A novel heterobifunctional linker for facile access to bio-conjugates

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I. Melting studies

The melting curves (absorbance *versus* temperature) were recorded at 260 nm on a UV–Visible spectrophotometer equipped with a temperature controller. The melting experiments were carried out by mixing the equimolar amount of two oligonucleotide strands in a solution of sodium phosphate buffer (10 mM; pH=7.0) containing EDTA (1 mM) and NaCl (100 mM). The ODN concentration was kept at 12 μM. The absorbance was recorded in the temperature range of 10–80 °C at a sweep rate of 1 °C min^{-1}. All experiments were done in triplicate. The respective melting temperatures obtained are collected below in Table 1.

Table 1. Melting temperature of the duplex formed by the unmodified ODN and conjugate with the complementary sequence[a].

Oligonucleotides	T_m ±0.5 °C
Unmodified 11-mer + complementary sequence	61.0
Conjugate **4** + complementary sequence	62.1

[a] The ODN sequence is 5'-CGCACACACGC-3' and the complementary sequence is 5'-GCGTGTGTGCG-3'.

II. Circular dichroism (CD) studies

The CD experiments were carried out by mixing the equimolar amount of two-oligonucleotide strands in a 10 mM sodium phosphate buffer (pH = 7.0) containing EDTA (1mM) and NaCl (100 mM). The ODN concentration was kept at 12 μM.

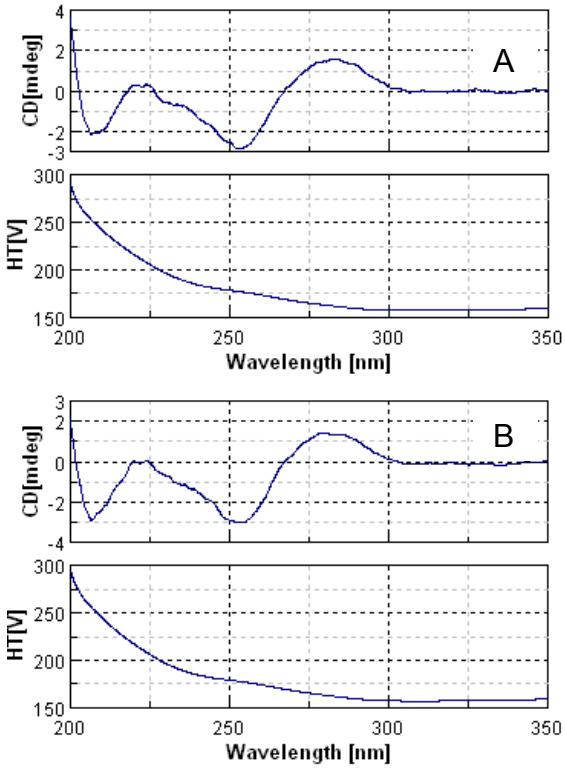

Fig 1. CD spectra for the duplexes formed by unmodified 11-meroligonucleotide (A) and peptide–oligonucleotide conjugate **4** (B) with the complementary sequence.

III The spectroscopic characterisation data for heterobifunctionnal linker 1

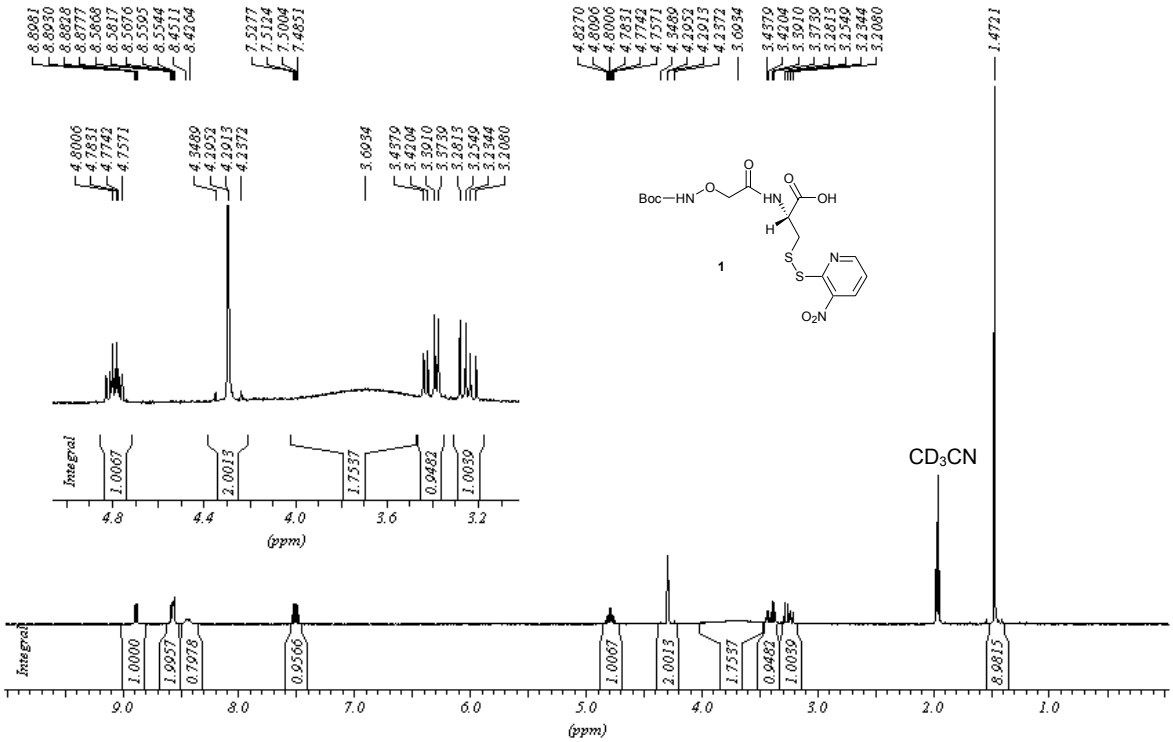

Fig 2. ^{1}H NMR (CD_3CN, 300 MHz) spectrum of heterobifunctionnal linker **1**.

