S-BENZYLOXYMETHYLCYSTEINE, ITS PROPERTIES AND APPLICATION IN THE SYNTHESIS OF PORCINE BRAIN NATRIURETIC PEPTIDE (pBNP)[1,2]

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The properties of S-benzyloxymethylcysteine, Cys(Bom), were examined. The S-Bom group is stable to trifluoroacetic acid (TFA), but is easily cleaved by treatment with silver trifluoromethanesulfonate (AgOTf)/TFA or 1 M trimethylsilyl trifluoromethanesulfonate (TMSOTf)-thioanisole/TFA. Cys(Bom) can be converted to cystine by treatment with thallium(III) trifluoroacetate.

Cys(Bom) was successfully applied to the Fmoc-based solid phase synthesis of porcine brain natriuretic peptide (pBNP), a 26-residue peptide with one disulfide bond [Fmoc=9-fluorenylmethyloxycarbonyl]. In the final step, the peptide-resin was first treated with AgOTf/TFA, then with 1 M trimethylsilyl bromide-thioanisole/TFA. After dithiothreitol treatment and subsequent air-oxidation, a homogeneous pBNP was obtained in 17% yield, based on the first amino acid loaded on the resin. The result was compared with those produced by other alternative deprotecting procedures.

KEYWORDS S-benzyloxymethylcysteine; silver trifluoromethanesulfonate; thallium(III) trifluoroacetate; S-protected cysteine sulfoxide; trimethylsilyl trifluoromethanesulfonate deprotection; trimethylsilyl bromide deprotection; porcine brain natriuretic peptide synthesis; Fmoc-based solid phase peptide synthesis

N^{im}-Benzyloxymethylhistidine, His(Bom), introduced by Brown *et al.*,[3] is a useful derivative in peptide synthesis. We have introduced the Bom group to the sulfhydryl group of cysteine and examined its properties.

H-Cys(Bom)-OH was easily prepared by the reaction of cysteine with Bom-Cl in the presence of 4N NaOH in an ice-bath for 2 h and obtained, after recrystallization from hot water, as a crystalline compound. Its N^{α}-protected derivatives, Boc-Cys(Bom)-OH·CHA, Z(OMe)-Cys(Bom)-OH, and Fmoc-Cys(Bom)-OPfp, were prepared by the usual respective standard procedures. The physical constants and analytical data of H-Cys(Bom)-OH and its derivatives are listed in Table I.

Table I. Physical Constants and Analytical Data of Cys(Bom) and Its Derivatives

	Yield (%)	mp (°C)	$[\alpha]_D^{24}$ (solvent)	Formula	Analysis (%) Calcd (Found) C	H	N
H-Cys(Bom)-OH	69.2	202-204	+ 6.0° (1N HCl)	$C_{11}H_{15}O_3NS$	54.75 (54.99	6.27 6.17	5.81 5.67)
Z(OMe)-Cys(Bom)-OH	89.2	62-63	-32.2° (DMF)	$C_{20}H_{23}O_6NS$	59.24 (59.02	5.72 5.63	3.45 3.44)
Boc-Cys(Bom)-OH.CHA	90.8	113-116	+ 6.0° (DMF)	$C_{22}H_{36}O_5N_2S$	59.97 (59.94	8.24 8.47	6.36 6.16)
Fmoc-Cys(Bom)-OPfp	71.5	132-133	-37.0° (DMF)	$C_{32}H_{24}O_5NSF_5$	61.05 (61.28	3.84 3.82	2.23 2.28)

H-Cys(Bom)-OH was stable under various conditions required for peptide synthesis, i.e., TFA (0°C, 4 h), 80% hydrazine hydrate (25°C, 18 h), and 20% piperidine/DMF (25°C, 18 h). Z(OMe)-Cys(Bom)-OH was not sensitive to $NaBO_3$-oxidation.[4] The sulfoxide formed (after 18 h, at 25°C)

with this oxidant (1.1 eq) remained in less than 20% yield, while Z(OMe)-Cys(MBzl)-OH and Z(OMe)-Cys(Ad)-OH[5] were quantitatively oxidized to the corresponding sulfoxides. Next, H-Cys(Bom)-OH was treated with soft metals or acids in an ice-bath for 1 h and the cysteine regenerated or cystine formed was quantified by an amino acid analyzer. As summarized in Table II, the Bom group was found to be easily cleaved by silver trifluoromethanesulfonate (AgOTf)[6] or 1 M TMSOTf-thioanisole/TFA.[7] However, after treatment with 1 M TFMSA-thioanisole/TFA[8] or 1 M TMSBr-thioanisole/TFA,[9] recovery of cysteine was not quantitative. It was found that H-Cys(Bom)-OH could be converted to cystine by treatment with thallium(III) trifluoroacetate [$Tl(TFA)_3$][10] in TFA in nearly 90% yield but not with iodine.[11]

