Electronic Supplementary Information for Dalton Transactions

A technetium intermediate specifically promotes deprotection of trifluoroacetyl HYNIC during radiolabelling under mild conditions

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Electronic Supplementary Information

1. Oligomerisation of HYNIC active ester – experimental and LCMS data

(a) Experimental: To a solution of HYNIC in DMSO (15 mg in 1 ml, 98 μmol) was added dicyclohexylcarbodiimide (DCC,1 eq.). After stirring for 5 min, N-hydroxysuccinimide (NHS, 1 eq.) was added. The resulting solution was left stirring at room temperature for 3 hours. A 20 μl aliquot of the reaction mixture was sampled and diluted with 380 μl of a mixture of DMSO/water (1:1; v/v). 100 μl of the resulting solution was injected for analysis by RP LC-MS using the following HPLC Method: mobile phase: linear gradient of increasing acetonitrile (ACN) in 0.05% aqueous TFA: 0-10 min 5% ACN, 5-40 min 10%-90% ACN, 40-45 min 90%-100% ACN, 40-45 min 100%-5% ACN; flow rate: 0.2 ml/min; detection: UV absorbance at 214 nm and 254 nm).

(b) Selected LCMS data up to m/z = 1000 showing formation and oligomerisation of **4**. Other peaks were observed corresponding to HYNIC oligomers incorporating dicyclohexylcarbodiimide adducts and N-hydroxysuccinimide ring opening reactions.

M+ ion m/z	Calc $(M+H)^+$	Solution species	Elution time/min	
251.1	251	4	14-19	
386.1	386	13, n = 1	15	
521.1	521	13, n = 2	15	
656.0	656	13, n = 3	15	
791.1	791	13, n = 4	15	
926.1	926	13, n = 5	15	
694.7	694	14, n = 4	16-17	
829.7	829	14, n = 5	16-17	

2. ESMS of products of labelling of 7 and 9 with Tc/tricine

In a screw top 2.5 ml polypropylene Corning vial, 3 µg of HYNIC compound in water was incubated with 0.5 ml of a solution of *tris*(hydroxymethyl)methylglycine (tricine; 100 mg/ml in water), 0.5 ml of $^{99m}TcO_4^-$ solution (>200 MBq), and 10 µl of stannous chloride dihydrate solution (3 mg/ml in ethanol) for 30 min at 97°C or 15 min at 20°C. To meet MS requirements, the labeling experiment was scaled up as follows: In a screw top 2.5 ml polypropylene corning vial, 14 µg of HYNIC compound in water was incubated with 100 µl of a solution of tricine (100mg/350 µl in water), 50 µl $^{99}TcO_4^-$ solution (1 x 10^{-8} moles), 5 µl $^{99m}TcO_4^-$ solution (3 MBq), and 5 µl stannous chloride dihydrate solution (6 mg/ml in ethanol) for 30 min at 97°C or 15 min at 20°C.

LCMS methods as above.

HYNIC derivative	***m/z* (intensity) and elution times**				
	Method A (ES-)	**Method B (ES+)**		**Method C (ES+)**	
	1 tricine Tc-complex	**1 tricine Tc-complex**	**2 tricines Tc-complex**	**1 tricine Tc-complex**	**2 tricines Tc-complex**
7	-	776.0 (100%) 30.4, 30.7 min	955.0 (60%) 29.3, 29.6 min	776.0 (10%) 30.4, 30.7 min	955.0 (100%) 29.3, 29.6 min
9	-	776.0 (100%) 30.4, 30.7 min	955.0 (60%) 29.3, 29.6 min	776.0 (10%) 30.4, 30.7 min	955.0 (100%) 29.3, 29.6 min

Similar results were obtained at 97°C and at room temperature.