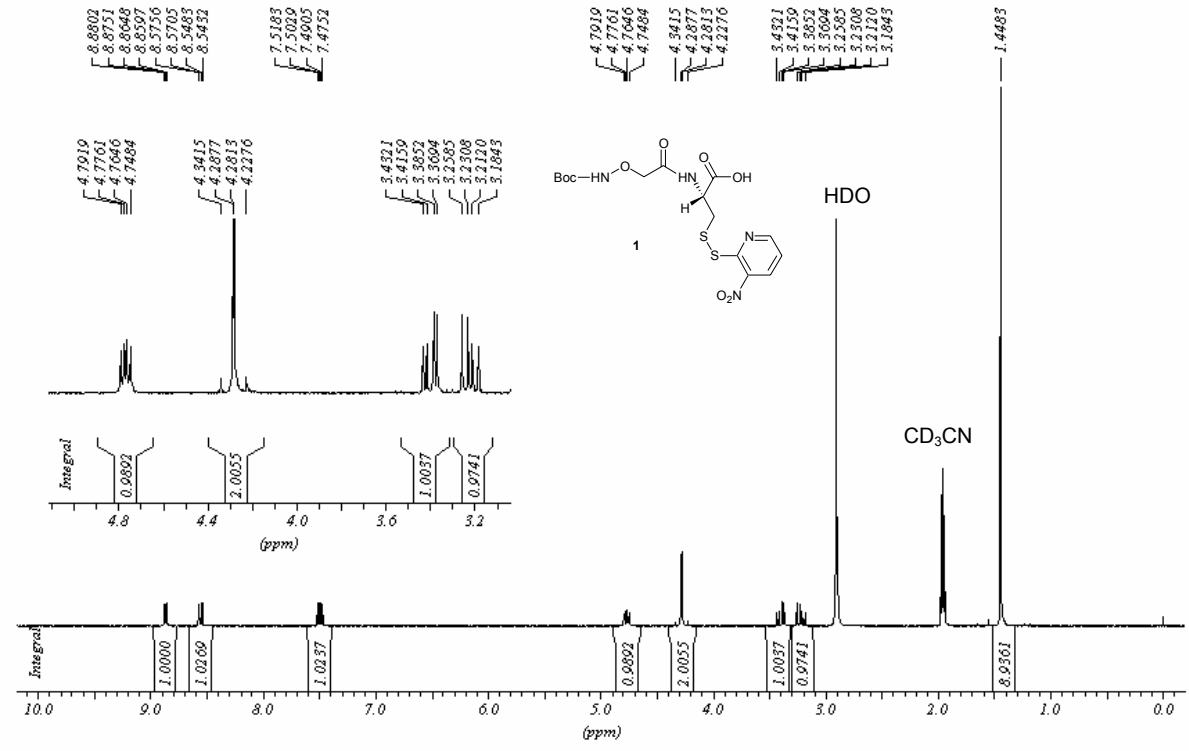

Fig 3. ^{1}H NMR (CD_3CN/D_2O 6:1, 300 MHz) spectrum of heterobifunctionnal linker **1**.

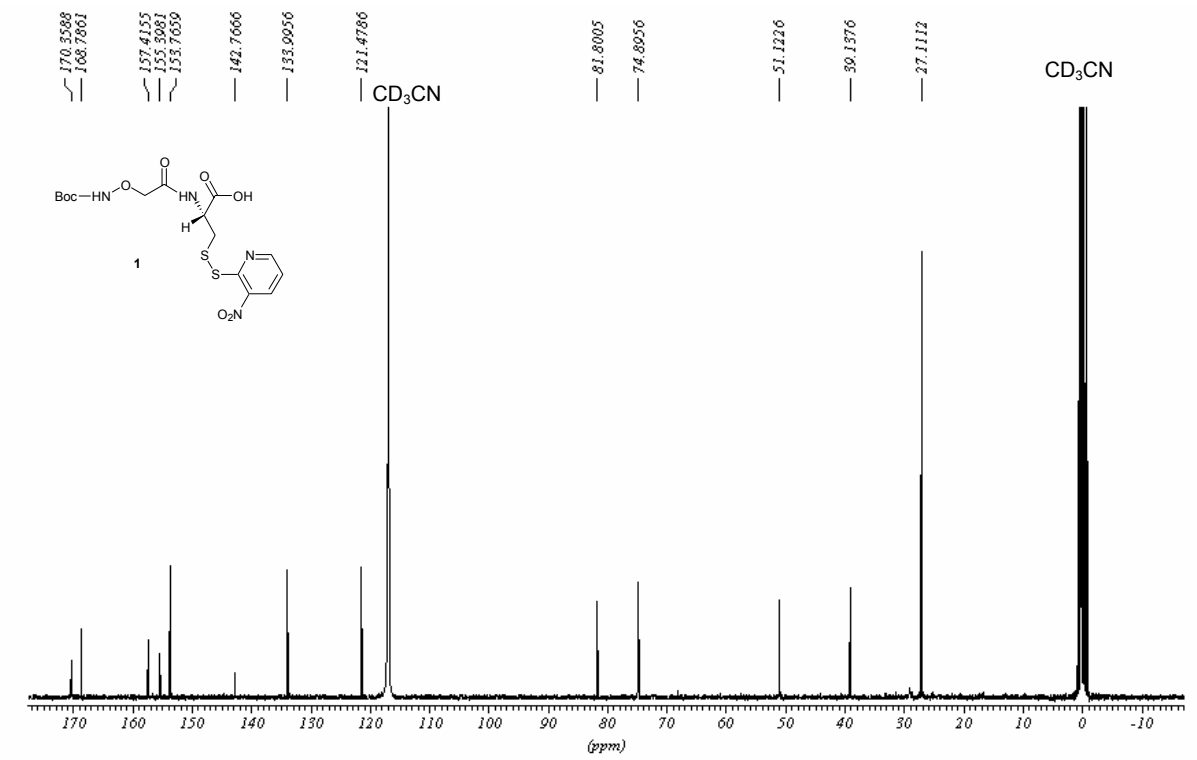

Fig 4. ^{13}C NMR (CD_3CN, 300 MHz) spectrum of heterobifunctionnal linker **1**.

