JOM 23723PC

Preliminary Communication

The use of $[(C_6H_7)Fe(CO)_3]^+$ as a recoverable protecting group in peptide synthesis

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(Received March 10, 1993)

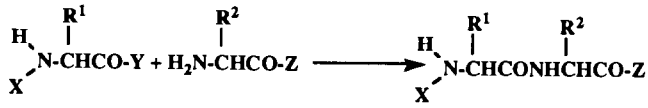
Abstract

The dienyl complex $[(\eta^5\text{-}C_6H_7)Fe(CO)_3]^+$ (**1**) has been shown to function as a novel and efficient SH protecting group in peptide synthesis, as illustrated for the synthesis of the tripeptide NAc–cys·gly·ala·OEt. An advantage over current methods is the ease of recovery intact of the cationic protecting group (**1**).

Methodologies for peptide synthesis include as essential ingredients (i) the protection (X) of reactive functional groups on the amino acids and (ii) the activation (Y) of a carboxyl group for the coupling reaction (Scheme 1; in solid phase peptide synthesis Z is conveniently a polymer support) [1,2]. Following removal of the blocking group X from the amine centre of the peptide product, repeated coupling/deprotection cycles are then carried out.

The most commonly employed groups for the repeated "temporary" protection of amino acid α-NH_2 groups are t-BOC (t-butoxycarbonyl) and Fmoc (9-fluorenylmethyloxycarbonyl). Other reagents have been developed for the "permanent" protection of reactive side chain functional groups such as SH (cys), imidazole (his) and COOH (glu, asp). An undesirable feature of these widely employed protecting groups is that they are typically destroyed by the deprotection step or converted to a form that is not readily recyclable.

We have reported elsewhere [3,4] that the dienyl complex **1** reacts rapidly and reversibly with a range of amino acid esters to give adducts of the type $[\{RO_2C\text{-}CH(R^1)NH\cdot C_6H_7\}Fe(CO)_3]$ (**2**). More recently we have found [5] that cation **1** has a very high affinity for

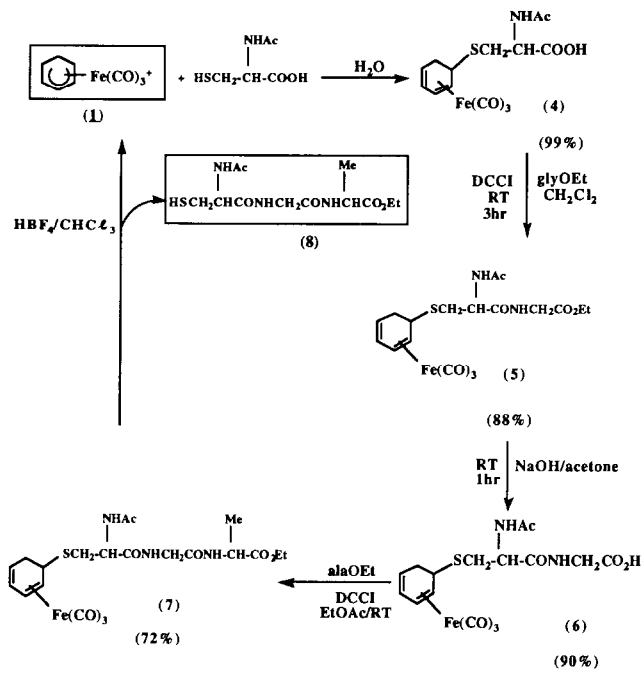


Scheme 1.

the sulphydryl group of cysteine and the tertiary imidazole nitrogen of histidine, giving related adducts such as $[\{HOOC\cdot CH(NH_2)CH_2S\cdot C_6H_7\}Fe(CO_3)]$ (**3**). In each of these cases the amino acid could be rapidly removed from the neutral adduct and the cation **1** recovered quantitatively by treatment with dilute trifluoroacetic acid (TFA). These unique properties of cation **1** of rapid addition to amino acids and facile recovery suggested that it might provide a novel and, in some aspects, superior protecting group for peptide synthesis.

As summarized in Scheme 2 below, we have now demonstrated the efficacy of cation **1** as an SH protecting group in peptide synthesis.

The initial adduct **4** is quantitatively precipitated as an off-white solid from the reaction of **1** with N-acetylcysteine in water. The subsequent coupling reac-



Scheme 2.

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0022-328X/93/$6.00

tion with glycine ethylester ($DCCI/CH_2Cl_2$) appears to be complete within 2 h at room temperature, and may be facilitated by the known [6] electron-withdrawing character of the (diene)$Fe(CO)_3$ moiety in **4**. Base hydrolysis (NaOH/acetone) of the ester group in the dipeptide adduct **5** gives the corresponding acid derivative **6**, which undergoes further facile coupling with alanine ethylester to give the tripeptide adduct **7**. Removal of the tripeptide and recovery of the dienyl protecting group **1** is conveniently achieved by brief treatment of **1** with HBF_4 in $CHCl_3$, which leads to separation of $[(C_6H_7)Fe(CO)_3]^+$ as its BF_4^- salt.

Each of the species **4**–**7** in Scheme 2 has been fully characterized by element analysis, ^1H NMR and IR spectroscopy, and electrospray (ES) mass spectrometry. Unlike FAB or CI mass spectrometry, which typically show only fragment ions ([M – peptide]) for the diene adducts, we have generally found [7] strong molecular ions in the ES mass spectra of adducts such as **4**–**7**. The dipeptide structure of **5** is confirmed from its ^1H NMR spectrum in dry DMSO-d^6, which reveals two discrete signals for the two amide α-NH protons (a doublet at 8.16 and a triplet at 8.52 ppm). The α-CH proton of the N-acetylcysteine group in **4** is also moved downfield (to 4.44 ppm), as expected, upon coupling with glycine ethylester to give **5**. Similarly, the tripeptide adduct **7** shows the expected three amide proton signals, at 8.40 (t), 8.18 (d), and 8.07 (d) ppm, corresponding to the glycine, cysteine and alanine amino acids, respectively. From 2D NMR experiments, **7** and the resultant tripeptide **8** have the desired structure and amino acid sequence. COSY and TOCSY spectra lead to the assignment of the ^1H NMR spectrum and a ROESY spectrum links the amino acids together via nOe's from the α-NH of one amino acid to the α-CH of the preceding amino acid [8].

A further advantage of complex **1** over existing protecting groups, apart from ease of recovery, is the strong yellow colour and lipophilicity that it imparts to peptides. This facilitates the use of simple column (silica) chromatography and high-resolution hexane-containing solvents in the purification procedures at each stage of Scheme 2.

Further studies are in progress to examine the utility of cation **1** as a more general protecting group for α-amino (NH_2) functionalities in peptide synthesis.

Acknowledgement

The Australian Research Council is thanked for financial support.

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