[Chem. Pharm. Bull.]
35(9)3880-3883(1987)

Studies on Peptides. CLV.^{1,2)} Evaluation of Trimethylsilyl Bromide as a Hard-Acid Deprotecting Reagent in Peptide Synthesis

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(Received March 2, 1987)

Trimethylsilyl bromide (TMSBr) in trifluoroacetic acid (TFA) was found to have the ability to cleave benzyl-type protecting groups, *i.e.*, benzyloxycarbonyl (Z), benzyl (O-Bzl) and *p*-methoxybenzyl (S-MBzl). The reaction was best accelerated by addition of thioanisole, compared with other soft nucleophiles so far examined. The rate of the cleavage reaction with TMSBr/TFA was judged to be somewhat slower than that with trimethylsilyl trifluoromethanesulfonate/TFA. However, TMSBr/TFA reduced Met(O) efficiently and gave almost no side reaction of Asp (succinimide formation). This deprotecting procedure was applied to the synthesis of human gastrin-releasing peptide.

Keywords—trimethylsilyl bromide; hard acid deprotecting reagent; soft nucleophile; thioanisole; trimethylsilyl trifluoromethanesulfonate; trifluoromethanesulfonic acid; Met sulfoxide; Asp-side reaction; human gastrin-releasing peptide

Removal of Bzl-type protecting groups by HBr/AcOH was first demonstrated by Ben-Ishai and Berger³⁾ in 1952. This deprotecting procedure was applied to the synthesis of α -melanocyte-stimulating hormone by Guttmann and Boissonnas⁴⁾ in 1958 and later, to the solid phase peptide synthesis by Merrifield⁵⁾ in 1963. However, this procedure did not gain wide acceptance in practical peptide synthesis, since HF was found to be useful for the same purpose by Sakakibara *et al.*⁶⁾ in 1967. From a practical standpoint, preparation and handling of HBr/AcOH reagent are rather laborious. If the HF deprotecting procedure had not been available, presumably the solid phase synthesis would not have gained its present degree of applicability. However, in spite of the attractive features of the HF procedure, the HBr/TFA deprotecting procedure still appears to be superior to HF for suppressing acid-catalyzed succinimide formation of the Asp residue linked to particular amino acids, as reported by Bodanszky *et al.*⁷⁾

We wish to report that Bzl-type protecting groups can be cleaved, as by HBr/TFA, when the trimethylsilyl group is supplied as a source of hard acid, in lieu of the proton of HBr in TFA, and thioanisole is used as a soft nucleophile.⁸⁾ The efficiency of 1 M TMSBr–thioanisole/TFA as a peptide deprotecting reagent was examined and the results were compared with those of 1 M TMSOTf–thioanisole/TFA.⁹⁾

Amino acid derivatives were treated with 1 M TMSBr/TFA in the presence of various cation scavengers in an ice-bath for 60 min and amino acids regenerated were determined quantitatively by using an amino acid analyzer. As shown in Table I, in this system, thioanisole was found to be most effective to accelerate the cleavage reaction, compared with other scavengers examined. The Bzl group was cleaved from Ser, Thr, Glu and Asp and the Z group from Lys in nearly quantitative yields by 1 M TMSBr–thioanisole/TFA. In addition, the Cl₂–Bzl group¹⁰⁾ from Tyr and the Mts group¹¹⁾ from Trp and Arg were cleaved in nearly

TABLE I. Removal of Various Protecting Groups by 1 M Me₃SiBr/TFA in the Presence of a Soft Base

Treated amino acid deriv. (0 °C, 60 min)	Parent amino acid regenerated (%)			
	Anisole	Thioanisole	MeSMe	PhSPh
Z(OMe)-Lys(Z)-OH		87.8		
Z(OMe)-Ser(Bzl)-OH	64.1	85.7	82.3	82.3
Boc-Thr(Bzl)-OH		100.0		
Z(OMe)-Glu(OBzl)-OH		93.9		
Boc-Asp(OBzl)-OH	27.6	100.5	45.2	46.0
Boc-Asp(OChp)-OH		4.2	3.9	5.0
Boc-Tyr(Cl ₂ -Bzl)-OH	32.9	96.9	74.7	76.2
Boc-Trp(Mts)-OH	3.3	86.1	13.6	11.4
Z(OMe)-Arg(Mts)-OH	23.4	87.0	19.0	40.8
H-Cys(MBzl)-OH		98.7		
Z(OMe)-Cys(Ad)-OH		0		
Boc-Cys(<i>tert</i> -Bu)-OH		7.6		
Z(OMe)-Met(O)-OH		91.3		

90% yield under these conditions. However, removal of the Chp group¹²⁾ from Asp was unsuccessful. Of various S-protecting groups of cysteine, the MBzl group¹³⁾ was cleaved completely, but others were not cleaved under the conditions stated above. Thus, the rate of cleavage by 1 M TMSBr-thioanisole/TFA was judged to be much slower than that by 1 M TMSOTf-thioanisole/TFA. However, it seems worthwhile to note that Met(O)¹⁴⁾ was reduced back to Met in 91% yield by this TMSBr/TFA system, while the 1 M TMSOTf-thioanisole/TFA system reduced Met(O) in only 27% yield under identical conditions.

