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Studies on Peptides. CLII.^{1,2)} Hard Acid Deprotecting Procedure for Peptide Synthesis

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The use of trimethylsilyl trifluoromethanesulfonate as a peptide deprotecting reagent was examined, together with the role of soft bases employed. A new deprotecting procedure based on the hard acid principle is presented.

Keywords—new peptide deprotecting procedure; hard acid principle; trimethylsilyl trifluoromethanesulfonate; trimethylsilyl methanesulfonate; trimethylsilyl trifluoroacetate; hard-soft acid-base concept; thioanisole-mediated deprotection; ²⁹Si-NMR chemical shift; human glucose-dependent insulinotropic polypeptide synthesis

The use of silyl compounds, such as Me₃SiI³⁾ and recently MeSiCl₃ or SiCl₄,⁴⁾ as peptide deblocking reagents has been examined. However, somewhat elevated temperature or prolonged treatment was required for complete removal of the Bzl group and even the Z group. We found that trimethylsilyl trifluoromethanesulfonate (TMSOTf), a powerful trimethylsilylating reagent,⁵⁾ which can be classified as a super hard acid,⁶⁾ has an ability to cleave, not only the Boc group,⁷⁾ but also readily various other protecting groups currently employed in peptide synthesis, including the Z and Bzl groups. The resulting trimethylsilylated compounds can be hydrolyzed easily with water or ammonium fluoride⁸⁾ to afford free peptides. The properties of several trimethylsilyl compounds were examined, together with the role of soft bases⁹⁾ employed in this hard acid deprotecting reaction.

Various benzyl-type (N^ε-Z, O-Bzl, O-Cl₂-Bzl¹⁰⁾) and phenylsulfonyl-type (N^G-Mts,¹¹⁾ N^G-MBS,¹²⁾ and Nⁱⁿ-Mts¹³⁾) protecting groups, which are stable to TFA, were cleaved by treatment with 1 M TMSOTf/TFA in an ice-bath within 30 min. As a cation scavenger, thioanisole¹⁴⁾ (at a concentration of 1 M) was employed and amino acids regenerated were determined on an amino acid analyzer as shown in Table I. Several S-protecting groups of cysteine (MBzl,¹⁵⁾ Ad,¹⁶⁾ and *tert*-Bu¹⁷⁾) were found to be cleaved similarly. The other protecting groups, N^{im}-Tos,¹⁸⁾ N^{im}-Bom,¹⁹⁾ and O-Chp,²⁰⁾ were also cleaved smoothly from His, Trp and Asp, respectively, under the above conditions. Cys(Bzl)²¹⁾ and Cys(Acm)²²⁾ resisted the action of this hard acid. Complete removal of two N^G-protecting groups of Arg, Tos²³⁾ and NO₂,²⁴⁾ could not be achieved under the above conditions. However, recovery of Arg from Arg(Tos) reached 86% after a 60 min treatment. From these data, the rate of this cleaving reaction was judged to be much faster than that of 1 M TFMSA/TFA.²⁵⁾

Thioanisole is a good scavenger to suppress side reactions in TFMSA/TFA deprotection, *i.e.*, Lys(Bzl) formation¹⁴⁾ and 3-Bzl-Tyr formation²⁶⁾ and is known to play a role as a soft base^{9,14)} to accelerate the cleaving reaction.²⁷⁾ This thioether was judged to be still a better soft base than diphenylsulfide (PhSPh)²⁸⁾ and dimethylsulfide (MeSMe)²⁹⁾ in the present

