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Studies on Peptides. CXXXIV.[1,2] Evaluation of S-1-Adamantylcysteine for Peptide Synthesis

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The S-1-adamantyl (Ad) group of cysteine is more stable to TFA treatment than the S-*p*-methoxybenzyl (MBzl) group, but is cleavable by 1 M trifluoromethanesulfonic acid–thioanisole in trifluoroacetic acid at 0 °C within 60 min or by $(CF_3COO)_3Tl$ under similar conditions. S-Ad-cysteine is less susceptible to sulfoxide formation than the S-MBzl group. Trimethylphenylthiosilane is an effective reducing reagent of the sulfoxide.

Keywords——S-1-adamantylcysteine; S-1-adamantylcysteine sulfoxide; sodium perborate oxidation; benzeneselenol; trimethylphenylthiosilane

The 1-adamantyl (Ad) group was first introduced for cysteine by Nishimura *et al.*[3] in 1978 as an S-protecting group removable by $(CH_3COO)_2Hg$ in TFA. However, this new derivative has never been applied in practical peptide synthesis. We found that the Ad group has several advantages over the MBzl group,[4] one of the most widely used S-protecting groups currently employed in peptide synthesis.

First, we found that the Ad group can be removed quantitatively by 1 M TFMSA–thioanisole in TFA[5] in an ice-bath within 60 min or by a soft acid, $(CF_3COO)_3Tl$, under similar conditions (Fig. 1). These new findings prompted us to evaluate the usefulness of this group for practical peptide synthesis.

The S-Ad group of cysteine was found to be more stable to TFA than the S-MBzl group. The latter was cleaved partially by TFA (*ca.* 10% after 2.5 h at 0 °C), whereas the former group remained intact under these conditions. Thus, when Cys(Ad) was employed, the risk of

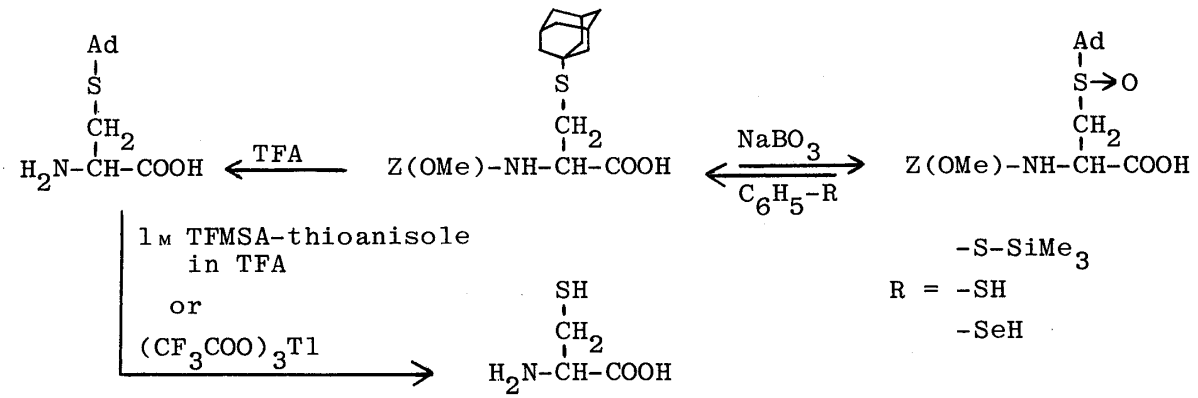

Fig. 1. Properties of S-1-Adamantylcysteine

partial conversion of the S-protecting group, such as from Cys(MBzl) to Cys(*tert*-Bu) during TFA treatment of Boc–Cys(MBzl)–OH,[6] can be eliminated. This side reaction is known to occur by partial cleavage of the S-MBzl group followed by attack of the *tert*-butyl cation derived from the Boc group. In the case of TFA treatment of Z(OMe)–Cys(MBzl)–OH, apparent conversion of the S-protecting group is not observed. However, even in this case, the possibility can not be excluded that partial conversion of the MBzl group by the *p*-methoxybenzyl cation derived from the Z(OMe) group may take place at the sulfur atom of cysteine.

