# Supplementary Material (ESI) for Chemical Communications


# Trifluoromethyldiazirine: an Effective Photo-induced Cross-linking Probe for Exploring Amyloid Formation

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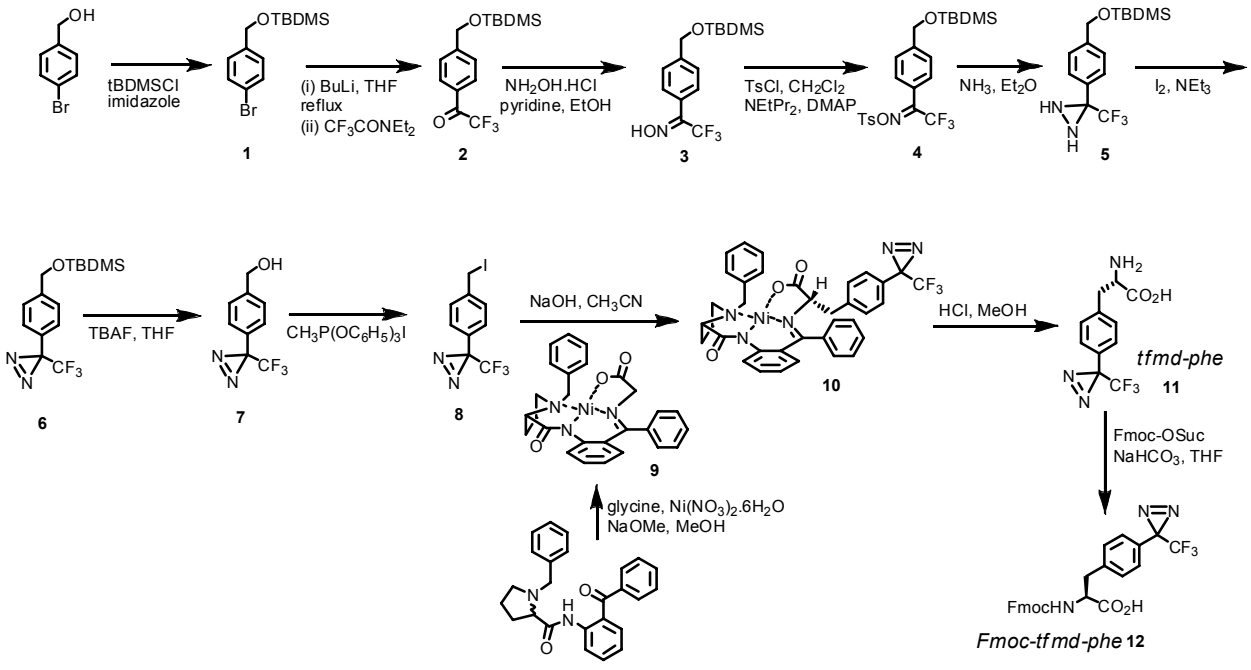
## Supporting Information

### Synthesis

All solvents and reagents were purchased from Aldrich or Alfa-Aesar unless otherwise stated and used without further purification. Peptide coupling reagents and protected amino acids were sourced from Novabiochem. Yields were not optimized. Purification by column chromatography was carried out using Merck Kieselgel 60 silica gel. Analytical thin layer chromatography (TLC) was conducted using Merck Kieslegel 0.25 mm silica gel pre-coated aluminium plates with fluorescent indicator active at $UV_{245}$. NMR spectra were acquired on Bruker AMD300 or Bruker DMX500 spectrometers (Bruker, Bremen, Germany); $^{1}H$ at 500 or 300 MHz and $^{13}C$ at 125, or 75 MHz, respectively. Chemical shifts are expressed as parts per million downfield of TMS as a standard, and coupling constants are expressed in Hz. The following abbreviations are used: s for singlet; d for doublet; t for triplet; q for quartet; m for multiplet; and br for broad. Anhydrous solvents were obtained in the following manner: THF by standing over 4 Å self-indicating molecular sieves followed by direct distillation; ether by direct distillation from sodium/benzophenone; chloroform by distillation from calcium sulphate; dichloromethane by direct distillation from calcium hydride; acetonitrile by standing overnight over 3 Å molecular sieves; dimethyl formamide by drying over magnesium sulphate and then standing over 4 Å molecular sieves; methanol by standing over 3 Å molecular sieves; ethanol by standing over 4 Å molecular sieves; and pyridine by standing overnight over potassium hydroxide followed by direct distillation and standing over 4 Å molecular sieves.