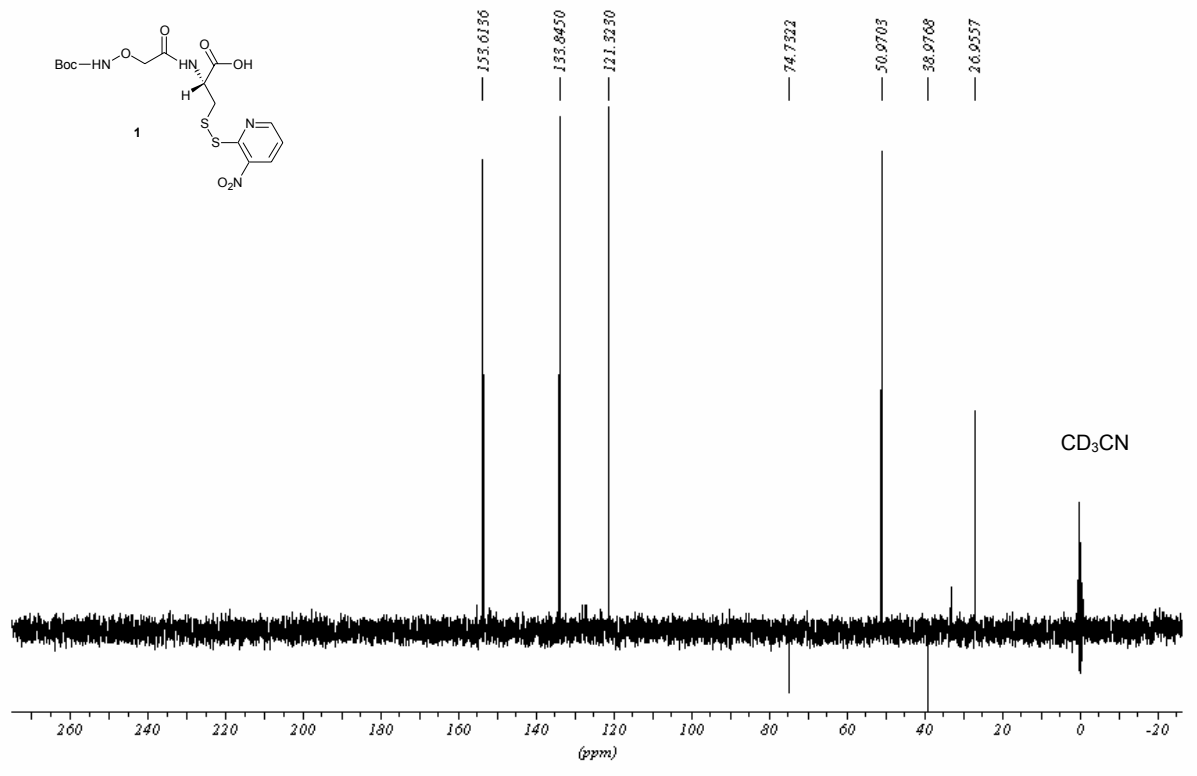

Fig 5. DEPT ^{13}C NMR (CD_3CN, 300 MHz, θ= 120 °) spectrum of heterobifunctionnal linker **1**.

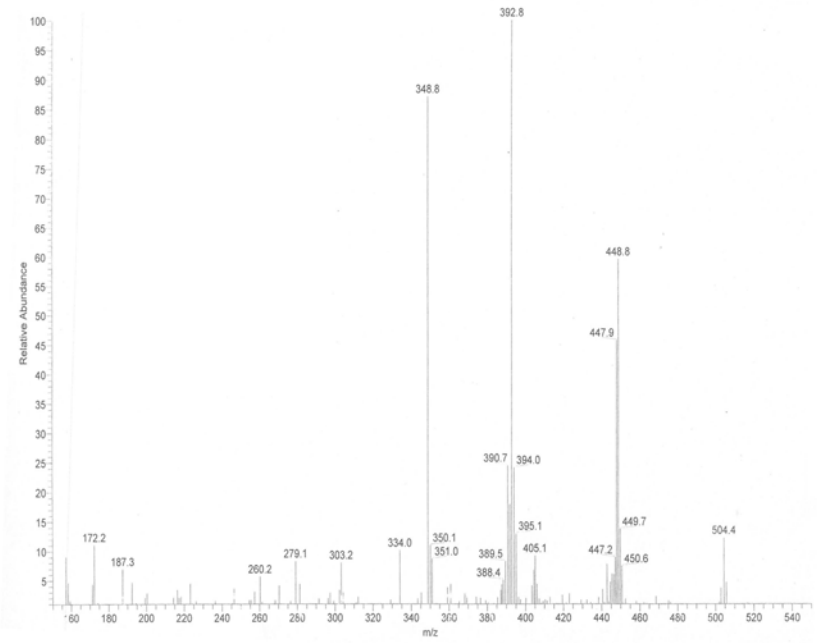

Fig 6. DCI mass spectra of heterobifunctionnal linker **1** (*m*/*z*: calcd 448.5).

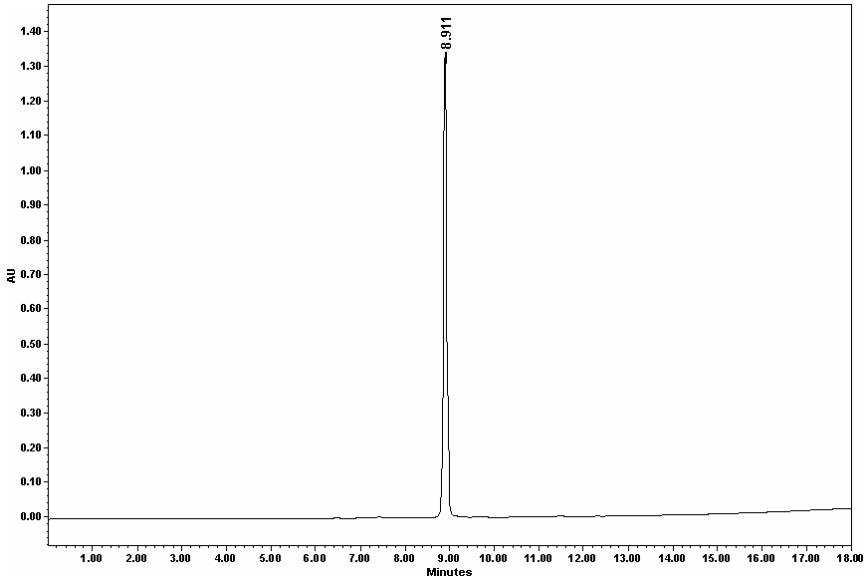

Fig 7. RP-HPLC profile (λ_{abs} = 250 nm) of heterobifunctional linker **1**.

IV. Characterisation data for peptide–oligonucleotide conjugate 4

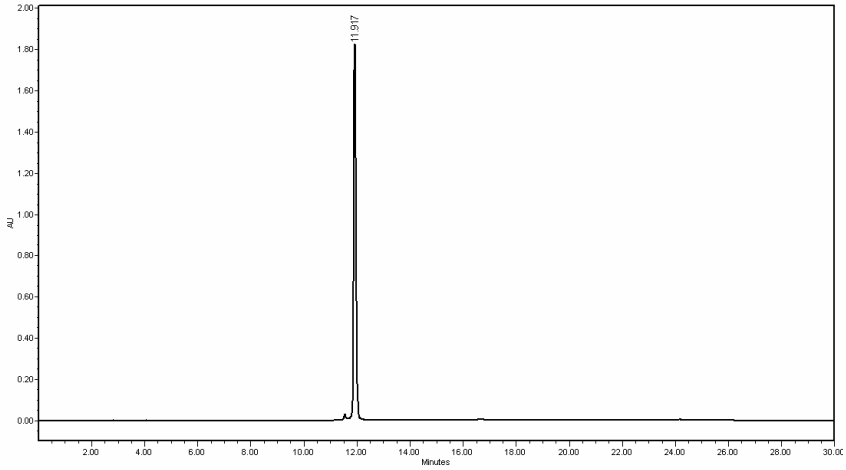

Fig 8. RP-HPLC profile (λ_{abs} = 260 nm) of purified peptide–oligonucleotide conjugate **4** synthesised from **7**.