Method A: Peptide analytical mode, negative mode ionization with tube lens offset (skimmer) and capillary voltage set at -50 V and -16 V respectively; *Method B:* Peptide analytical mode, positive mode ionization with tube lens offset (skimmer) and capillary voltage set at +30 V and +19 V respectively; *Method C:* organic analytical profile mode, positive mode ionization with tube lens offset (skimmer) and capillary voltage set at 0 V and +15 V respectively;

3. Reaction of Fmoc-(trifluoroacetylHYNIC)-lysine 9 with stannous chloride, pertechnetate, and tricine; experimental methods and LCMS data

(a) Experimental:

Sodium pertechnetate challenge: To an aqueous solution of sodium pertechnetate [200 µl of a 0.03 M solution, pH 5.0] was added a 0.05 M solution of Fmoc-(trifluoroacetylHYNIC)-lysine **9** in DMSO (50 µl, 2.5 µmol) and the resulting solution was stirred at 20 ˚C for 2 h. A 40 µl aliquot of the reaction mixture was sampled and diluted with 360 µl of a mixture of DMSO/water (1:1; v/v). 25 µl of the resulting solution was injected for analysis by RP LC-MS, using Method 2.

Tricine challenge: To an aqueous solution of tricine [250 µl of a 0.19 M solution, pH 4.5] was added a 0.05 M solution of Fmoc-(trifluoroacetylHYNIC)-lysine **9** in DMSO (20 µl, 1 µmol) and the resulting solution was stirred at 20 ˚C for 2 h. A 20 µl aliquot of the reaction mixture was sampled and diluted with 380 µl of a mixture of DMSO/water (1:1; v/v). 25 µl of the resulting solution was injected for analysis by RP LC-MS, using Method 1.

Stannous chloride challenge 1: To an aqueous solution of stannous chloride [30 µl of a 0.1 M solution (pH 2), added to 100 µl of phosphate buffer (pH 8), overall pH 7.0] was added a 0.05 M solution of Fmoc-(trifluoroacetylHYNIC)-lysine **9** in DMSO (20 µl, 2.5 µmol) and the resulting solution was stirred at 20 ˚C for 2 h. A 20 µl aliquot of the reaction mixture was sampled and diluted with 480 µl of a mixture of DMSO/water (1:1; v/v). 50 µl of the resulting solution was injected for analysis by RP LC-MS, using Method 1.

Stannous chloride challenge 2: To an aqueous solution of stannous chloride [30 µl of a 0.1 M solution (pH 2), added to 100 µl of water] was added 30 µl of a 1.6 M solution of tricine. The resulting solution was titrated with drops of an aqueous solution of 0.1 M NaOH until pH 6. Then a 0.05 M solution of Fmoc-(trifluoroacetylHYNIC)-lysine **9** in DMSO (20 µl, 2.5 µmol) was added and the resulting solution was stirred at 20 ˚C for 2 h. A 20 µl aliquot of the reaction mixture was sampled and diluted with 480 µl of a mixture of DMSO/water (1:1; v/v). 50 µl of the resulting solution was injected for analysis by RP LC-MS, using Method 1. This procedure was then repeated using stannic chloride instead of stannous chloride.