Next, Asp-succinimide formation by this deprotecting reagent was examined and the result was compared with that in the case of 1 M TMSOTf/TFA. A model peptide, Z(OMe)-Ala-Asp(OBzl)-Gly-OBzl¹²⁾ was treated with 1 M TMSBr/TFA or 1 M TMSOTf/TFA in the presence of thioanisole or diphenylsulfide in an ice-bath for 120 min and the succinimide formed was quantitated with a dual-wavelength TLC (thin-layer chromatography) scanner. The amount of the side product formed by TMSBr/TFA treatment was judged to be negligible, while TMSOTf/TFA treatment gave the side product in 4.6% yield. In this respect, the TMSBr/TFA system seems to be superior to the TMSOTf/TFA system. The deprotecting procedure based on the hard acid principle was found not to be entirely free from the ring closure reaction of the Asp residue, depending on the nature of hard acid employed, as we had observed in human pancreatic polypeptide synthesis.¹⁾

The above results suggested that this TMSBr/TFA deprotecting procedure can be applied to the synthesis of relatively small peptides containing Asp or Met. As a model experiment, protected human gastrin releasing polypeptide (hGRP)¹⁵⁾ containing two Met(O) residues was deblocked by treatment with TMSBr/TFA. In the previous synthesis, these Met(O) residues were reduced by treatment with phenylthiotrimethylsilane,¹⁶⁾ prior to TFMSA/TFA deprotection.¹⁷⁾ However, in the present experiment, protected hGRP was directly treated with 1 M TMSBr-thioanisole/TFA in an ice-bath for 3 h to ensure the complete deprotection of the three protecting groups, Z from Lys and two Mts groups from Arg and Trp, and at the same time the complete reduction of the two Met(O). The crude product obtained after gel-filtration was examined by high-performance liquid chromatography (HPLC). As shown in Fig. 1, the product possessing an identical retention time with that of an authentic sample of hGRP emerged from the column as the main component. The desired product was isolated by preparative HPLC in 52.4% yield. Thus, the desired peptide

Z(OMe)-Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys(Z)-Met(O)-
 Tyr-Pro-Arg(Mts)-Gly-Asn-His-Trp(Mts)-Ala-Val-Gly-His-Leu-Met(O)-NH₂
 ↓ 1) 1 M TMSBr-thioanisole/TFA
 ↓ 2) gel-filtration on Sephadex G-25
 crude hGRP
 ↓ 3) HPLC
 purified hGRP

Fig. 1. HPLC of the Crude Sample of Synthetic hGRP

a) Gel-filtered sample. b) Gel-filtered sample (20 µg) + synthetic hGRP¹⁵⁾ (25 µg).

was obtained in a much better yield than before (30%), without particular treatment for the reduction of Met(O), before or after deprotection.

Some advantageous features of the 1 M TMSBr-thioanisole/TFA deprotecting procedure have thus been demonstrated. We intend to evaluate further whether this hard acid deprotecting procedure with TMSBr can be applied to the synthesis of more complex peptides by increasing its concentration in TFA.

Experimental

Amino acid analysis and HPLC were conducted with a Hitachi 835-02 instrument and a Waters 204 compact model, respectively. Optical rotation and ultraviolet absorption (UV) were determined with Union PM-101 and Hitachi 100-20 instruments, respectively. *R_f* values in TLC on silica gel (Kieselgel G, Merck) refer to the following solvent systems: *R_{f1}* *n*-BuOH-pyridine-AcOH-H₂O (3:1:1:1), *R_{f2}* *n*-BuOH-AcOH-pyridine-H₂O (4:1:1:2), *R_{f3}* *n*-BuOH-AcOH-AcOEt-H₂O (1:1:1:1), and the ninhydrin color intensity was measured with a Shimadzu dual-wavelength TLC scanner, model CS-900. Leucine-aminopeptidase was purchased from Sigma Chem. Co. (Lot. No. 62F-8000).