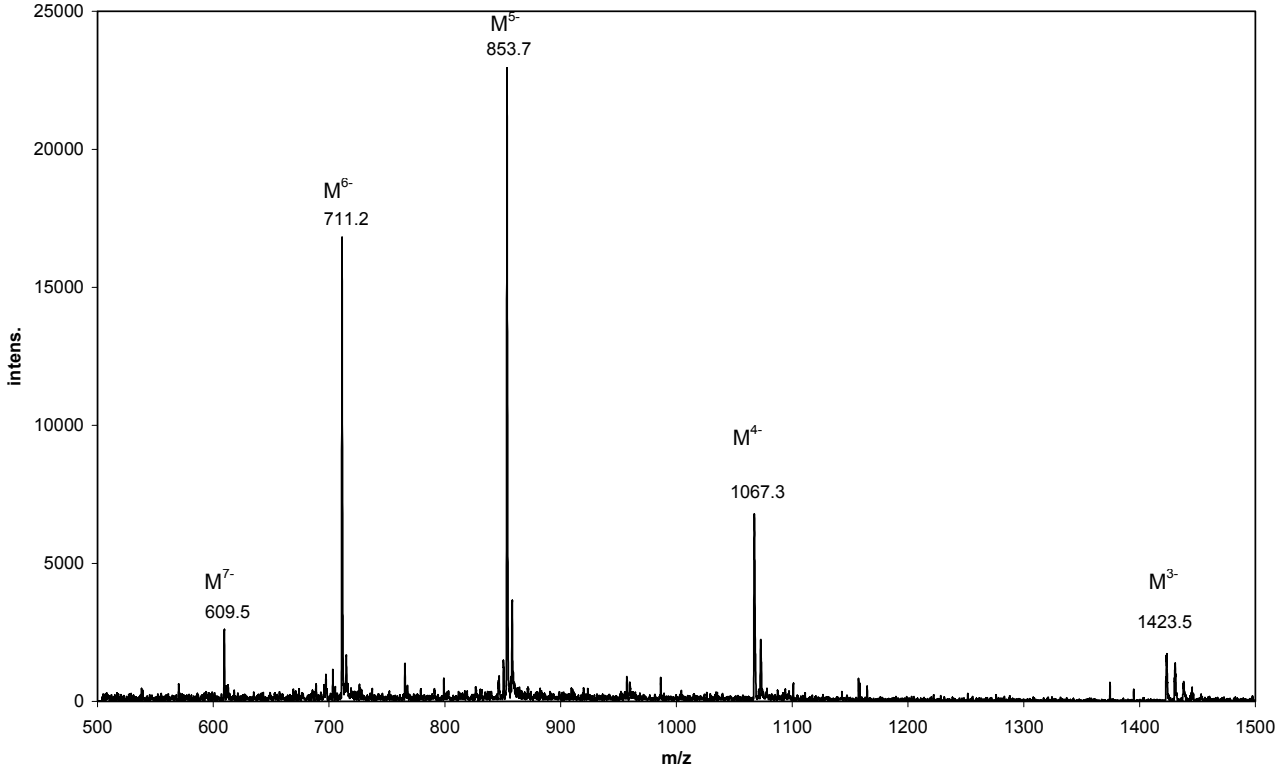

Fig 9. ESIMS (negative mode) of purified peptide–oligonucleotide conjugate **4** synthesised from **7** (*m*/*z*: calcd = 4274.2).

V. RP-HPLC profile of cysteine RGD peptide 5

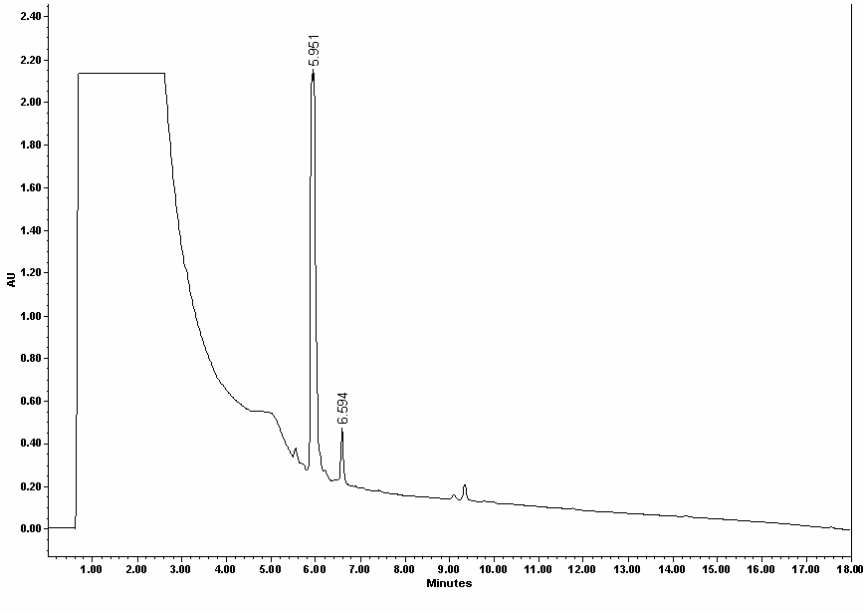

Fig 10. RP-HPLC profile (λ_{abs} = 260 nm) of purified RGD-cysteine peptide **5**.