The preparation of (S)-3-[4-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenyl]alanine was accomplished using minor adaptations to the method of Fishwick and co-workers (Fishwick, C. W. G.; Sanderson, J. M.; Findlay, J. B. C. Tetrahedron Lett. **1994** 35, 4611-4614) in the following manner:




**Supplementary Scheme 1.** Synthesis of Fmoc-tfmd-phe (**12**)

***N,N*-diethyltrifluoroacetamide** (Robson, J. H.; Reinhart, J. *J. Am. Chem. Soc.* **1955** *77*, 498-499) To a stirred solution of diethylamine (24.77 mL, 17.412 g, 238.1 mmol) in anhydrous ether (100 mL) at -6 °C (cyclohexane/dry ice) under an atmosphere of nitrogen was added trifluoroacetic anhydride (25.00 g, 119.0 mmol) dropwise over 30 minutes. The reaction mixture was allowed to warm to room temperature and stirred for a period of two and a half hours. The reaction mixture was then washed with water until the washings were neutral, and then the organic layer was dried over magnesium sulphate, filtered and concentrated to leave a red oil. Distillation under reduced pressure yielded the title compound (14.00 g, 70 %) as a pale yellow liquid: $\delta_H$ (500 MHz, $CDCl_3$), 1.22 (6H, m, $CH_3$), 3.45 (4H, m, $CH_2$); $\delta_C$ (125 MHz, $CDCl_3$), 12.22, 14.10, 41.65, 42.03, 110.98, 114.80, 118.61, 122.42, 155.69, 156.16, 156.63, 157.09.

**4-Bromobenzyl alcohol-O-(*tert*-butyldimethylsilyl) ether 1**
A stirred solution of 4-bromobenzylalcohol (28.990 g, 155 mmol), tert-butyldimethylsilylchloride (25.590 g, 175 mmol) and imidazole (25.327 g, 372 mmol) in anhydrous DMF (65 mL) under an atmosphere of nitrogen was heated to 40 ºC. After stirring for twenty-one hours, the reaction mixture was poured into ether (180 mL) and washed with water (3 x 50 mL), dried over magnesium sulphate, filtered and concentrated. Distillation of the resulting oil afforded the title compound (41.089 g, 88 %) as a colourless liquid: b.p., 160 °C/ 0.3 mm (lit. 105-108 °C/ 0.12 mm); $\delta_H$ (300 MHz, $CDCl_3$), 0.09 (6H, s, 2 x $CH_3$), 0.93 (9H, s, 3 x $CH_3$), 4.75 (2H, s, $CH_2$), 7.19 (2H, d, $J$ = 8.2, ArCH), 7.45 (2H, d, $J$ = 8.7, ArCH); $\delta_C$ (75 MHz, $CDCl_3$), -4.84, 18.81, 26.35, 64.72, 120.98, 128.12, 131.68, 140.86.

**4-[(*tert*-butyldimethylsiloxy)methyl]-2,2,2-trifluoroacetonphenone 2**
To a stirred solution of 4-bromobenzyl alcohol-O-(*tert*-butyldimethylsily) ether (19.69 g, 63.7 mmol) in anhydrous ether (380 mL) was added butyl lithium (77.3 mmol, 45.00 mL, 1.7 M in hexanes) at -78 °C (acetone/dry ice) under a nitrogen atmosphere over a period of ten minutes. The reaction mixture was allowed to stir under these conditions for a further one hour and 30 minutes (TLC on a mini-workup indicated good conversion to the aryl lithium). *N,N*-diethyltrifluoroacetamide (14.00 g, 83.0 mmol) in ether (55 mL) was then added dropwise over 30 minutes and stirring continued for a further one hour and thirty minutes. Saturated aqueous ammonium chloride (250 mL) was then added and the reaction mixture allowed to warm to room temperature. The reaction mixture was then diluted with ether (150 mL), and then separated and the organic layer washed with saturated aqueous ammonium chloride (2 x 125 mL) and water (2 x 75 mL). The combined aqueous layers were washed with ether (150 mL) and then the combined organics dried over magnesium sulphate, filtered and concentrated. Distillation of the resulting liquid afforded the title compound (15.68 g, 78 %) as a colourless liquid: b.p. 145 °C/ 0.8 mm (lit. 120-123 °C/ 0.7 mm)[1]; $\delta_H$ (300 MHz, $CDCl_3$) 0.04 (6H, s, 2 x $CH_3$) 0.87 (9H, s, 3 x $CH_3$), 4.75 (2H, s, $CH_2$), 7.42 (2H, d, $J$ = 8.7, ArCH), 7.97 (2H, d, $J$ = 8.2, ArCH).

