lys-Val-Leu-Arg-Arg-His-OH, R = Acm or Tacm] was prepared by the combination of Fmoc-based solid-phase peptide synthesis[12] using Wang resin[13] (0.48 mmol g^{-1} resin) and HBF_4 deprotection method[14] followed by FPLC purification. The purified *S*-protected hBNP (2.2 μmol) in TFA (8.4 ml) was treated with methyltrichlorosilane (100 equiv.) in the presence of diphenylsulphoxide (10 equiv.) at 25 °C for 10 min to form the disulphide bond. The product was purified by FPLC using the same conditions as described for oxytocin syntheses. The homogeneous peptide§ was obtained in 12% yield based on the starting *C*-terminal resin and had physicochemical properties identical with those of an authentic sample.[15]

Thus, using the silylchloride–sulphoxide system, the disulphide bond was formed directly by the reduction–oxidation reaction from *S*-protected cysteine in TFA without any solubility problem[16] within a relatively short time. These results show the potential of this new reagent system for the synthesis of cystine-containing peptides.

Received, 29th October 1990; Com. 0/04848H

§ Amino acid ratios in HCl (6 mol dm^{-3}) hydroylsate and leucine aminopeptidase (LAP, Sigma) digestion (in parentheses); Asp 1.03 (N.D.), Ser 4.66 (5.46), Glu 0.92 (N.D.), Gly 4.88 (4.81), Val 1.93 (1.95), Cys 0.40 (0.97), Met 1.87 (2.01), Ile 1.00 (1.07), Leu 2.00 (2.00), Phe 1.03 (1.01), Lys 2.85 (2.90), His 0.95 (0.96), Arg 3.85 (3.88), Pro 0.97 (0.87); HPLC [YMC AM302, 4.6 × 150 mm, MeCN (10–60%, 30 min) in aq. 0.1% TFA, 0.7 ml min^{-1}], retention time 13.20 min.

References

1 R. G. Hiskey, in *The Peptides*, ed. E. Gross and J. Meienhofer, Academic Press, New York, 1981, vol. 3, p. 37.
2 B. Kamber, A. Hartmann, K. Eisler, B. Riniker, H. Rink, P. Sieber and W. Rittel, *Helv. Chim. Acta*, 1980, **63**, 899; M. Yoshida, T. Tatsumi, Y. Fujiwara, S. Iinuma, T. Kimura, K. Akaji and Y. Kiso, *Chem. Pharm. Bull.*, 1990, **38**, 1551.
3 T. Numata, H. Togo and S. Oae, *Chem. Lett.*, 1979, 329.
4 D. F. Veber, J. D. Milkowski, S. L. Varga and R. Hirschmann, *J. Am. Chem. Soc.*, 1972, **94**, 5456.
5 Y. Kiso, M. Yoshida, Y. Fujiwara, T. Kimura, M. Shimokura and K. Akaji, *Chem. Pharm. Bull.*, 1990, **38**, 673.
6 P. K. Chakravarty and P. K. Olsen, *J. Org. Chem.*, 1978, **43**, 1270.
7 F. M. Callahan, G. W. Anderson, R. Paul and J. E. Zimmerman, *J. Am. Chem. Soc.*, 1963, **85**, 201.
8 S. Akabori, S. Sakakibara, Y. Shimonishi and Y. Nobuhara, *Bull. Chem. Soc. Jpn.*, 1964, **37**, 433.
9 B. W. Erickson and R. B. Merrifield, *J. Am. Chem. Soc.*, 1973, **95**, 3750.
10 S. Kumagaye, H. Kuroda, K. Nakajima, T. X. Watanabe, T. Kimura, T. Masaki and S. Sakakibara, *Int. J. Peptide Protein Res.*, 1988, **32**, 519.
11 T. Sudoh, K. Maekawa, M. Kojima, N. Minamino, K. Kangawa and H. Matsuo, *Biochem. Biophys. Res. Commun.*, 1989, **159**, 1427.
12 E. Atherton and R. C. Sheppard, in *The Peptides*, ed. S. Udenfriend and J. Meienhofer, Academic Press, New York, 1987, vol. 9, p. 1.
13 S. S. Wang, *J. Am. Chem. Soc.*, 1973, **95**, 1328.
14 K. Akaji, M. Yoshida, T. Tatsumi, T. Kimura, Y. Fujiwara and Y. Kiso, *J. Chem. Soc., Chem. Commun.*, 1990, 288.
15 M. Yoshida, K. Akaji, T. Tatsumi, Y. Fujiwara, T. Kimura and Y. Kiso, in *Peptide Chemistry 1989*, ed. N. Yanaihara, Protein Res. Foundation, Osaka, 1990, p. 33.
16 N. Fujii, A. Otaka, S. Funakoshi, K. Bessho and H. Yajima, *J. Chem. Soc., Chem. Commun.*, 1987, 163.