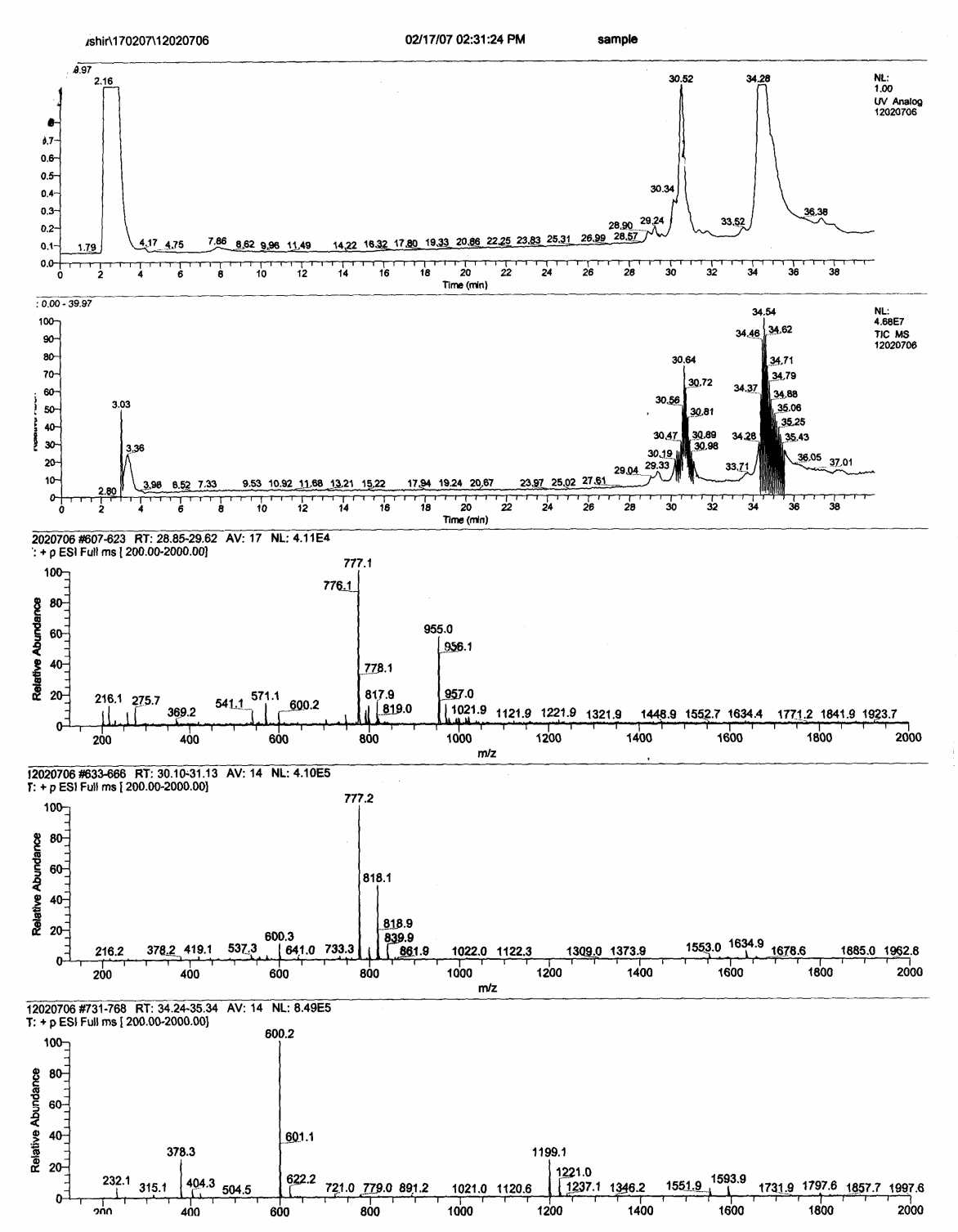
"Labelling" of Fmoc-(trifluoroacetylHYNIC)-lysine **9** with ^{99}Tc

To a 3 mM aqueous solution of sodium pertechnetate (84 µl, 0.25 µmol) was added 15 µl of stannous chloride dihydrate solution (6 mg/ml in ethanol) followed by 40 µl of a solution of tricine (100mg/350 µl in water). The solution obtained was incubated at 20 ˚C for 15 min then titrated with drops of a 0.1 M solution of NaOH till pH 5.5 before addition of a 0.05 M solution of Fmoc-(trifluoroacetylHYNIC)-lysine **8** in DMSO (50 µl, 2.5 µmol). The resulting solution was stirred at 20 ˚C for 15 min. A 60 µl aliquot of the reaction mixture was sampled and diluted with 340 µl of a mixture of DMSO/water (1:1; v/v). 100 µl of the resulting solution was injected for analysis by RP LC-MS, using Method 1. This procedure was then repeated with an increased concentration of sodium pertechnetate (50 µl of a 30 mM aqueous solution, 1.5 µmol).