**4-[(*tert*-butyldimethylsiloxy)methyl]-2,2,2-trifluoroacetonphenone oxime 3**
To a stirred solution of 4-[(*tert*-butyldimethylsiloxy)methyl]-2,2,2-trifluoroacetophenone (10.51 g, 34.6 mmol) in anhydrous pyridine (100 mL) and anhydrous ethanol (47 mL) under nitrogen was added hydroxylamine hydrochloride (2.64 g, 38.0 mmol). The reaction mixture was then brought to 80 °C and heating continued overnight. The reaction mixture was then concentrated, suspended in ether (250 mL), washed with water (4 x 100 mL), dried over magnesium sulphate, filtered and concentrated. The resulting residue was purified by column chromatography (70 g silica, 2 cm diameter, dichloromethane) to yield the title compound (7.835 g, 72 %) as a colourless viscous liquid: $\delta_H$ (300 MHz, $CDCl_3$), 0.11 and 0.12 (6H, s, 2 x $CH_3$), 0.95 and 0.96 (9H, s, 3 x $CH_3$), 4.78 and 4.79 (2H, s, $CH_2$), 7.40 (4H, m, ArCH), 9.66 and 10.02 (1H, brs, OH); $\delta_C$ (75 MHz, $CDCl_3$), -4.88, 18.84, 26.33, 64.89, 124.67, 126.29, 128.70, 129.01, 137.75, 144.15, 144.48, 149.15; (ESI-MS) *m/z* 356 $[M+Na]^+$.

**4-[(*tert*-butyldimethylsiloxy)methyl]-2,2,2-trifluoroacetonphenone-O-(4-toluenesulphonyl) oxime 4**

To a stirred solution of 4-[(*tert*-butyldimethylsiloxy)methyl]-2,2,2-trifluoroacetonphenone oxime (4.000 g, 12.01 mmol), anhydrous diisopropylethylamine (2.720 mL, 2.018 g, 15.60 mmol) and 4-dimethylaminopyridine (0.132 g, 1.08 mmol) in anhydrous dichloromethane (25 mL) at 0 °C under an atmosphere of nitrogen was added 4-toluenesulphonyl chloride (2.750 g, 14.40 mmol). Following addition the reaction was allowed to warm to room temperature and stirred for a further two hours. The reaction mixture was then washed with water (3 x 25 mL), dried over magnesium sulphate, filtered and concentrated. Column chromatography (60 g silica, 2cm diameter, 3:4 dichloromethane:hexane) on the resulting red oil afforded the product (5.327 g, 91%) as a pale green oil: $\delta_H$ (300 MHz, $CDCl_3$), 0.11 and 0.12 (6H, s, 2 x $CH_3$), 0.95 and 0.96 (9H, s, 3 x $CH_3$), 2.46 and 2.48 (3H, s, $CH_3$), 4.77 and 4.78 (2H, s, $CH_2$), 7.40 (6H, m, ArCH), 7.90 (2H, m, ArCH); (ESI-MS), *m/z* 510 $[M+Na]^+$, 526 $[M+K]^+$.

**3-[α-(*tert*-butyldimethylsiloxy)-4-tolyl]-3-trifluoromethyl diaziridine 5**

In a three-neck flask fitted with a potassium carbonate guard tube and acetone/dry ice condenser under an atmosphere of nitrogen, a stirred solution of 4-[(*tert*-butyldimethylsiloxy)methyl]-2,2,2-trifluoroacetonphenone-O-(4-toluenesulphonyl) oxime (3.943 g, 8.096 mmol) in anhydrous ether (20 mL) was cooled to -78 °C (acetone/dry-ice bath). Ammonia gas was bubbled through the solution until sufficient liquid ammonia had condensed (~60 mL). The reaction mixture was then allowed to stir at this temperature for a further three hours before being allowed to warm to room temperature over two hours. Following evaporation of ammonia, the reaction mixture was filtered and concentrated to give the title compound (2.534 g, 95 %) as a translucent paste that was used in subsequent transformations without further purification: $\delta_H$ (300 MHz, $CDCl_3$), 0.10 (6H, s, 2 x $CH_3$) 0.94 (9H, s, 3 x $CH_3$), 2.22 (1H, d, $J$ = 8.2, NH), 2.79 (1H, d, $J$ = 8.7, NH), 4.76 (2H, s, $CH_2$), 7.38 (2H, d, $J$ = 8.2, ArCH), 7.58 (2H, d, $J$ = 8.2, ArCH).