VI. Spectroscopic characterisation data for RGD peptide–linker conjugate 6 (Boc Protected)

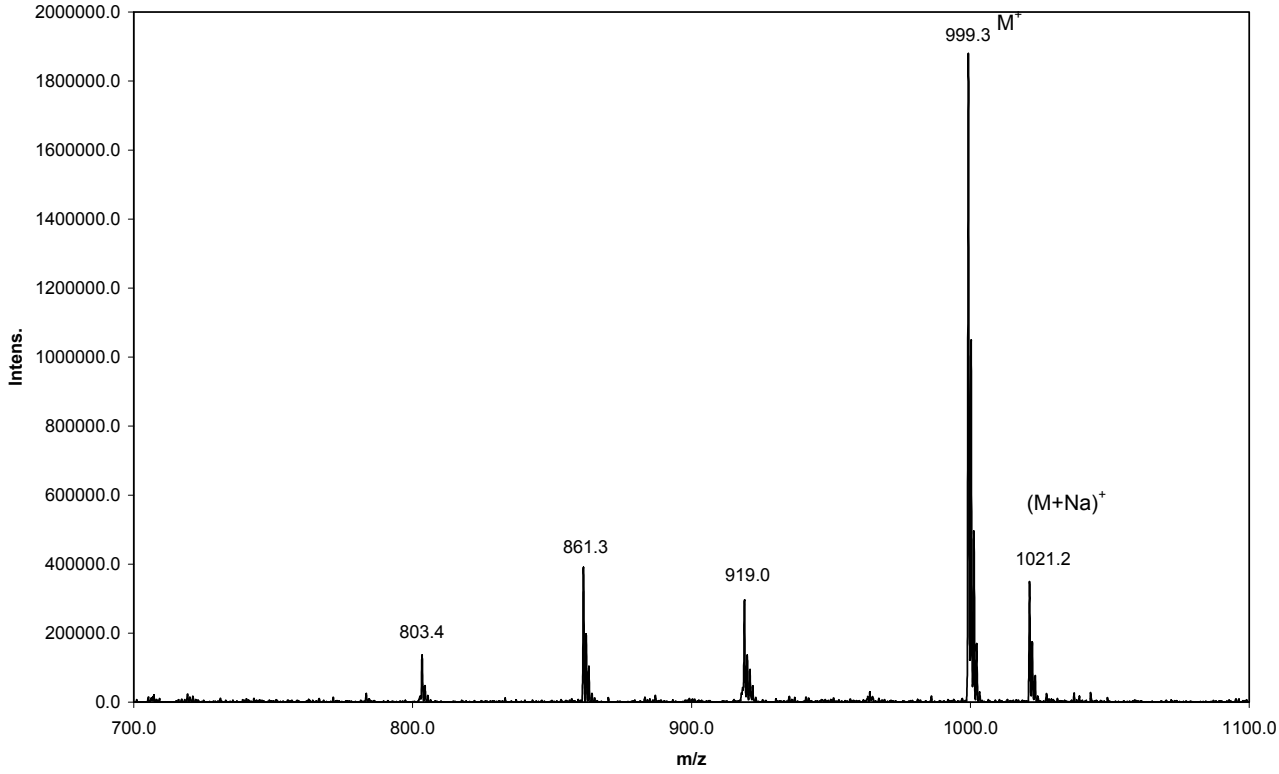

Fig 11. ESIMS (positive mode) of crude RGD peptide–linker conjugate **6** (*m*/*z*: calcd = 1000.1).

VII. Spectroscopic characterisation data for RGD peptide–linker conjugate 7 (Boc free)

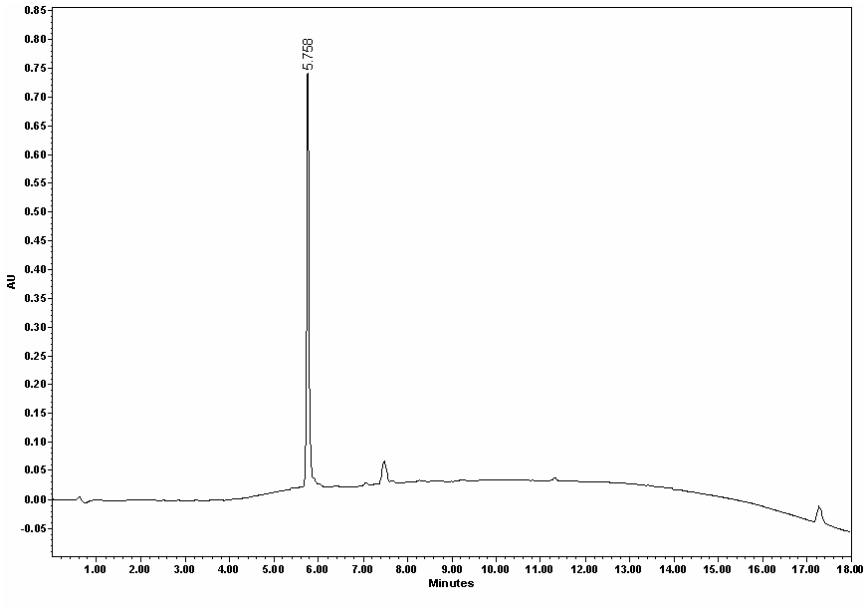

Fig 12. RP-HPLC profile (λ_{abs} = 700nm) of purified RGD peptide–linker conjugate **7**.