LCMS methods: Electrospray ionization mass spectra (ES-MS) were obtained with a Finnigan Mat LCQ ion trap mass spectrometer coupled to a Hewlett-Packard 1100 HPLC system for LCMS. Samples were analyzed by RP HPLC MS (ES+) using either a Phenomenex Polymer PRP-1 column (150 x 2 mm, 5 µm), using one of two gradient systems: Method 1: mobile phase: linear gradient of increasing acetonitrile (ACN) in 0.05% aqueous TFA: 0-10 min 5% ACN, 10-35 min 5%-90% ACN, 35-40 min 90%-100% ACN, 40-45 min 100%-5% ACN; flow rate: 0.2 ml/min; detection: UV absorbance at 214 nm and 254 nm; Method 2: mobile phase: linear gradient of increasing acetonitrile (ACN) in 0.05% aqueous TFA: 0-5 min 5% ACN, 5-30 min 5%-90% ACN, 30-40 min 90%-100% ACN, 40-45 min 100%-5% ACN; flow rate: 0.2 ml/min; detection: UV absorbance at 214 nm and 254 nm. ESMS analysis was performed using the following mass spectrometry methods: organic analytical profile, positive mode ionization with tube lens offset (skimmer) and capillary voltage set at 0 V and +15 V respectively

(b) Results

Number	Reagents	pH	Reaction time/min	% of 9 remaining	Product
1	Aqueous sodium pertechnetate	5.0	120	100	-
2	Aqueous tricine	4.5	120	100	-
3	Stannous chloride in phosphate buffer	7.0	120	100	-
4	Stannous chloride and tricine in water	6.0	120	100	-
5	Stannic chloride and tricine in water	6.0	120	100	-
6	0.1 eq. sodium pertechtenate, stannous chlorice and tricine in water (see LCMS report below)	5.5	15	~90	Tc-complexes **15, 16**
7	0.6 eq. sodium pertechtenate, stannous chlorice and tricine in water	5.5	15	~45	Tc-complexes **15, 16**