**3-[α-(*tert*-butyldimethylsiloxy)-4-tolyl]-3-trifluoromethyl diazirine 6**

In a darkened fume hood, to a stirred solution of 3-[α-(*tert*-butyldimethylsiloxy)-4-tolyl]-3-trifluoromethyl diaziridine (2.500 g, 7.53 mmol) and anhydrous triethylamine (1.745 mL, 1.267 g, 12.55 mmol) in anhydrous methanol (5 mL) was added iodine (1.594 g, 6.28 mmol) portionwise, until a red/orange colour persisted. The reaction mixture was then allowed to stir for a further twenty minutes before being neutralised with 10 % aqueous citric acid solution and then quenched with a few drops of 5 % aqueous sodium metabisulphite solution. The reaction mixture was then poured in ether (150 mL) dried over sodium sulphate, filtered and concentrated. Column chromatography (70 g silica, 2 cm column, hexane:dichloromethane, 2:1 (nb: column wrapped in newspaper)) afforded the title compound (1.514 g, 61 %) as a light yellow oil: $\delta_H$ (500 MHz, $CDCl_3$), 0.09 (6H, s, 2 x $CH_3$) 0.93 (9H, s, 3 x $CH_3$), 4.74 (2H, s, CH2), 7.16 (2H, d, $J$ = 7.7, ArCH), 7.35 (2H, d, $J$ = 7.7, ArCH); $\delta_C$ (75 MHz, $CDCl_3$), -4.94, 18.79, 26.30, 64.64, 120.79, 124.42, 126.64, 126.78, 127.98, 143.74.

**4-[3-trifluoromethyl-3H-diazirin-3-yl)benzyl alcohol 7**

In a darkened fume hood, 3-[α-(*tert*-butyldimethylsiloxy)-4-tolyl]-3-trifluoromethyl diazirine (1.500 g, 4.54 mmol) was treated with a solution of tetrabutylammonium fluoride (5.5 mL, 1.0 M in THF, 5.45 mmol) containing water (0.275 mL, 5 %). The solution was allowed to stir at room temperature for five hours, then diluted with ether (40 mL), washed with water (3 x 12 mL), dried over sodium sulphate, filtered and concentrated. Column chromatography on the resultant oil (23 g silica, 2 cm column, dichloromethane, (nb: column wrapped in newspaper)) yielded the title compound (0.791, 81 %) as a pale yellow oil: $\delta_H$ (500 MHz, $CDCl_3$), 2.39 (1H, brs, OH), 4.66 (2H, s, $CH_2$), 7.17 (2H, d, $J$ = 7.7, ArCH), 7.35 (2H, d, $J$ = 8.5, ArCH).

**3-(α-iodo-4-tolyl)-3-(trifluormethyl)-3H-diazirine 8**

To a stirred solution of 4-[3- trifluoromethyl-3H-diazirin-3-yl)benzyl alcohol (0.780 g, 3.611 mmol) in anhydrous acetonitrile (7 mL) under nitrogen was added methyltriphenoxyphosphonium iodide (3.666 g, 7.22 mmol). The reaction mixture was then allowed to stir for twenty hours before being diluted with ether (60 mL) and washed successively with sodium hydroxide (1 M, 2 x 40 mL) and water (2 x 50 mL). The organic layer was then dried with magnesium sulphate, filtered and concentrated. The resultant red oil was purified by column chromatography (28 g silica, 2 cm column, ether:pentane, 1:2 (nb: column wrapped in newspaper) to afford the title compound (0.741 g, 63 %) as a pale yellow solid: $\delta_H$ (500 MHz, $CDCl_3$), 4.42 (2H, s, $CH_2$), 7.12 (2H, d, $J$ = 7.7, ArCH), 7.40 (2H, d, $J$ = 8.5, ArCH); (ESI-MS) *m/z* 327 $[M+H]^+$.

