SYNTHESIS OF PORCINE BRAIN NATRIURETIC PEPTIDE-32 USING SILVER TETRAFLUOROBORATE AS A NEW DEPROTECTING REAGENT OF THE *S*-TRIMETHYLACETAMIDOMETHYL GROUP[1)]

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Silver tetrafluoroborate ($AgBF_4$) in trifluoroacetic acid (TFA) has been found to cleave the *S*-trimethylacetamidomethyl (Tacm) group or the *S*-acetamidomethyl (Acm) group without affecting other functional groups in the peptide chain. Newly isolated porcine brain natriuretic peptide-32 (pBNP-32) was synthesized using $AgBF_4$ and Cys(Tacm) derivatives. The synthetic pBNP-32 had the highest chick rectum relaxant activity among the ANP-BNP families.

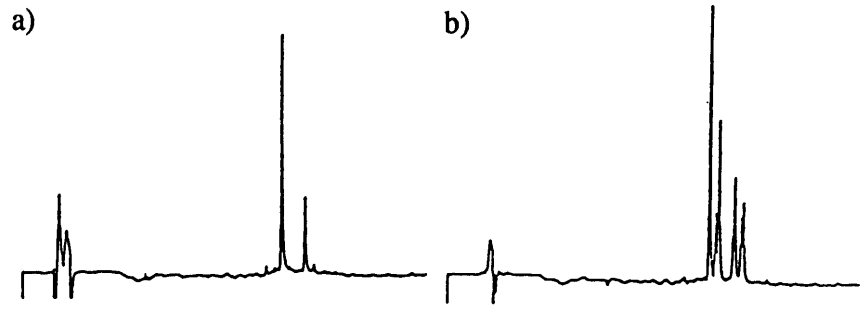
KEYWORDS *S*-trimethylacetamidomethylcysteine; silver tetrafluoroborate deprotection; tetrafluoroboric acid deprotection; iodine-oxidation; porcine brain natriuretic peptide-32; peptide synthesis; chick rectum relaxant activity

In the course of our investigation of the *S*-trimethylacetamidomethyl (Tacm) group,[2)] we have observed that partial oxidation of Met to Met(O) occurs during the removal of the *S*-Tacm group with I_2 in 90% aq. AcOH. We therefore sought a milder method for removal of the *S*-Tacm group and now report that silver tetrafluoroborate ($AgBF_4$) in trifluoroacetic acid (TFA) can remove the *S*-Tacm group without affecting other functional groups in the peptide chain. This deprotecting reagent has been successfully applied to the synthesis of newly isolated porcine brain natriuretic peptide-32 (pBNP-32),[3)] and we have found that synthetic pBNP-32 has the highest chick rectum relaxant activity among the atrial natriuretic peptide (ANP)-BNP families.

In the deprotection of the *S*-protecting group with monovalent silver ions, the anion counterparts play an important role since silver ions, in the form of nitrate[4)] or trifluoromethanesulfonate,[5)] remove several *S*-protecting groups, but in the form of acetate they do not.[6)] We have used silver ions in the form of tetrafluoroborate and examined its usefulness for deprotecting the *S*-protecting group. Boc-Cys(Tacm)-OH in TFA was treated with $AgBF_4$ (10 eq) in the presence of anisole (2 eq) in an ice-bath for 60 min. After treatment with dithiothreitol (DTT, 20 eq) at 25°C, the regenerated cysteine was quantified by an amino acid analyzer. As shown in Table I, cysteine was quantitatively recovered from this derivative. Under identical conditions, the *S*-Acm[7)] group was also cleaved quantitatively. As monovalent silver

Table I. Regenerated Cysteine after $AgBF_4$ Treatment in TFA (4°C, 60 min)

Cysteine derivatives	Regenerated Cys (%)	Cysteine derivatives	Regenerated Cys (%)
Boc-Cys(Tacm)-OH	107	Boc-Cys(Tmb)-OH	73
Boc-Cys(Acm)-OH	93	Boc-Cys(Bu)-OH	0
Boc-Cys(MBzl)-OH	87	Boc-Cys(MeBzl)-OH	0



a) Air-oxidation after Ag treatment
b) I_2 oxidation in 50% aq. AcOH

YMC AM 302 (4.6 x 150 mm); MeCN (10-60%, 30 min) in 0.1% aq. TFA; 0.7 ml/min [retention time: 19.7 min (same as that of authentic sample, purchased from Peptide Inst., Osaka, Japan)].