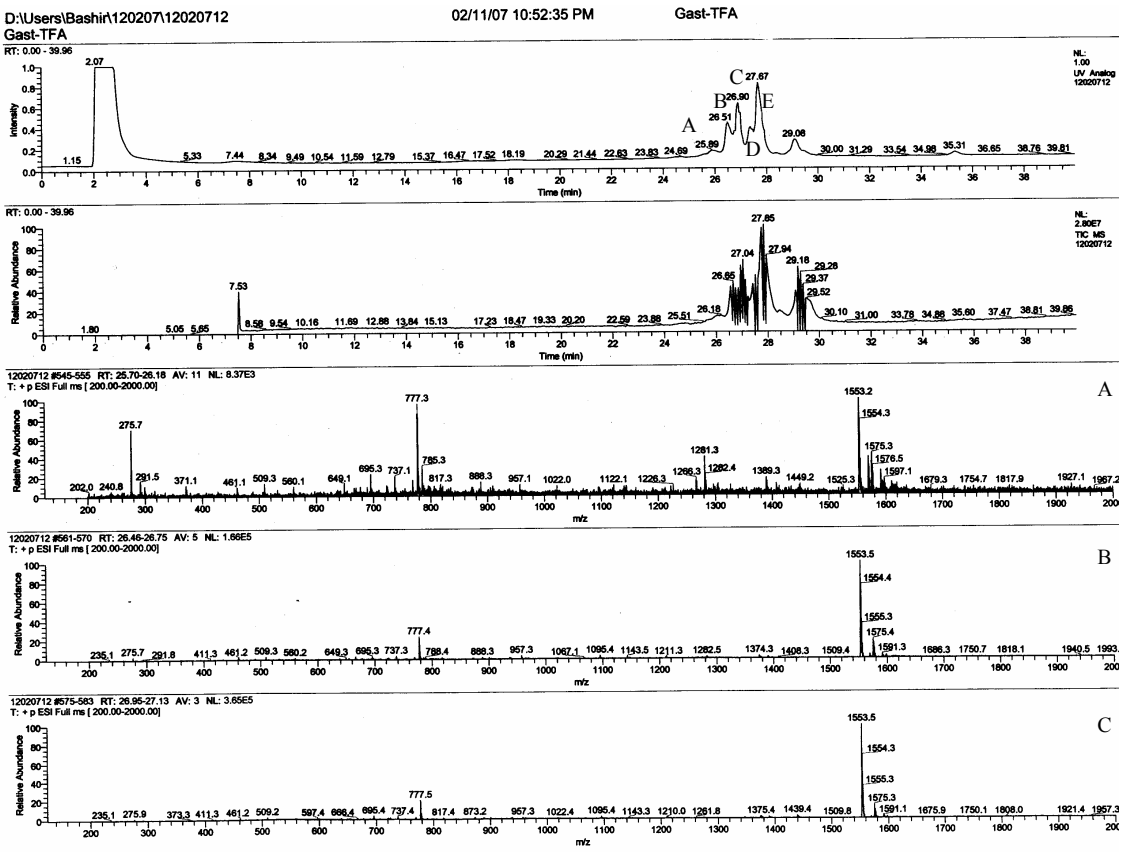


LCMS report for labelling of TFA-protected Fmoc-lysine-HYNIC (**9**) with 0.1 mole equivalents of technetium to give Tc-complexes **15** and **16**.

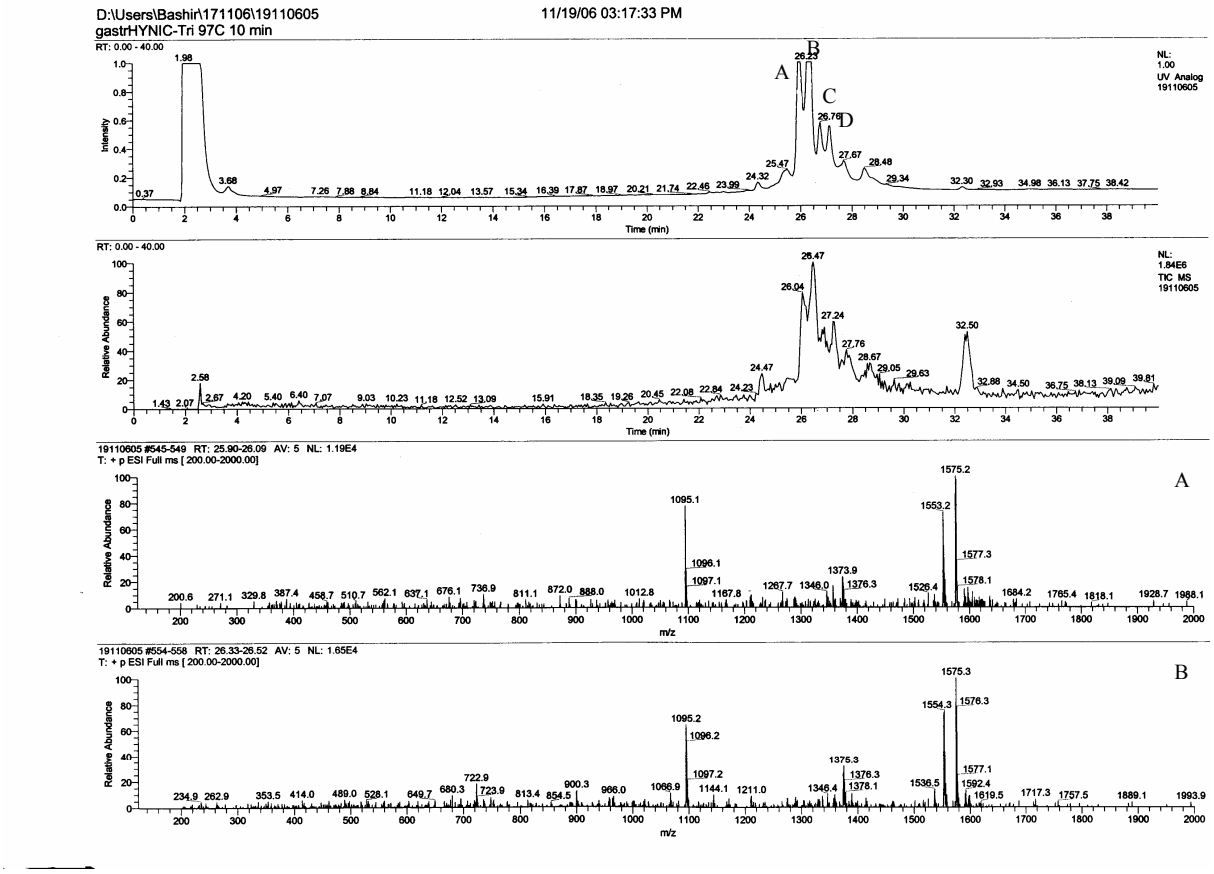
4. LCMS of peptides Tc-99 labelling of 10 and 12 with tricine as co-ligand

