Selective Labelling of Peptides using (Dienyl) Iron Tricarbonyl Cations

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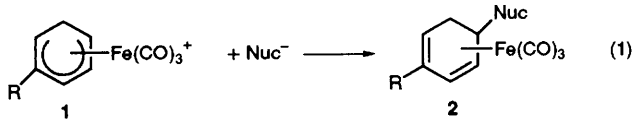
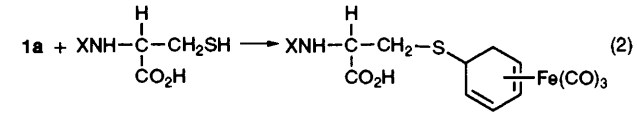
Dienyl cations of the type $[(\eta^5\text{-}R\cdot C_6H_6)Fe(CO)_3]^+$ **1** are shown to be versatile electrophilic reagents for the rapid and selective labelling of peptides and proteins, with the affinity for amino acid residues decreasing markedly in the order: cys (SH) > his (–N=) > glu (δ-CO_2^-) ≫ α-NH_2, lys (ε-NH_2), ser (γ-OH), asn (-CONH)

Dienyl iron tricarbonyl cations of the type $[(\eta^5\text{-}R\cdot C_6H_6)Fe(CO)_3]^+$ (**1**; R = H, 2-MeO) are chemically robust and very efficient electrophiles, attacking a wide range of nucleophilic substrates to give substituted diene products (**2**) [eqn. (1)].[1–3] Our kinetic studies[3,4] of such reactions have also shown cations **1** to be highly selective, exhibiting a reactivity range of over 10^9 towards some 40 nucleophiles examined to date. As summarised below, we report now that the unique reactivity patterns of cations **1** may be exploited to provide a new and versatile class of reagents for the selective labelling of amino acid residues in peptides and proteins.



1 **2** (1)

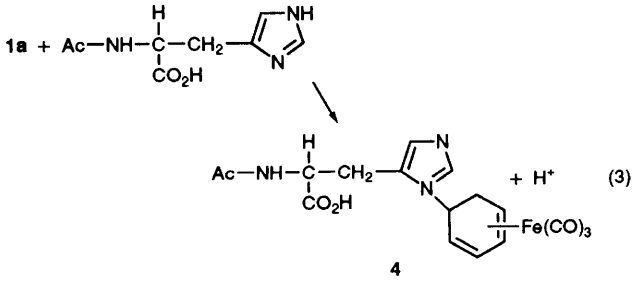
In situ ^1H and ^{13}C NMR and IR spectral studies reveal that cation (**1a**, R = H) adds in a very selective fashion to amino acids in D_2O. Rapid and quantitative addition of **1a** occurs on the sulfhydryl group of cysteine or *N*-acetylcysteine [eqn. (2), X = H or COMe] in the pH range 5–7. The cysteine adducts **3** precipitate in high yield as off-white solids, which have been fully characterised by elemental analyses, ^1H and ^{13}C NMR, IR [ν_{CO}/cm^{-1} 2055 and 1974 (Nujol)] and FAB mass spectrometry. A characteristic feature of the ^{13}C NMR spectrum of **3** [in $(CD_3)_2SO$] is the downfield shift of *ca.* 6 ppm for the β-CH_2 carbon upon attachment of cation **1** to the cysteine sulfur.



(2)

3

The reaction of cation **1a** with *N*-acetylhistidine is more pH dependent. At pH = 5.4 in D_2O, only *ca.* 20% formation of the adduct **4** is noted from *in situ* ^1H NMR and FTIR (circle cell) studies [eqn. (3)]. However, at pH 6.7 adduct **4** is predominantly formed (*ca.* 80%). The increased reaction at higher pH presumably arises from partial deprotonation of the imidazole sidechain which enhances its nucleophilicity. Attachment of cation **1a** to *N*-acetylhistidine in eqn. (3) is accompanied by a characteristic downfield shift of 0.4 ppm for the H-2 signal of the histidine imidazole group. We have previously noted[5] similar spectroscopic changes in the related addition of **1a** to free imidazole. Although adduct **4** did not precipitate from solution, its formation was further supported by the appearance of intense bands at ν_{CO}/cm^{-1} 2056 and 1982 in D_2O characteristic of neutral (diene) iron tricarbonyl adducts, and the large drop in pH to *ca.* 5 during reaction.