Fig. 1. HPLC Pattern of the Crude Somatostatin

ions in the form of tetrafluoroborate is moderately activated compared with other forms, two other *S*-protecting groups, 4-methoxybenzyl (MBzl) and 2,4,6-trimethylbenzyl (Tmb),[8] were cleaved incompletely (87% and 73%, respectively). *tert*-Butyl (Bu) and 4-methylbenzyl (MeBzl) were not affected. The results indicated that the mild reagent, $AgBF_4$ in TFA, is suitable for peptide synthesis using Cys(Tacm) or Cys(Acm) derivatives.

As Trp is known to be susceptible to modification during I_2-aq. AcOH oxidation,[9] we have compared the amounts of this side reaction during $AgBF_4$ and I_2 treatments on high performance liquid chromatography (HPLC) using somatostatin as a model peptide. The di-Tacm somatostatin [H-Ala-Gly-Cys(Tacm)-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys(Tacm)-OH], prepared by the Fmoc-based solid-phase method and deprotection with 1M HBF_4-thioanisole in TFA,[10] was dissolved in TFA and treated with $AgBF_4$ (20 eq) in the presence of anisole (10 eq) in an ice-bath for 60 min. The peptide-Ag salt precipitated with ether was treated with DTT (40 eq) in 1N AcOH at 25°C for 3 h. After centrifugation, the supernatant was gel-filtered on Sephadex G-15 using 1N AcOH. The desired eluate was diluted with water, then subjected to air-oxidation at pH 7.5. On the other hand, the *S*-Tacm group was oxidatively cleaved with I_2 in aq. AcOH in essentially the same manner described for the *S*-Acm group.[9] As shown in Fig. 1, the crude product obtained using I_2-aq. AcOH gave a more complex elution pattern on HPLC than that obtained using $AgBF_4$. The same results were obtained in the comparison of the deprotecting reagents using the *S*-Acm somatostatin as a starting derivative.

Using this new *S*-deprotecting reagent, we have synthesized a newly isolated Met-containing peptide pBNP-32 (Fig.2), which consists of 32 amino acids extending from the *N*-terminus of pBNP (26