Table II. Removal of the S-Bom Group under Various Conditions

Reagent (0°C, 1h)	Cysteine regenerated (%) (Cystine formed)
1 M TMSOTf-thioanisole / TFA	91.0
1 M TFMSA-thioanisole / TFA	84.1
1 M TMSBr-thioanisole / TFA	76.5
AgOTf-anisole / TFA	≒100
I_2 / 50% AcOH (r.t.)	(2.5)
$Tl(TFA)_3$-anisole / TFA	(88.8)

Next, in order to examine the usefulness of Cys(Bom) for practical peptide synthesis, the Fmoc-based solid phase synthesis[12] of porcine brain natriuretic peptide (pBNP), a 26-residue peptide with one disulfide bridge,[13] was conducted (Fig. 1). Starting with the p-alkoxybenzyl alcohol resin,[14] the peptide chain was elongated manually by successive two-step cycle reactions, according to the principle of Sheppard et al.,[12] *i.e.*, removal of the Fmoc group[15] by 20% piperidine/DMF treatment (two treatments for 5 min and 20 min each) and condensation of the respective amino acids by diisopropylcarbodiimide (DIPCDI) + N-hydroxybenzotriazole (HOBT)[16] or by the pentafluorophenyl (Pfp) ester procedure[17] in DMF. Each coupling reaction was continued until the resin became negative to ninhydrin. Besides Fmoc-Cys(Bom)-OPfp, the following Fmoc amino acids were employed: Asp(OBu), Ser(Bu), Arg(Mtr),[18] and Tyr(Bu).

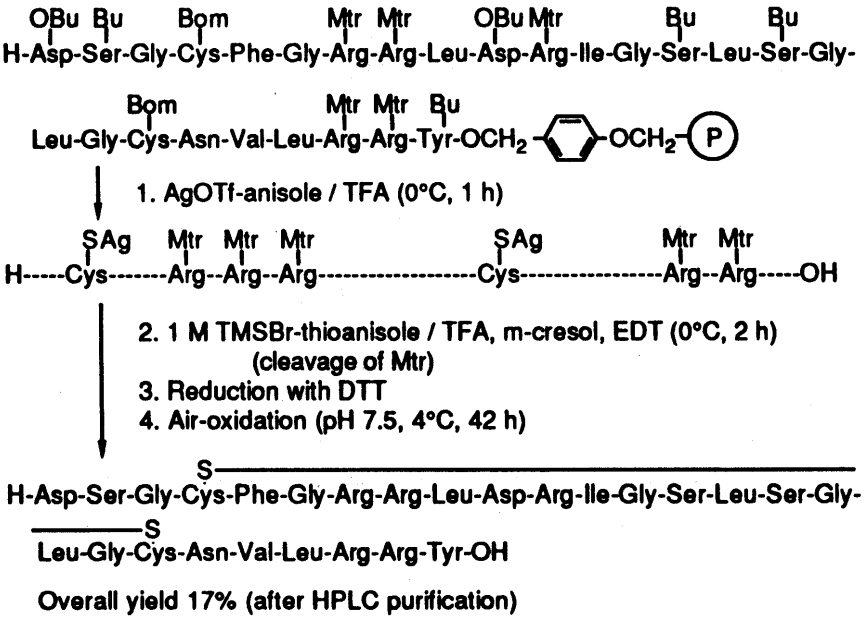

Fig. 1. Fmoc-Based Solid Phase Synthesis of pBNP (AgOTf-TMSBr deprotection)

In the final step, the peptide resin was treated with AgOTf [10 eq/Cys(Bom)] in TFA in the presence of anisole (0°C, for 1 h) to remove the two Bom groups. Under these conditions, the peptide was cleaved off from the resin and at the same time, all Bu type protecting groups were judged to be cleaved by TFA. After filtration, the liberated peptide was further treated with 1 M TMSBr-thioanisole / TFA (0 °C, for 2 h) in the presence of m-cresol and EDT to remove the Mtr groups from the 5 Arg residues involved. The deprotected peptide was treated with dithiothreitol to ensure the complete removal of the Ag salt. After being gel-filtered, the sulfhydryl peptide was submitted to air-oxidation in 0.25 M ammonium acetate buffer at pH 7.5 (peptide conc. 2.8×10^{-5}M, 4°C, for 42 h) until the Ellman test[19] reached the minimal plateau value. After lyophilization, the product was purified to apparent homogeneity by high performance liquid chromatography (HPLC) on a TSK-gel column, which was eluted with a gradient of MeCN (25-35% for 45 min) in 0.1% TFA. The purity of the product thus obtained (17% yield from the starting amino acid resin) was confirmed by analytical HPLC on an Asahipak ODP-50 column (4.6 x 150 mm): retention time 21 min, when eluted by a gradient of MeCN (20-40%, for 30 min) in 0.1% TFA at a flow rate of 1.0 ml/min. Acid hydrolysis with 6 N HCl and digestion with leucine-aminopeptidase (numbers in parentheses) gave amino acids in ratios predicted by theory (numbers in blanket): Asp + Asn 2.89(1.87 + N.D.)[2+1], Ser 2.33(N.D.)[3], Gly 5.00(5.00)[5], Cys N.D.(0.86)[1], Val 1.05(1.06)[1], Ile 0.98(1.05)[1], Leu 4.13(4.13)[4], Tyr 1.05(1.17)[1], Phe 1.03(1.03)[1], Arg 4.86(5.12)[5], (recovery of Gly, 82% and 79% respectively).