**Nickel complexation of (S)-2-[*N*-(*N'*-benzylprolyl)amino]-benzophenone and glycine 9**

**(**Belokon, Y. N.; Bulychev, A. G.; Vitt, S. V.; Struchkov, Y. T.; Batsanov, A. S.; Timofeeva, T. V.; Tsyryapkin, A. A.; Ryzhov, M. G.; Lysova, L. A.; Bakhmutov, V. I.; Belikov, V. M. *J. Am. Chem. Soc.* **1985** *107*, 4252-4259**):**

(S)-2-[*N*-(*N'*-benzylprolyl)amino]-benzophenone (0.9635 g, 2.51 mmol), glycine (0.9410 g, 12.53 mmol) and nickel (II) nitrate hexahydrate (1.4721 g, 5.06 mmol) were stirred together in anhydrous methanol (16 mL) at 55 °C for 10 minutes. Sodium methoxide (4.9 M in methanol, 4.35 mL, 21.30 mmol) was then added and the mixture allowed to heat at this temperature for a further one hour. Acetic acid (1.7 mL) was then added and the reaction mixture diluted with water (80 mL) and left in the fridge overnight. The resulting red crystals were filtered and subjected to column chromatography (70 g silica, 5:1 chloroform: acetone) to afford a red solid which was re-crystallised from a minimum amount of methanol with a small amount of water to afford the product (0.794 g, 64%) as red crystals: m.p., decomposes 219-222°C (lit. 208-212°C); (300MHz, $CDCl_3$), 2.13 (2H, m), 2.45 (1H, m), 2.56 (1H, m), 3.27 (1H, m), 3.47 (1H, m), 3.71 (4H, m), 4.50 (1H, d, $J$ = 12.8), 6.71 (1H, m), 6.81 (1H, m), 6.98 (1H, m), 7.11 (1H, m), 7.27 (3H, m), 7.40-7.52 (5H, m), 8.07 (2H, d, $J$ = 7.2), 8.28 (1H, d, $J$ = 8.7); $\delta_C$ (125 MHz, $CDCl_3$), 24.14, 31.14, 57.86, 61.73, 63.51, 70.25, 121.30, 124.69, 125.61, 126.08, 129.37, 129.56, 129.78, 130.04, 130.19, 132.17, 132.66, 133.62, 133.69, 125.02, 142.91, 177.79, 181.79; (ESI-MS), *m/z* 498 $[M+H]^+$.

**Alkylation of Nickel complex with 3-(α-iodo-4-tolyl)-3-(trifluormethyl)-3H-diazirine 10**

In a darkened fume hood, to a stirred suspension of nickel complex (0.7 g, 1.41 mmol) and powdered sodium hydroxide (0.23 g, 5.96 mmol) in anhydrous acetonitrile (3 mL) at -10 °C (salt/ ice bath) under an atmosphere of nitrogen was added a solution of 3-(α-iodo-4-tolyl)-3-(trifluormethyl)-3H-diazirine (0.353 g, 1.08 mmol) in anhydrous acetonitrile (1 mL). The mixture was warmed to room temperature and stirred for a further twenty hours. The reaction mixture was then diluted with dichloromethane (50 mL) and washed successively with aqueous acetic acid (0.2 M, 30 mL) and water (2 x 40 mL). The organic layer was then dried over magnesium sulphate, filtered and concentrated. Purification of the resulting red paste by column chromatography (35 g silica, 1.5 cm column, chloroform:acetone, 5:1 (nb: column wrapped in newspaper)) afforded the product (0.440 g, 58 %) as a red glassy solid that was used in subsequent steps without further purification: $\delta_H$ (300MHz, $CDCl_3$), 1.72 (1H, m , Pro C<u>H</u>H'), 1.88 ( 1H, m, Pro CH<u>H</u>'), 2.24 (2H, m, Pro$CH_2$), 2.37 (1H, m, Pro alkyl), 2.83 (1H, dd, $J$ = 13.8 and 5.6, Phe C<u>H</u>H'), 3.06 (2H, m, Pro $CH_2$), 3.33 (1H, dd, $J$ = 10.2 and 6.1, Phe CH<u>H</u>'), 3.49, (1H, d, $J$ = 12.8, Bzl C<u>H</u>H'), 4.29 (2H, m, Bzl CH<u>H</u>' and PheCH), 6.66 (2H, m, ArCH), 6.88 (1H, s, ArCH), 7.24 (9H, ArCH), 7.47 (1H, m, ArCH), 7.56 (2H, m, ArCH), 7.99 (2H, d, $J$ = 7.2, ArCH), 8.22 (1H, d, $J$ = 8.7, ArCH); $\delta_C$ (75 MHz, $CDCl_3$), 22.58, 28.25 (Q), 30.51, 38.98, 56.88, 63.19, 68.56, 70.10, 77.42, 116.51, 120.15, 120.55, 123.26, 123.79, 125.85, 126.66, 127.00, 127.43, 127.58, 128.30, 128.67, 128.83, 129.11, 129.80, 130.63, 120.86, 131.31, 131.56, 132.44, 133.11, 133.41, 133.97, 137.60, 142.76, 171.38, 178.00, 180.00; (ESI-MS), *m/z* 697 $[M+H]^+$.