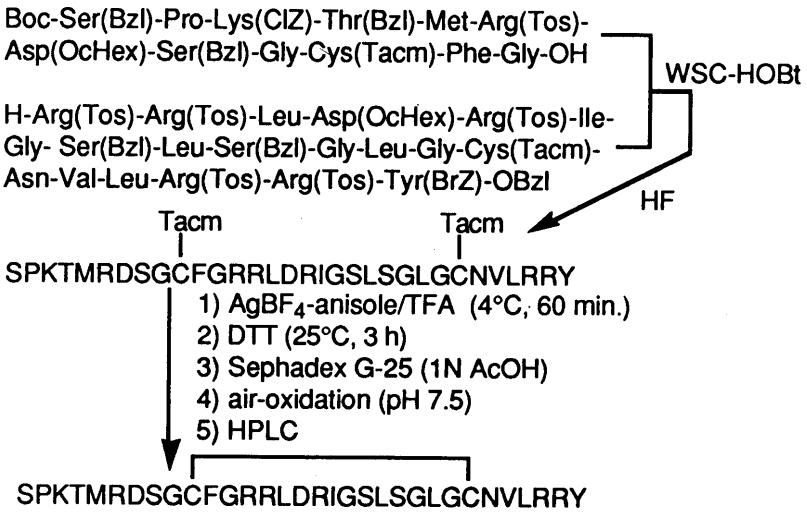


Fig. 2. Synthetic Route to Porcine BNP-32

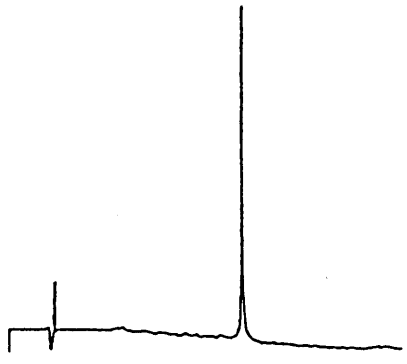


Fig. 3. HPLC of Synthetic pBNP-32

amino acid residues). Di-Tacm-pBNP-32 was prepared using an intermediate in the previous synthesis of pBNP[2a,b] in solution, followed by deprotection with HF-*m*-cresol-dimethylsulfide (0°C, 1 h). After gel-filtration on Sephadex G-25, the product was purified by fast protein liquid chromatography (FPLC) on a YMC-gel ODS AQ-300 (S-50) column [eluted with a gradient of 60% aq. MeCN (0-100%, 400 min) in 0.1% aq. TFA, 3.0 ml/min], then treated with $AgBF_4$ (40 eq) in the presence of anisole (10 eq), followed by DTT (80 eq) as above. After air-oxidation at pH 7.5, the product was purified by HPLC on a Cosmosil 5C18 P-300 (10 x 250 mm) column using a gradient of MeCN (20-40%, 90 min) in 0.3% aq. TFA to give a homogeneous peptide: yield 10.5% (calculated from the protected peptide), $[\alpha]_D^{29}$-60° (c=0.1, 1N AcOH). The purity of synthetic pBNP-32 was confirmed by analytical HPLC on a YMC AM-302 (4.6 x 150 mm) [retention time: 17.4 min, on a gradient elution with MeCN (10-60%, 30 min) in 0.1% aq. TFA, 0.7 ml/min] (Fig. 3) and by amino acid analysis after acid hydrolysis with 6N HCl {amino acid ratios (theoretical numbers in parentheses): Asp 3.04 (3), Thr 0.90 (1), Ser 3.63 (4), Pro 0.88 (1), Gly 5.05 (5), Val 0.94 (1), Cys 0.74 (1), Met 0.94 [0.98 in leucine-aminopeptidase (LAP, Sigma) digest] (1), Ile 0.94 (1), Leu 4.00 (4), Tyr 0.96 (1), Phe 0.94 (1), Lys 0.92 (1), Arg 5.98 (6) (recovery of Leu 87%)}. The purified product proved to be a monomer by gel-permeation HPLC on a YMC Pack Diol 60 (8 x 500 mm) [eluted with 0.1M phosphate buffer (pH 7.2): MeCN=4:1, 0.5 ml/min, the retention time (28.6 min) was between those of bovine insulin (24.6 min, Mw. 5733) and adrenorphin (33.4 min, Mw. 984)] and by fast atom bombardment mass spectrometry [the observed mass value 3569.9 $(MH)^+$ agreed well with the theoretical value 3569.819]. In an alternative oxidative cleavage of di-Tacm pBNP-32 using I_2 in aq. AcOH, we observed a large amount of Met(O) derivative on HPLC (retention time:17.2 min) and the yield of the purified pBNP-32 was 2.1%. Synthetic pBNP-32 showed approximately twice as much potent chick rectum relaxant activity as synthetic pBNP.[2a,b]

These excellent results show that $AgBF_4$ in TFA is a useful mild reagent for removal of the *S*-Tacm group in peptide synthesis. It is noteworthy that synthetic pBNP-32 obtained in highly purified form had approximately 5 times more potent chick rectum relaxant activity than α-rat ANP.[11] To our present knowledge, the synthetic pBNP-32 was the most potent peptide among the ANP-BNP families in chick rectum relaxant activity.

REFERENCES AND NOTES

1) Abbreviations: Boc=*tert*-butoxycarbonyl, Bu=*tert*-butyl, Bzl=benzyl, BrZ=2-bromobenzyloxycarbonyl, cHex=cyclohexyl, ClZ=2-chlorobenzyloxycarbonyl, Tos=*p*-toluenesulfonyl.
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