For comparison, the above peptide resin was treated with 1 M TMSOTf-thioanisole/TFA in the presence of m-cresol and EDT (0°C for 1 h) remove all protecting groups attached, including the two Bom groups. After air-oxidation, followed by HPLC purification, the desired product was obtained in 7% yield. This lower yield than the above experiments gave seems to be due to incomplete cleavage of the Bom group under the acid conditions employed. A longer treatment seems to be required, when Cys(Bom) is involved in the peptide chain.

For further comparison, a part of the above peptide resin was first treated with $Tl(TFA)_3$/TFA (0°C, for 1 h) to establish the disulfide bond, then with 1 M TMSBr-thioanisole/TFA as stated above. After HPLC purification, the desired peptide was obtained in 5% yield. This lower yield seems to be due to difficulty in forming the disulfide bond on the resin.

From these experimental results, we conclude that Cys(Bom) can be successfully applied to the synthesis of Cys-containing peptides, when AgOTf is used as a final deprotecting reagent.

REFERENCES AND NOTES

1) The content of this paper was presented to the 26th Symposium on Peptide Chemistry held in Tokyo (Oct. 24, 1988).
2) Abbreviations: Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bu=*tert*-butyl, Mtr=4-methoxy-2,4,6-trimethylbenzenesulfonyl, MBzl=*p*-methoxybenzyl, Ad=1-adamantyl, TFA=trifluoroacetic acid, DMF=dimethylformamide, TFMSA=trifluoromethanesulfonic acid, TMSOTf=trimethylsilyl trifluoromethanesulfonate, TMSBr=trimethylsilyl bromide, CHA=cyclohexylamine,EDT=ethanedithiol, DTT=dithiothreitol, Pfp=pentafluorophenyl.
3) T. Brown, J.H. Jones, and J.D. Richard, *J. Chem. Soc., Perkin* Trans, 1, 1982, 1553.
4) S. Funakoshi, N. Fujii, K. Akaji, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, 27, 2151 (1979).
5) N. Fujii, A. Otaka, S. Funakoshi, H. Yajima, O. Nishimura, and M. Fujino, *Chem. Pharm. Bull.*, 34, 869 (1986).
6) N. Fujii, A. Otaka, T. Watanabe, A. Okamachi, H. Tamamura, H. Yajima, Y. Inagaki, M. Nomizu, and K. Asano, *J. Chem. Soc., Chem. Commun.*, in press.
7) N. Fujii, A. Otaka, O. Ikemura, K. Akaji, S. Funakoshi, Y. Hayashi, Y. Kuroda, and H. Yajima, *J. Chem. Soc., Chem. Commun.*, 1987, 274.
8) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc., Chem. Commun.*, 1974, 107.
9) N. Fujii, A. Otaka, N. Sugiyama, M. Hatano, and H. Yajima, *Chem. Pharm. Bull.*,35, 3880 (1987).
10) N. Fujii, A. Otaka, S. Funakoshi, K. Bessho, and H. Yajima, *J. Chem. Soc., Chem. Commun.*, 1987, 163.
11) B. Kamber, A. Hartmann, K. Eisler, B. Riniker, H. Rink, P. Sieber, and W. Rittel, *Helv. Chim. Acta*, 63, 899 (1980).
12) A. Dryland and R.C. Sheppard, *J.Chem. Soc., Perkin* Trans 1, 1986, 125.
13) T. Sudoh, K. Kangawa, N. Minamino, and H. Matsuo, *Nature*, 332, 78 (1988); Its synthesis was presented by Y. Kiso, M. Yoshida, Y. Fujiwara, T. Kimura, and M. Shimokura at the 26th Symposium on Peptide Chemistry held in Tokyo (Oct. 24, 1988).
14) S.S. Wang, *J. Am. Chem. Soc.*, 95, 1328 (1973).
15) L.A. Carpino and G.Y. Han, *J. Am. Chem. Soc.*, 92, 5748 (1970).
16) W. König and R. Geiger, *Chem. Ber.*, 103, 788 (1970).
17) J. Kovacs, L. Kisfaludy, and M.Q. Ceprini, *J. Am. Chem. Soc.*, 89, 183 (1967).
18) M. Fujino, M. Wakimasu, and C. Kitada, *Chem. Pharm. Bull.*, 29, 2825 (1981).
19) G.L. Ellman, *Arch. Biochem. Biophys.*, 82, 70 (1959).

(Received November 24, 1988)