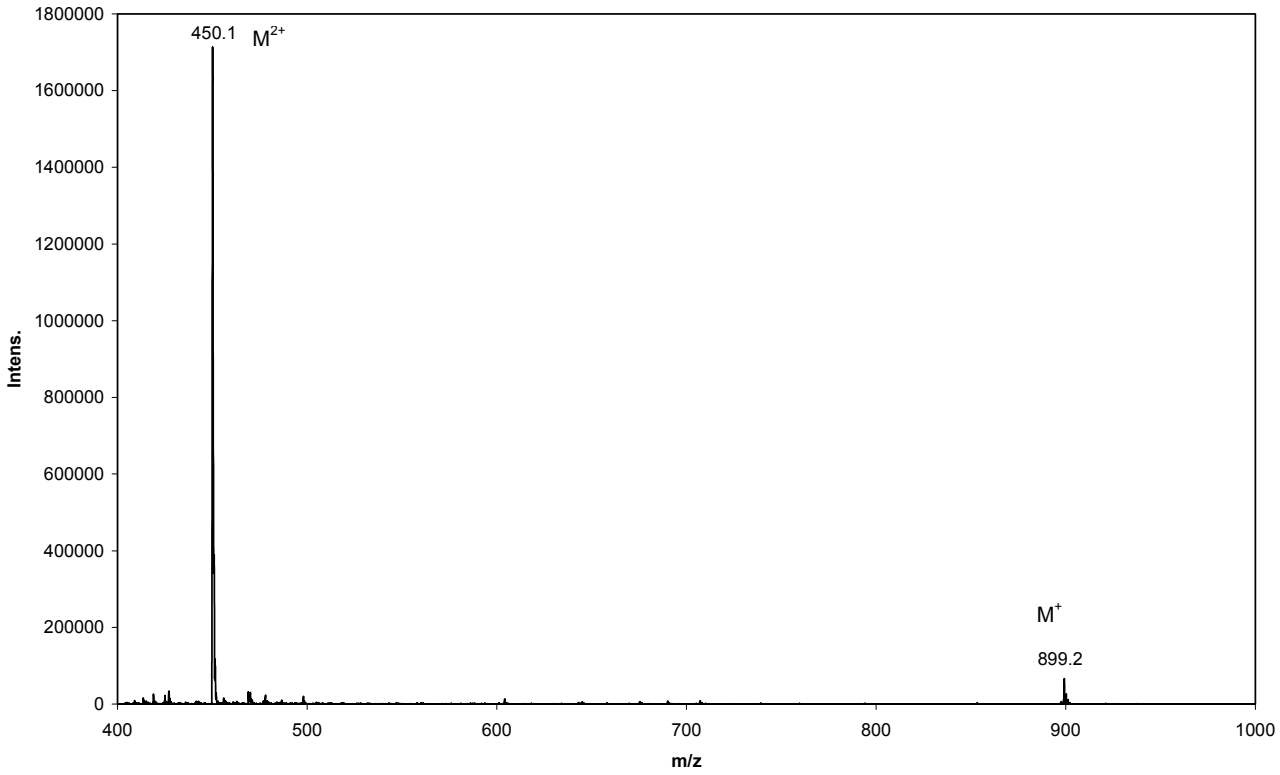

Fig 13. ESIMS (positive mode) of purified RGD peptide **7** (*m*/*z*: calcd = 899.0).

VIII. Spectroscopic characterisation data for oligonucleotide 3'-aldehyde 8

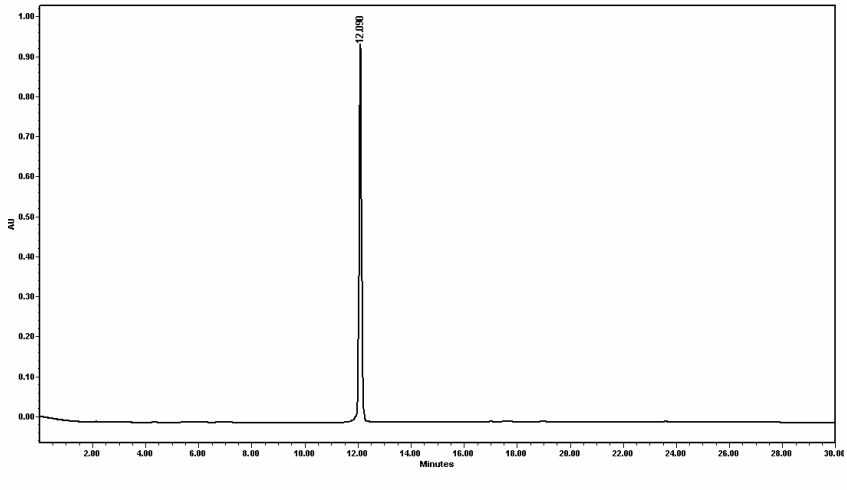

Fig 14. RP-HPLC (λ_{abs} = 260 nm) of purified oligonucleotide 3'-aldehyde **8**.

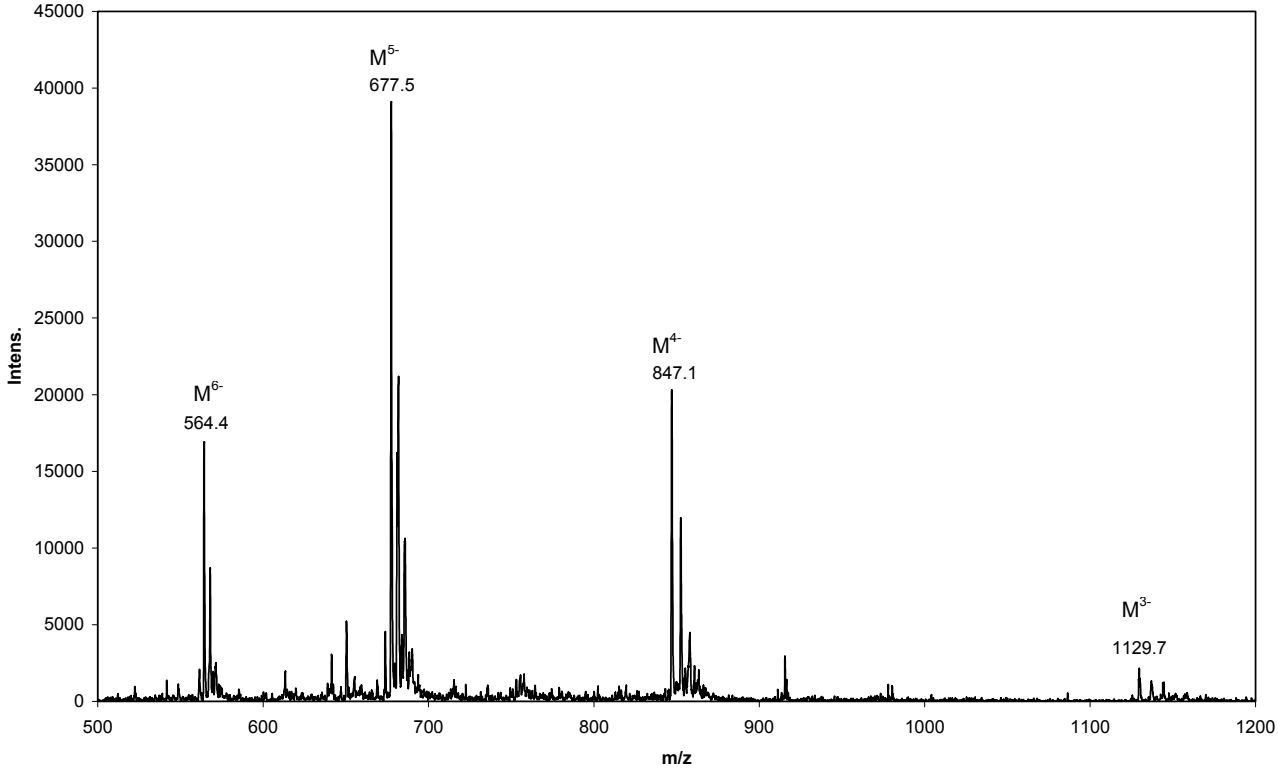

Fig 15. ESIMS (negative mode) of purified oligonucleotide 3'-aldehyde **8** (*m*/*z*: calcd = 3392.2).