Next, Z(OMe)–Cys(Ad)–OH was found to be less susceptible to sulfoxide formation[7] as compared to Z(OMe)–Cys(MBzl)–OH. The latter was oxidized to the corresponding sulfoxide by $NaBO_3$ within 18 h, whereas complete oxidation of the former required 28 h. The results suggest that peptide synthesis with Cys(Ad) may be less susceptible to air-oxidation, as compared to Cys(MBzl)-containing peptides. Thus, the use of Z(OMe)–Cys(Ad)–OH seems to be advantageous for the synthesis of relatively large peptides. As we pointed out previously, the sulfoxide, if formed, has to be reduced before deprotection, since otherwise a satisfactory recovery of cysteine can not be achieved.[7] Recently, we found that Se compounds or trimethylphenylthiosilane[8] are more effective reducing reagents for Met(O) than thiophenol.[9] Kiso *et al.*[10] recommended the use of dimethylselenide, trichloromethylsilane or chlorotrimethylsilane for this purpose. As described briefly in our paper,[9] such compounds are also effective reducing reagents for S-substituted cysteine sulfoxides. Indeed, Z(OMe)–Cys(Ad)(O)–OH and Z(OMe)–Cys(MBzl)(O)–OH were both reduced back to the parent derivatives quantitatively by trimethylphenylthiosilane at 40 °C within 3 h. However, benzeneselenol was not effective to reduce Z(OMe)–Cys(Ad)(O)–OH, while Z(OMe)–Cys(MBzl)(O)–OH was smoothly reduced with this reagent within 2 h.

In parallel with these experiments, we examined the properties of various S-protecting groups, such as Dbs,[11] Bzh[12] and Dpe,[13] in respect of stability to TFA, susceptibility to 1 M TFMSA–thioanisole in TFA and resistance to air-oxidation. Among the groups so far examined, the Ad group was judged to fulfill satisfactorily several criteria required for practical peptide synthesis. Thus, we demonstrated its usefulness in the synthesis of a calcitonin gene-related peptide as will be reported in the subsequent paper.

Experimental

Thin layer chromatography (TLC) was performed on silica gel (Kieselgel G, Merck) using $CHCl_3$–MeOH–AcOH (9 : 1 : 0.5).

Z(OMe)–Cys(Ad)–OH·DCHA——H–Cys(Ad)–OH[3] was acylated according to Weygand and Hunger,[14] and the product was converted to the corresponding DCHA salt as usual. It was recrystallized from MeOH and ether; yield 74%, mp 146—148 °C, $[\alpha]_D^{20} -7.1°$ ($c=0.9$, MeOH), *Rf* 0.70. *Anal.* Calcd for $C_{22}H_{29}NO_5S \cdot C_{12}H_{23}$ N: C, 67.73; H, 9.03; N, 4.65. Found: C, 67.99; H, 8.85; N, 4.66.

Treatment of Z(OMe)–Cys(Ad)–OH with TFA——In an ice-bath, the Z(OMe) group was cleaved by TFA in the presence of anisole as usual within 60 min. Besides H–Cys(Ad)–OH, no other spot was detected on TLC even after 150 min. Other S-protecting groups, R = Dbs, Bzh and Dpe, were partially cleaved within 150 min at 0 °C, when examined by TLC.

Treatment of H–Cys(Ad)–OH with 1 M TFMSA–Thioanisole in TFA——In the presence of *m*-cresol (10 eq), the sample (100 mg) was treated with 1 M TFMSA–thioanisole in TFA (7.8 ml) in an ice-bath for 120 min, then ether was added. The resulting powder was subjected to amino acid analysis; recovery of cysteine was 86.9%. No spot corresponding to the starting material was detected on TLC. The other protecting groups, Dbs, Bzh and Dpe, were also cleaved under the same conditions.

Treatment of H–Cys(Ad)–OH with $(CF_3COO)_3Tl$——In the presence of anisole (0.1 ml), H–Cys(Ad)–OH (100 mg) was treated with $(CF_3COO)_3Tl$ (1.0 eq) in an ice-bath for 60 min. The excess TFA was removed by evaporation *in vacuo* under 25 °C, then dry ether was added. The residue was dissolved in H_2O and the solution, after being adjusted to pH 7.5 with dil. NH_4OH, was incubated with EDT (0.33 ml, 10 eq) at 40 °C for 5 h. The resulting precipitate was filtered off. The filtrate was concentrated and the residue was subjected to amino acid analysis;