**_(S)_-3-[4-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenyl]alanine 11**

In a darkened fume hood, a solution of the alkylated nickel complex (0.250 g, 0.359 mmol) in methanol (10 mL) and aqueous hydrochloric acid (1 N, 6 mL) was heated at reflux for 15 minutes during which period the red colour faded to a pale yellow. The organic solvent was removed by rotary evaporation and then the aqueous residue washed with chloroform (2 x 25 mL). The combined organics were washed with water (2 x 15 mL) and then the combined aqueous residues were brought to pH 2.5 by addition of aqueous ammonia (0.88) and absorbed onto Dowex50 (W2) $H^+$ exchange resin (30 g). The column was washed with water (100 mL), then methanol (80 mL) and then the product eluted with methanolic ammonia (2 M, 140 mL). Fractions active against ninhydrin stain were concentrated and re-crystallised from the minimum amount of methanol/ water. This was unsuccessful and the resulting crystals and concentrated filtrate were combined to yield the product (0.042 g, 42 %) as a white solid: (300MHz, $CF_3CO_2D$), 3.43 (1H, m, CHH') 3.82 (1H, m CHH'), 4.60 (1H, m, CH), 7.19 (2H, m ArCH), 7.31 (2H, m, ArCH); (ESI-MS), *m/z* 697 $[M+H]^+$; $[\alpha]_D$ (c = 0.12, MeOH), -65.0 °C (lit. -70.1 ºC).