IX. Spectroscopic characterisation data for free aminoxy linker 9

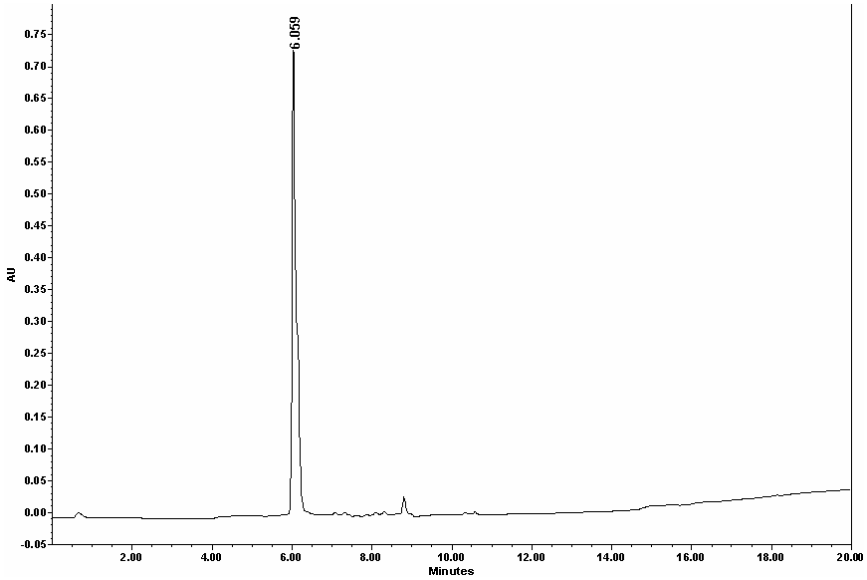

Fig 16. RP-HPLC profile (λ_{abs} = 250 nm) of crude free aminooxy linker **9**.

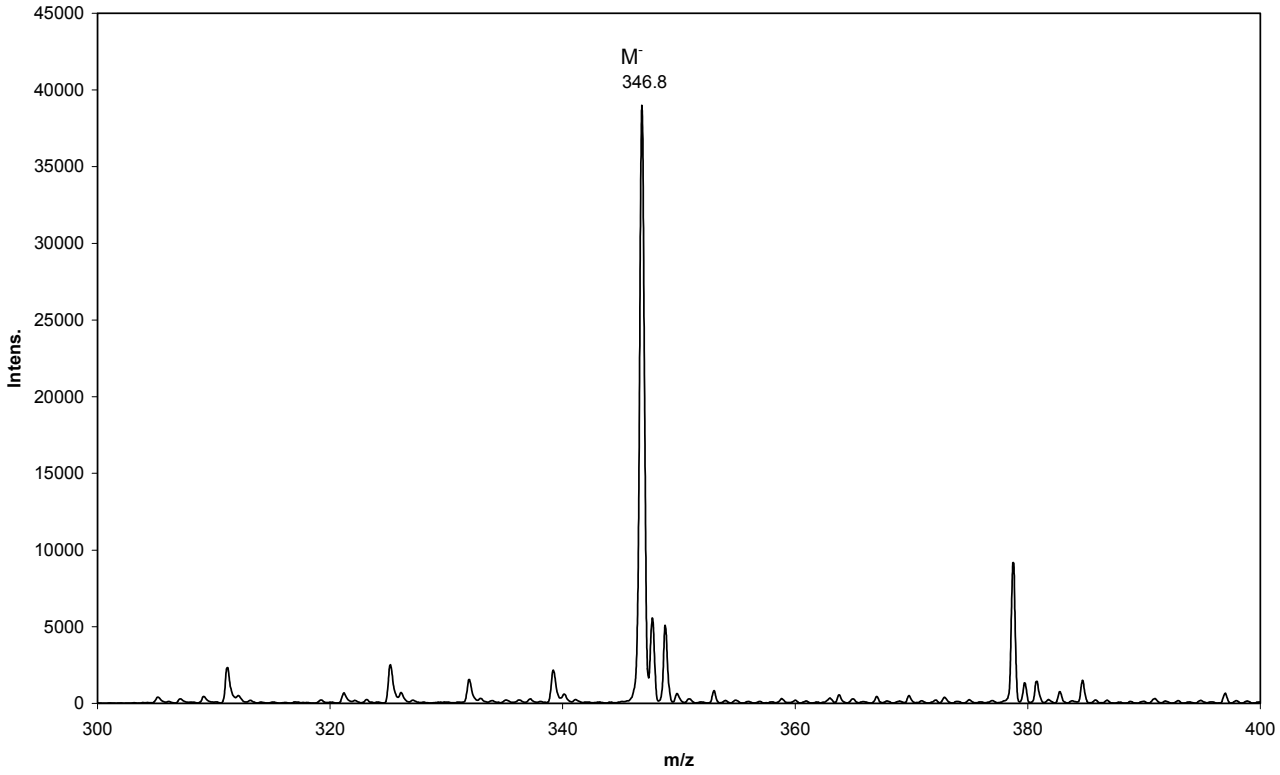

Fig 17. ESIMS (negative mode) of crude free aminooxy linker **9** (*m*/*z*: calcd = 347.4).

X. Spectroscopic characterisation data for oligonucleotide–linker conjugate 10

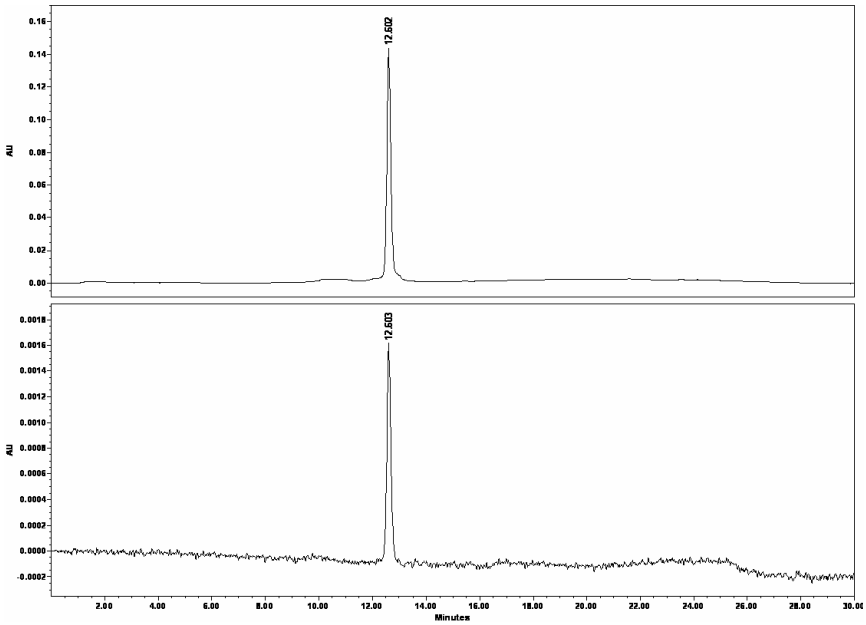

Fig 18. RP-HPLC profile (λ_{abs} = 260 and 400 nm) of purified oligonucleotide–linker conjugate **10**.