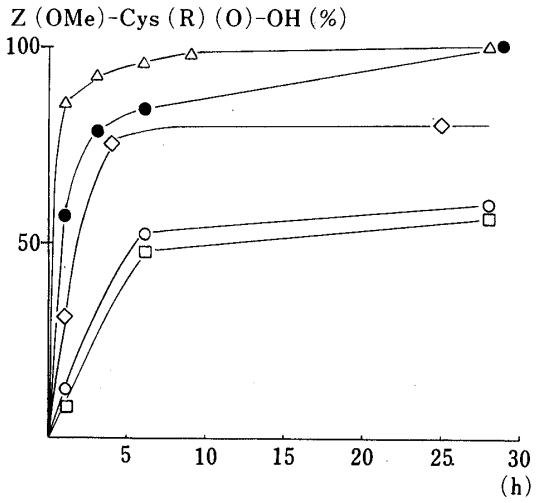

Fig. 2. Oxidation of Z(OMe)–Cys(R)–OH with $NaBO_3$

R=MBzl △, Ad ●, Dbs ◇, Dpe ○, Bzh □.

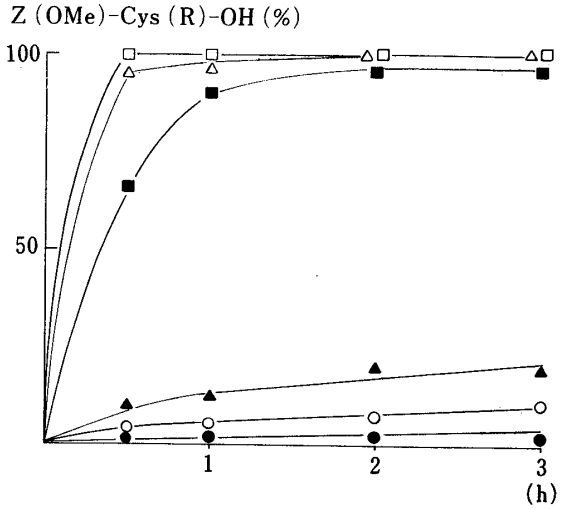

Fig. 3. Reduction of Z(OMe)–Cys(R)(O)–OH

R=Ad: PhSH ●, PhSeH ▲, $PhSSiMe_3$ ■.
R=MBzl: PhSH ○, PhSeH △, $PhSSiMe_3$ □.

recovery of cysteine was 89%. No spot corresponding to the starting material was detected on TLC.

Z(OMe)–Cys(Ad) (O)–OH——A solution of Z(OMe)–Cys(Ad)–OH (1.40 g, 3.33 mmol) in a mixture of AcOEt and H_2O (1 : 1, 30 ml) was stirred in the presence of $NaBO_3 \cdot 4H_2O$ (615 mg, 4 mmol) at room temperature for 24 h; loss of the starting material was followed by TLC. The solution was acidified with citric acid. The separated AcOEt layer was washed with H_2O–NaCl, dried over Na_2SO_4 and concentrated. The residue was recrystallized from MeOH and ether; yield 1.20 g (83%), mp 81—84 °C, $[\alpha]_D^{25} -30.0°$ ($c=0.8$, MeOH), *Rf* 0.47. *Anal.* Calcd for $C_{22}H_{29}NO_6S \cdot 1/2H_2O$: C, 59.43; H, 6.80; N, 3.15. Found: C, 59.66; H, 6.72; N, 3.18.

For comparison, Z(OMe)–Cys(R)–OH derivatives (R=MBzl, Dbs, Bzh and Dpe) (0.25 mmol each) in AcOEt–H_2O (1 : 1, 2 ml) were oxidized with $NaBO_3 \cdot 4H_2O$ (1.1 eq). The progress of the oxidation was monitored with a Shimadzu dual-wavelength TLC scanner and the results are shown in Fig. 2. The derivatives of Bzh and Dpe suffered less oxidation than the others. In these cases, a small amount of Z(OMe)–Cys–OH was detected on TLC.

Reduction of Z(OMe)–Cys(R)(O)–OH——Samples of Z(OMe)–Cys(R)(O)–OH (R=MBzl and Ad) (0.23 mmol) in DMF (1 ml) were incubated at 40 °C in the presence of various reducing reagents; thiophenol, benzeneselenol, trimethylphenylthiosilane (10 eq each). The progress of the reduction was monitored with a Shimadzu dual-wavelength TLC scanner and the results are shown in Fig. 3.

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2) Cysteine is of the L-configuration. The following abbreviations are used: Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Bu=*tert*-butyl, MBzl=*p*-methoxybenzyl, Dbs=dibenzosuberyl, Bzh=benzhydryl, Dpe=1,1-diphenylethyl, TFMSA=trifluoromethanesulfonic acid, TFA=trifluoroacetic acid, DMF=dimethylformamide, DCHA=dicyclohexylamine, EDT=ethanedithiol.
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