**_(S)-N_-(9-Fluorenylmethoxycarbonyl)-[4-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenyl]alanine 12**

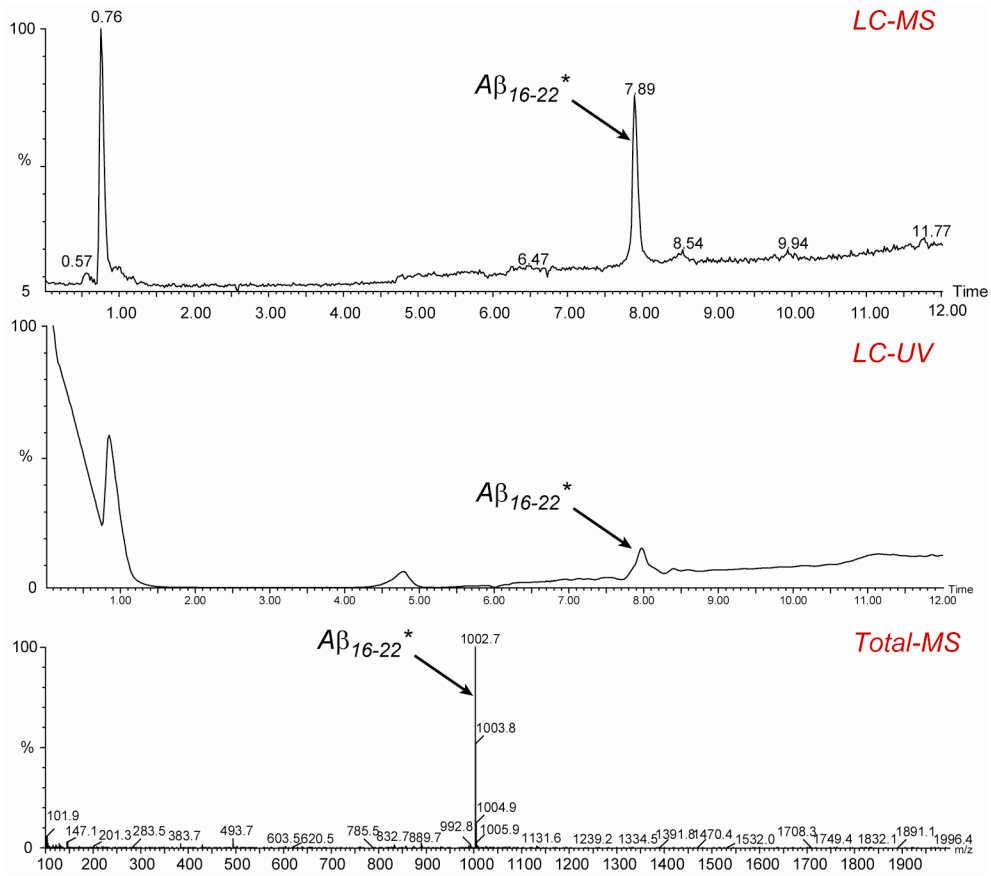
In a darkened fume hood, to a stirred suspension of (S)-3-[4-[3-(trifluoromethyl)-3H-diazirin-3-yl]phenyl]alanine (171 mg, 0.6 mmol) and sodium carbonate (177 mg, 1.7 mmol) in acetone (2 mL) and water (8.5 mL) at 0 °C was added *N*-(9-fluorenylmethoxycarbonyloxy) succinimide (209 mg, 0.6 mmol) portion wise. The reaction mixture was then returned to room temperature and stirred for a further 48 hours. The reaction mixture was brought to pH 1.0 by addition of aqueous hydrochloric acid (1.2 M) then extracted with ethyl acetate (10 mL) and washed successively with aqueous hydrochloric acid (1.2 M; 10 mL) and water (2 x 10 mL). The organic layer was then dried over sodium sulphate and concentrated. Column chromatography (methanol:dichloromethane, 5:95) on the resulting solid gave the title compound (145 mg, 49 %) as a off-white solid: m.p., 123-125 °C (lit. 119-121 °C); $^1H$ (300 MHz, $CDCl_3$), 3.12 (1H,dd, *J* = 5.7 and 13 .5, CHH' ), 3.21 (1H, dd, *J* = 5.1 and 13.8, CHH' ), 4.20 (1H, t, *J* = 6.5 CH Fmoc), 4.40 (1H, m, CHH Fmoc ), 4.50 (1H, m, CHH), 4.70 (1H, m, CH), 5.19 (1H, d, *J* = 7.7, NH), 7.09 (2H, d, *J* = 7.9, Ar Phe), 7.13 (2H, d, *J* = 7.9, Ar Phe), 7.30 – 7.33 (2H, m, Ar Fmoc), 7.41 (2H, t, *J* = 7.4, Ar Fmoc), 7.54-7.59 (2H, m, Ar Fmoc), 7.78 (2H, d, *J* = 7.3, Ar Fmoc); (ESI-MS), *m/z* 494 $[M+H]^+$.

**Synthesis of Ac-Lys-Leu-Val-Phe-Phe-Ala-Glu-$NH_2$ ($A\beta_{16-22}$) and Ac-Lys-Leu-Val-Phe-(tfmd-Phe)-Ala-Glu-$NH_2$ ($A\beta^*_{16-22}$):**

Aβ16-22 samples were synthesized manually using standard Fmoc synthesis and cleavage protocols, a Rink amide MBHA resin (Novabiochem), and HCTU activation in the presence of triisopropylethylamine, with the exception of coupling the first amino acid to the resin which was achieved using DCC and HOBt. Briefly, following resin swelling for 1 hour in DMF, each coupling step involved gentle agitation for 2 hrs followed by filtration, 5 x 2 minute washes with DMF, 5 x 2 minute washes with 20 % piperidine in DMF and 5 x 2 minute washes with DMF. The peptides were cleaved from the resin using TFA with triisopropyl silane over 1 hour followed by concentration of the filtrate. Samples were purified by high-performance liquid chromatography (HPLC), using a water/acetonitrile gradient with 0.1 % trifluoroacetic acid and a C18 reverse phase column. HPLC traces for Aβ*16-22 are shown in Fig. S1. Final purities were greater than 95 %, as determined by ESI and HPLC.