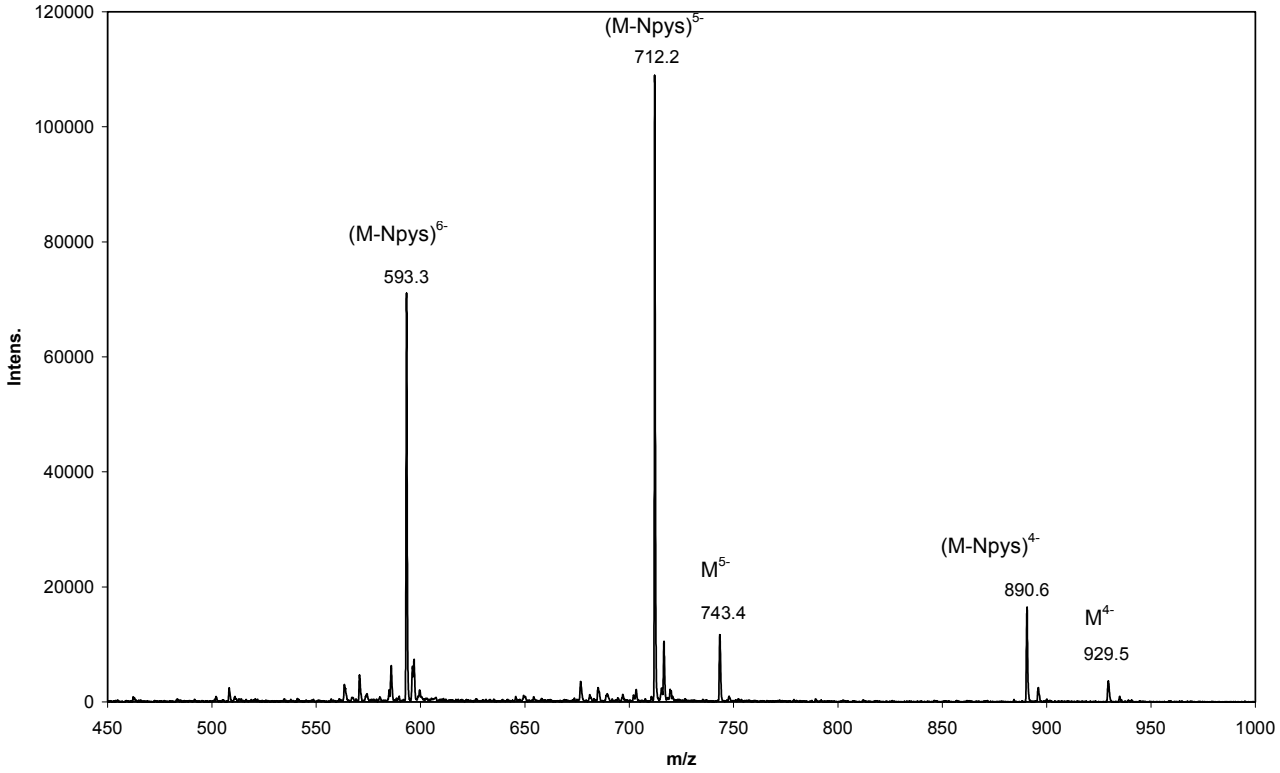

Fig 19. ESIMS (negative mode) of purified oligonucleotide–linker conjugate **10** (*m*/*z*: calcd = 3722.6).

XI. DTT treatment[1] of oligonucleotide–linker conjugate 10

Oligonucleotide–linker conjugate **10** (10 nmol, 35 µL) was suspended in a solution of DTT (10 mM) and the reaction was followed by RP-HPLC. Complete dispartition of the peak corresponding to the NPys protection was observed after a night's reaction. The chromatogram recorded after 15 min of reaction shows that peaks corresponding to Npys protected (RT = 13.1 min) and unprotected (RT = 11.9 min) oligonucleotide–linker conjugates are well resolved and hence must not have been collected together during the HPLC purification of the conjugate **10**. The NPys loss observed during the ESIMS analysis of the conjugate therefore must be due to the triethylamine solution added during the mass analysis.

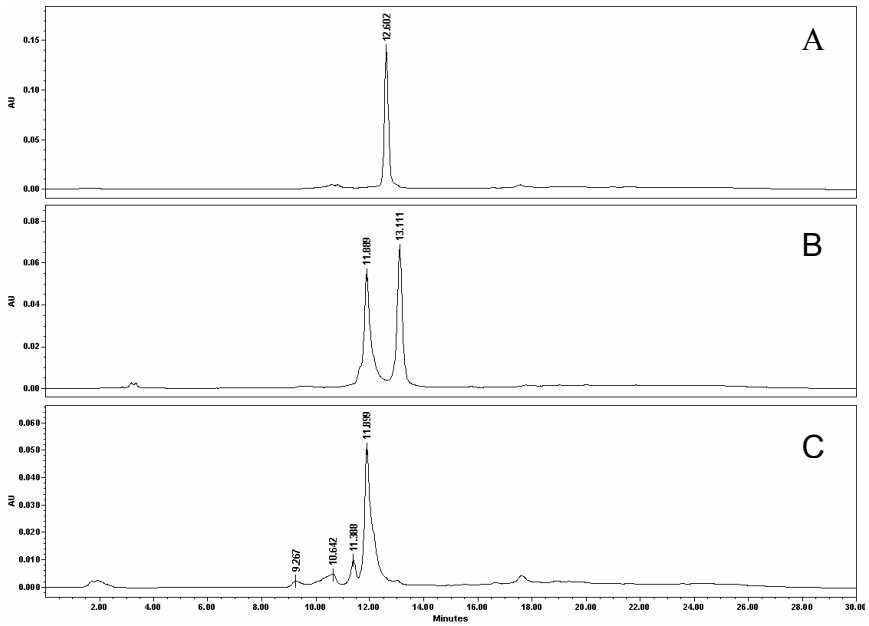

Fig 20. RP-HPLC profile (λ_{abs} = 260 nm) of purified oligonucleotide–linker conjugate **10** during the DTT treatment. (A) before treatment; (B) after 15 min of treatment and (C) overnight treatment.

XII. Notes and references

1 F. Maurel, F. Debart, F. Cavelier, A. R. Thierry, B. Lebleu and J.–J. Vasseur, *Bioorg. Med. Chem.,* 2005, **15**, 5084-5087.