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

Treated amino acid derivative (0 °C, 30 min)	Parent amino acid regenerated			
	None	Thioanisole	MeSMe	PhSPh
Z(OMe)-Lys(Z)-OH	48.4	98.7	87.0	73.1
Z(OMe)-Ser(Bzl)-OH	84.0	91.7	90.2	97.8
Boc-Thr(Bzl)-OH	91.8	98.0	94.6	100.0
Z(OMe)-Glu(OBzl)-OH	92.2	99.3	97.3	100.0
Z(OMe)-Asp(OBzl)-OH	100.0	99.2	97.7	100.0
Boc-Asp(OChp)-OH	91.4	100.0		100.0
Boc-Tyr(Bzl)-OH	63.3	100.0		87.5
Boc-Tyr(Cl ₂ -Bzl)-OH	81.5	100.0	88.3	86.6
Boc-His(Tos)-OH	88.5	94.5	96.3	100.0
Boc-His(Bom)-OH	88.5	88.9	50.5	90.2
Boc-Trp(Mts)-OH	74.7	100.0	25.6	100.0
Boc-Trp(For)-OH ^{a)}		100.0		
Z(OMe)-Arg(Mts)-OH	93.3	97.6	67.2	100.0
Z(OMe)-Arg(MBS)-OH	80.6	93.5		84.3
Z-Arg(Tos)-OH	3.4	62.1	0	8.3
Z-Arg(NO ₂)-OH		11.0		10.8
H-Cys(MBzl)-OH	100.0	100.0		100.0
Boc-Cys(<i>tert</i> -Bu)-OH	≡0	87.3	32.9	97.1
Z(OMe)-Cys(Ad)-OH	91.7	100.0	11.0	100.0
Boc-Cys(Acm)-OH	0	0		
H-Cys(Bzl)-OH	0	0		
Z(OMe)-Met(O)-OH	0	27.6	4.0	≡0
Boc-Ile-PAM-resin ^{b)}	15.4	81.9	12.4	23.8

a) In the presence of ethanedithiol. b) 60 min treatment.

Fig. 1. Possible Mechanism of TMSOTf-Soft Base/TFA Deprotection

deprotecting reaction, and a possible mechanism of its action is shown in Fig. 1. When PhSPh (at a concentration of 0.5 M, due to its lower solubility in TFA) was employed, the recoveries of Lys and Tyr were somewhat low. MeSMe (at a concentration of 1 M) was not effective enough to cleave the Mts and MBS groups (Table I). The results suggested the existence of a subtle relationship between soft acids (protecting groups) and soft bases. Reduction of Met(O)³⁰⁾ by 1 M TMSOTf/TFA treatment with the aid of these soft bases was unsuccessful. Trp(For)³¹⁾ was deprotected by this TMSOTf/TFA treatment in the presence of ethanedithiol, presumably through a thioacetal intermediate.^{29b)}

It seems noteworthy that Ile, possessing a bulky side chain, could be cleaved from Boc-Ile-O-CH₂-PAM-resin³²⁾ by treatment with 1 M TMSOTf-thioanisole/TFA in fairly good

yield. The result seems to demonstrate the usefulness of this deprotecting procedure for solid-phase peptide synthesis.

When the ^{29}Si -NMR signal³³⁾ of three trimethylsilyl compounds in TFA were measured, using Me_4Si as an internal standard, the signal of TMSOTf appeared at the lowest magnetic field, compared with those of trimethylsilyl methanesulfonate (TMSOMs) and trimethylsilyl trifluoroacetate (TMSOTa). These values seem to correlate well with cleaving efficiencies of these trimethylsilyl compounds tested as sources of a hard acid. For example, the protecting groups of four compounds, Ser(Bzl), Asp(OBzl), Lys(Z) and Arg(Mts), were cleaved by TMSOMs by increasing the concentration and elevating the temperature (22 °C), but complete

TABLE II. Removal of Protecting Groups by Trimethylsilyl Compounds in the Presence of Thioanisole

Hard acid (22 °C, 60 min)	^{29}Si ppm ^{a)}	Ser(Bzl)	Lys(Z)	Arg(Mts)	Asp(OBzl)
1 M TMSOTf/TFA ^{b)}	46.1 ^{c)}	91.7	98.7	97.6	99.2
2 M TMSOMs/TFA	37.9	93.5	92.4	80.0	99.5
3 M TMSOTa/TFA	35.6	7.1	16.9	0	1.6

a) Me_4Si was used as an internal standard. b) Data taken from Table I (conducted at 0 °C for 30 min). c) Lit.³³⁾ 44.6 ppm in benzene.