Method: In a screw top 2.5 ml polypropylene Corning vial, 10 µl of a solution of a 1 mM solution of nanogastrin-HYNIC peptide **(10** or **12)** in water (1.5×10^{-8} moles) was incubated with 0.3 ml of a 0.5 M solution of *tris*(hydroxymethyl)methylglycine (tricine; 1.5×10^{-4} moles), 0.1 ml of a 2 mM solution of $Na^{99}TcO_4$ solution (2×10^{-8} moles), and 10 µl of stannous chloride dihydrate solution (6 mg/ml in ethanol) for 15 min at 20°C or 30 min at 97°C in a water bath. 100 µl of the resulting solution was injected for analysis by RP LC-MS. Electrospray ionization mass spectra (ES-MS) were obtained with a Finnigan Mat LCQ ion trap mass spectrometer coupled to a Hewlett-Packard 1100 HPLC system for LCMS. A Phenomenex Polymer PRP-1 column (150 x 2 mm, 5 µm) was used with the following HPLC method: mobile phase: linear gradient of increasing solvent B (ACN)/water; 70/30% v/v) in 0.05% aqueous TFA: 0-5 min 5% solvent B, 5-35 min 5%-90% solvent B, 35-40 min 90%-100% solvent B, 40-45 min 100%-5% solvent B; flow rate: 0.2 ml/min; detection: UV absorbance at 214 nm and 254 nm); ESMS analysis of technetium complexes was performed using the following mass spectrometry method: organic analytical profile mode, positive mode ionization with tube lens offset (skimmer) and capillary voltage set at 0 V and +15 V respectively.

Peptide labelled	Main m/z (obs)	Elution time (min)	assignment
10	1553.2	25.5 – 28.0 (all peaks give similar ESMS)	[10+Tc+tricine-4H]+
	1575.2		[10+Tc+Tricine-5H+Na]+
12	777.3	25.5 - 28.0 (all peaks give similar ESMS)	[10+Tc+tricine-3H]2+
	1553.2		[10+Tc+tricine-4H]+
	1575.3		[10+Tc+Tricine-5H+Na]+



LCMS report for labelling of protected peptide **12** for 30 min at 97°C. Similar results were obtained after 15 min incubation at room temperature. Peaks A, B, C and E contain technetium. Peak E gave the same ESMS as B and C. Peak D is unlabelled peptide.



LCMS report for labelling of unprotected peptide **10** for 30 min at 97°C. Similar results were obtained on incubation at room temperature for 15 min. Peaks A and B contain technetium. Peaks C and D gave ESMS with peaks corresponding to unlabelled peptide **10.** Peak C gave M^+ - 15 as the base peak, and peak D gave M^+ – (2x15) as the base peak. These correspond respectively to loss of NH and 2 x NH from the unprotected HYNIC group during incubation. These peaks are not observed when **12** is labelled.

The peak at 1095.2 in A and B is attributed to a peptide fragment formed during labelling or during ionisation in the mass spectrometer. Such fragment peaks do not occur when protected peptide **12** is treated similarly (see p. 8)