(3)

4

In situ spectroscopic studies indicate less favourable adduct formation between cation **1a** and *N*-acetylglutamic acid (10% completion in D_2O at pH 6.7). After 15 min a precipitate forms, the IR spectrum of which [ν_{CO}/cm^{-1} 2045, 1973 (Nujol)] and ^1H NMR in $(CD_3)_2SO$ (downfield shift of 0.7 ppm for γ-CH_2 protons) are consistent with a neutral adduct in which the *N*-acetylglutamate has attached to **1a** *via* the δ-carboxyl group. In contrast, no reaction is observed between **1a** and the amino acids *N*-acetylglycine, *N*-acetyllysine, *N*-acetylserine, *N*-acetylglutamine, *N*-acetylasparagine and *N*-acetylarginine at pH 6.7. The failure to observe a reaction between **1a** and α- and ε-NH_2 of amino acids is presumably because they are protonated at pH 6.7, since facile addition of **1** to the α-NH_2 of a range of amino acid esters has been reported by us in organic solvents.[3,6]

The results suggest that the affinity of cation **1** for amino acid residues in peptides and proteins should vary in the order: cys (SH) > his (-N=) > glu (CO_2^-) ≫ gly (α-NH_2), lys (ε-NH_2), ser (γ-OH), asp (CONH). This prediction has been confirmed from 2D ^1H NMR spectroscopic studies of the reactions of a series of peptides and proteins with **1a** in D_2O (Table 1).

With the biologically important tripeptide glutathione, γ-glu-cys-gly **5**, an adduct precipitates upon addition of **1a** at pH 3.1 (the natural pH of glutathione). Its ^1H, COSY and ^{13}C NMR spectra in $(CD_3)_2SO$ confirm attachment of **1a** at the cysteine sulfur. For example, the β-CH_2 of the cys residue is shifted downfield by 0.6 ppm (^1H) and 6.3 ppm (^{13}C) compared with free glutathione. This adduct has been further characterised by elemental analysis, IR (see Table 1) and FAB negative ion mass spectrometry ($[M - H]^-$ at *m/z* 525).

Reaction of **1a** with the tripeptides, *p*-glu-his-gly-NH_2 **6** and *p*-glu-his-pro-NH_2 **7**, is quantitative at pH 6.8–7.0, although no precipitate forms. Binding of **1a** to the histidine residue is clearly shown in each case by the large downfield shift (0.4–0.6 ppm) for the H-2 signal of the histidine imidazole group. Interestingly, highly selective and almost quantitative addition of cation **1a** to the sole histidine residue (his-15) of lysozyme **8**, an enzyme containing 129 amino acids, has also been established by 2D ^1H NMR studies using TOCSY (total

Table 1 Selective labelling of peptides with **1a**[a]

Peptide	pH	Residue targetted by **1a**	ν_{CO} of adduct/ cm^{-1}[a]
Glutathione (γ-glu-cys-gly) **5**	3.1	Cys	2050, 1966[b]
p-Glu-his-gly-NH_2 **6**	6.8–7.0	His	
p-Glu-his-pro-NH_2 **7**	6.8–7.0	His	2050, 1980[c]
Lysozyme[d] **8**	6.8	His-15	2050, 1978[c]
p-Glu-his-trp-ser-tyr-gly-leu-arg-pro-gly-cys **9**[e]	6.8	Cys	

[a] Cation **1a** itself has ν_{CO} at 2118 and 2059 cm^{-1} in D_2O. [b] In Nujol. [c] In D_2O (using a circle-cell). [d] 129 Amino acid protein, containing one histidine residue (his-15). [e] Cys-11 derivative of luteinizing hormone releasing hormone.

correlation spectroscopy) in D_2O (Table 1). As expected, a large downfield shift (0.6 ppm) occurs for the H-2 signal of the imidazole group of his-15 upon treatment with **1a**. His-15 is on the surface of the molecule at the end of a helix. A TOCSY spectrum indicates that the conformation of lysozyme is unaltered by addition of **1a**, apart from some small changes in the vicinity of his-15.

The cys-11 derivative **9** of luteinizing hormone releasing hormone, which contains one cysteine and one histidine residue at different ends of the polypeptide, provides a useful substrate for determining the relative affinity of **1a** for these two amino acids. TOCSY and ROESY spectra of the reaction mixture confirms that the cysteine residue is targetted in preference to histidine.

Similar, but less facile, reactions have been observed between amino acids and peptides and the related cation $[(2\text{-}MeOC_6H_6)Fe(CO)_3]^+$ **1b**. The lower electrophilicity of **1b** compared with **1a** has previously been noted in kinetic studies[3,4] of such cations, arising from the electron-donating behaviour of the 2-methoxy substituent on the dienyl ring. Higher reactivity of type **1** dienyl cations towards peptides could presumably be acheived by the use of electron-withdrawing dienyl ring substituents.

The above studies suggest that dienyl cations of type **1** may provide very useful new reagents for the rapid and selective labelling/modification of proteins. Studies are in progress with a variety of proteins to establish the ability of cation **1** to distinguish between similar amino acids in different environments.

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