Fig. 2. HPLC of Crude Synthetic hGIP

a) TMSOTf-treated sample. b) TMSOTf-gel-filtered sample. c) TFMSA-gel-filtered sample.

removal of these protecting groups by treatment with 3 M TMSOTa–thioanisole/TFA was unsuccessful, as shown in Table II. Thus, of the hard acids tested, TMSOTf was judged to be the most attractive reagent for deprotection.

Next, we wished to demonstrate the usefulness of this new deprotecting procedure in practical peptide syntheses. As an example, protected human GIP (glucose-dependent insulinotropic polypeptide)³⁴⁾ was treated with 1 M TMSOTf–thioanisole/TFA and periodically a part of the solution was examined by high-performance liquid chromatography (HPLC). As shown in Fig. 2a, the desired peak increased, with a decrease of other side peaks. After gel-filtration on Sephadex G-25, the crude deprotected peptide obtained at this stage exhibited much higher purity than the product obtained by 1 M TFMSA–thioanisole/TFA deprotection, when compared by HPLC (Fig. 2b, c). After subsequent HPLC purification, a 42-residue peptide corresponding to the entire amino acid sequence of hGIP³⁵⁾ was obtained in a better yield (28%) than before (10%).

We have described here a new deprotecting procedure by the use of a hard acid, which differs from the Brönsted acid deprotecting procedures currently employed in peptide synthesis. This procedure, which seems to have several attractive features, may gain wide applicability in solution-phase peptide synthesis, as well as in solid-phase peptide synthesis, particularly for large-scale preparation.

Experimental

Amino acid analysis and HPLC were conducted with a Hitachi 835-02 model and a Waters 204 compact model, respectively. Rotation and ultraviolet absorption (UV) were determined with a Union PM-101 model and a Hitachi 100-20 model, respectively. *R_f* values in thin layer chromatography (TLC) conducted on silica gel (Kieselgel G, Merck) refer to the following solvent systems: *R_{f1}* *n*-BuOH–AcOH–pyridine–H₂O (4:1:1:2) and *R_{f2}* *n*-BuOH–AcOH–AcOEt–H₂O (1:1:1:1). Leucine-aminopeptidase was purchased from Sigma Chem. Co. (Lot. No. L-6007).

Treatment of Amino Acid Derivatives with Trimethylsilyl Compounds—(a) A mixture of an amino acid derivative (0.1 mmol each) and Gly (internal standard) was treated with 1 M TMSOTf/TFA in the presence or the absence of a soft base in an ice-bath for 30 min. As a soft base, three thioether compounds were tested: thioanisole (at a concentration of 1 M), MeSMe (at a concentration of 1 M) and PhSPh (at a concentration of 0.5 M; this concentration was judged to be practical, since PhSPh is not freely soluble in TFA). A part of the solution was subjected to quantitative amino acid analysis and the results are listed in Table I. (b) To examine the cleaving ability of other trimethylsilyl compounds, four derivatives, Ser(Bzl), Lys(Z), Arg(Mts) and Asp(OBzl), were selected. A mixture of each derivative (0.8 mmol) and Gly (internal standard) was treated with 2 M TMSOMs/TFA or 3 M TMSOTa/TFA in the presence of thioanisole (at a concentration of 1 M) at room temperature (22 °C) for 60 min. An aliquot was examined on an amino acid analyzer and the results are listed in Table II, together with the ²⁹Si chemical shift values.

Cleavage of Ile from Boc-Ile-O-CH₂-PAM-resin—TMSOTf was added at a concentration of 1 M to a suspension of Boc-Ile-O-CH₂-PAM-resin (25 mg, Ile content, 0.80 mmol/g) and Gly (internal standard) in 1 M thioanisole/TFA, and the mixture was stirred in an ice-bath for 60 min, then a part of the solution was subjected to quantitative amino acid analysis. The results are shown in Table I.