Treatment of Amino Acid Derivative with 1 M TMSBr/TFA in the Presence of Various Scavengers—A mixture of an amino acid derivative (0.03 mmol each) and Gly (internal standard) was treated with 1 M TMSBr/TFA (0.6 ml, reagent 20 eq each) in an ice-bath for 60 min. As a scavenger, four compounds were tested: anisole, thioanisole, MeSMe (to a concentration of 1 M for each), and PhSPh (not freely soluble, but an amount equivalent to 1 M was added). A part of the solution was subjected to quantitative amino acid analysis and the results are listed in Table I.

Treatment of Z(OMe)-Ala-Asp(OBzl)-Gly-OBzl with 1 M TMSBr-Thioanisole/TFA—A sample (10 mg) was treated with 1 M TMSBr-thioanisole/TFA (0.4 ml) in an ice-bath for 120 min. The product was precipitated with *n*-hexane and examined by TLC with the scanner using authentic samples of H-Ala-Asp-Gly-OH (*R_{f1}* 0.20) and H-Ala-Asc-Gly-OH (*R_{f1}* 0.33) as references.

Deprotection of Protected hGRP by Treatment with 1 M TMSBr-Thioanisole/TFA—Protected hGRP (50 mg, 15.7 µmol) was treated with 1 M TMSBr-thioanisole/TFA (5 ml) in the presence of *m*-cresol (16.7 µl, 10 eq per Tyr) and EDT (13.4 µl, 10 eq per Trp) in an ice-bath for 3 h, then dry ether was added. The resulting powder was dissolved in H₂O (2 ml). The pH of the ice-chilled solution was adjusted to 8.0 with 5% NH₄OH and 1 M NH₄F (200 µl) was added. After 30 min, the pH was readjusted to 5.0 with 1 N AcOH and the solution was applied to a column of Sephadex G-25 (2.3 × 130 cm), which was eluted with 1 N AcOH. The fractions corresponding to the front main peak (tube Nos. 36–50, 6 ml each, determined by UV absorption measurement at 280 nm) were combined and the solvent was removed by lyophilization to give a crude deprotected peptide; yield 41 mg (94%). Its HPLC elution pattern on an ODS column (AM-302, Yamamura Chemical Co.) with 25% MeCN in 0.1% TFA is shown in Fig. 1a, in

comparison with that of a mixture of the crude sample and an authentic sample of synthetic hGRP (Fig. 1b).

The product (9.1 mg) was purified by HPLC using a Nucleosil 5C18 column (10 × 250 mm) with isocratic elution with MeCN (27%) in 0.1% TFA. The desired eluate (retention time 24 min) was collected, then the solvent was removed by evaporation and the residue was lyophilized to give a fluffy white powder. The rest of the sample was similarly purified; yield 23.6 mg (52.4% from the protected hGRP), R_f 0.45, R_f 0.48, $[\alpha]_D^{21} - 99.7^\circ$ ($c = 0.1$, 1 N AcOH), (lit.¹⁵) -99.9° in 1 N AcOH). Amino acid ratios in 4 N methanesulfonic acid¹⁸ hydrolysate: Asp 1.08, Thr 1.88, Pro 3.08, Gly 5.19, Ala 2.13, Val 3.00, Met 2.05, Leu 3.00, Tyr 0.98, Lys 1.07, His 1.98, Trp 0.85, Arg 1.00 (recovery of Leu, 82%). Amino acid ratios in a leucine-aminopeptidase digest (numbers in parentheses are theoretical): Thr 2.16 (2), Pro 2.93 (3), Gly 4.88 (5), Ala 2.08 (2), Val 2.92 (3), Met 1.95 (2), Leu 3.00 (3), Tyr 1.03 (1), Lys 1.12 (1), His 1.78 (2), Trp 0.88 (1), Arg 1.00 (1), Asn not determined (recovery of Leu, 77%).

References and Notes

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- 2) Amino acids used in this investigation are of the L-configuration. The following abbreviations are used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, Bzl = benzyl, Cl₂-Bzl = 2,6-dichlorobenzyl, MBzl = *p*-methoxybenzyl, Chp = cycloheptyl, Mts = mesitylenesulfonyl; 'Bu = *tert*-butyl, Ad = adamantyl, TFA = trifluoroacetic acid, TMSBr = trimethylsilyl bromide, TFMSA = trifluoromethanesulfonic acid, TMSOTf = trimethylsilyl trifluoromethanesulfonate, (O) = sulfoxide, Asc = aminosuccinimide, MeSMe = dimethylsulfide, PhSPh = diphenylsulfide, EDT = ethanedithiol, ODS = octadecylsilanol.
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