Deprotection of Protected GIP by Treatment with 1 M TMSOTf–Thioanisole/TFA—Protected GIP (50 mg, 74 μmol) was treated with 1 M TMSOTf–thioanisole/TFA (5 ml; the amount of TMSOTf was 45 eq/protecting group) in an ice-bath for 120 min to ensure complete deprotection. *m*-Cresol (15 μl, 20 eq) and ethanedithiol (12 μl, 20 eq) were added as additional scavengers to protect the Tyr and Trp residues. Periodically, an aliquot was examined by HPLC. The elution patterns obtained after 60 min and 120 min treatments are shown in Fig. 2a. Dry ether was added and the resulting powder was collected by centrifugation and washed with fresh ether. The deprotected peptide was dissolved in H₂O (*ca.* 5 ml) and the solution was adjusted to pH 8.0 with Et₃N. After addition of mercaptoethanol (200 μl) and 1 M NH₄F (400 μl), the solution was stirred in an ice-bath for 30 min, then the pH was adjusted to 5 with 1 N AcOH. Next, the solution was applied to a column of Sephadex G-25 (3.3 × 108 cm), which was eluted with 1 N AcOH. The desired fractions (8 ml each, tube Nos. 40–52; monitored by UV absorption measurement at 280 nm) were combined and the solvent was removed by lyophilization to give a fluffy powder; yield 35 mg (96%). Its HPLC elution pattern is shown in Fig. 2b in comparison with that of the gel-filtered sample obtained by 1 M TFMSA–thioanisole/TFA treatment.

The gel-filtered sample was next applied to a column of CM-Trisacryl M (2.0 × 5.0 cm), which was eluted first with pH 6.46, 0.01 M AcONH₄ buffer (120 ml), then with a gradient formed from 0.2 M NaCl in 0.01 M AcONH₄

(250 ml) through a mixing flask containing the starting buffer (250 ml). The desired fractions (5.8 ml each, tube Nos. 25–35; monitored by UV absorption measurement at 280 nm) were combined and the solvent was removed by lyophilization. The residue was desalted by gel-filtration on Sephadex G-25 using 1 N AcOH under the conditions stated above. Lyophilization of the desired fraction gave a fluffy white powder; yield 13.6 mg (37%).

The product (6.8 mg) was next purified by HPLC using a Nucleosil 5C18 column (4.0 × 150 mm) with a gradient of MeCN (30–35%) in 0.1% TFA aq. The desired eluates (retention time, 20 min) were collected and the residue was lyophilized to give a fluffy white powder. The rest of the sample was similarly purified; yield 5.1 mg (28% from the protected GIP), R_f 0.26, R_f 0.11. $[\alpha]_D^{18}$ –43.2° (c = 0.3, 1 N AcOH, lit.³⁴) –39.7° in 0.1 N AcOH). Amino acid ratios in a leucine-aminopeptidase digest (numbers in parentheses are theoretical values): Asp 3.68(4), Ser 1.75(2), Glu 1.09(1), Gly 2.19(2), Ala 3.21(3), Val 1.17(1), Met 0.98(1), Ile 3.98(4), Leu 2.00(2), Tyr 1.95(2), Phe 2.19(2), Lys 4.76(5), His 1.98(2), Trp 2.11(2) (recovery of Leu 78%; Thr, Gln and Asn were not determined).

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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, Tos = *p*-toluenesulfonyl, Mts = mesitylenesulfonyl, MBS = *p*-methoxybenzenesulfonyl, Bom = benzyloxymethyl, MBzl = *p*-methoxybenzyl, *tert*-Bu = *tert*-butyl, Ad = adamantyl, Acn = acetamidomethyl, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, (O) = sulfoxide, PAM = 4-(oxymethyl)phenylacetamidomethyl, Chp = cycloheptyl, For = formyl.
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