Ac-Lys-Leu-Val-Phe-Phe-Ala-Glu-$NH_2$ synthesised on a 0.063 mmol scale (resin loading 0.63 mmol/g) yielded 7 mg (13 %) product as a white solid: (ESI-MS), *m/z* 896 $[M+H]^+$.

Ac-Lys-Leu-Val-Phe-(tfmd-Phe)-Ala-Glu-$NH_2$ synthesised on a 0.063 mmol scale (resin loading 0.63 mmol/g) yielded 33.7 mg (53 %) as an off white solid: (ESI-MS), *m/z* 1001 $[M+H]^+$.



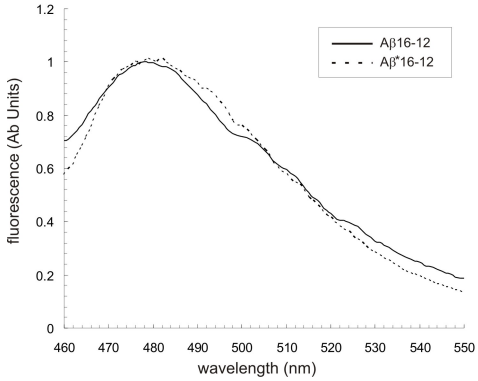

**Supplementary Figure 1.** HPLC purification of Ac-KLVF*FAE-$NH_2$ (Aβ*16-22).

## Fibrilisation

$Aβ_{16-22}$ and $Aβ^*_{16-22}$ fibrils where grown under identical conditions. Each peptide was dissolved as a 50x stock in DMSO. 100 µl of peptide at 400 µM in 25 mM sodium phosphate buffer, pH 7.0, 0.01 % sodium azide was incubated for 1 week at 25 °C without agitation. The final DMSO concentration was 2 %.

## Thioflavin T Measurements

For determination of ThT binding 20 µl of sample was removed and added to 1000 µl of a 20 µM ThT solution in 25 mM sodium phosphate pH 7.0. The ThT fluorescence was quantified using a PTI Quantamaster C-61 spectrofluorimeter by exciting at 444 nm and scanning the emission wavelengths from 460 to 550 nm with slit widths set at 5 nm. Data were normalized by subtracting the spectra of the buffer and taking the intensities at λmax of each sample as unity.



**Supplementary Figure 2.** Thioflavin T binding of $A\beta_{16-22}$ and $A\beta^*_{16-22}$

**Negative Stain Electron Microscopy**

Samples of aggregated peptide were pelleted by centrifugation on a desk top centrifuge at 16,300 *g* for 30 min before being resuspended in MilliQ water. Samples (10 μl) of aggregated peptide were spotted onto carbon-coated copper grids for 1 min (ProSciTech). The grids were blotted with filter paper to remove excess buffer and the samples stained with 0.5 % (w/v) uranyl acetate for 30-40 s. Grids were blotted again and air-dried before analysis on a Philips CM10 electron microscope operating at 100 keV.

**Cross-linking**

$A\beta_{16-22}$ and $A\beta^*_{16-22}$ fibrils were irradiated with light of ~350 nm for 1 hour using a 6W bench-top thin layer chromatography (TLC) bulb. The insoluble fibrils were then pelleted by centrifugation on a desk top centrifuge at 16,300 *g* for 30 min. The pellet was resuspended and fully dissociated in hexafluoroisopropanol for 24 h at room temperature.

**ESI-IMS-MS**

Dissociated peptide was diluted 25 times into methanol. Samples (1:1, v/v, MeOH:0.1% aqueous formic acid) were infused into the nanoESI source of a Synapt HDMS a hybrid quadrupole-IMS-orthogonal acceleration time-of-flight mass spectrometer (oa-TOF) (Waters UK Ltd, Manchester, UK) using a Triversa NanoMate (Advion Biosystems Inc, Ithica, NY, USA) automated nanoESI interface operating at a capillary voltage of 1.7 kV. Sample cone voltage was 50 V with a source temperature of 60 °C and a desolvation temperature of 100 °C. Nitrogen was used as both the nebulizing gas and the desolvation gas. The quadrupole was operated in non-resolving mode to transmit a wide *m/z* range (MS analyses) or in resolving mode for MS/MS experiments.

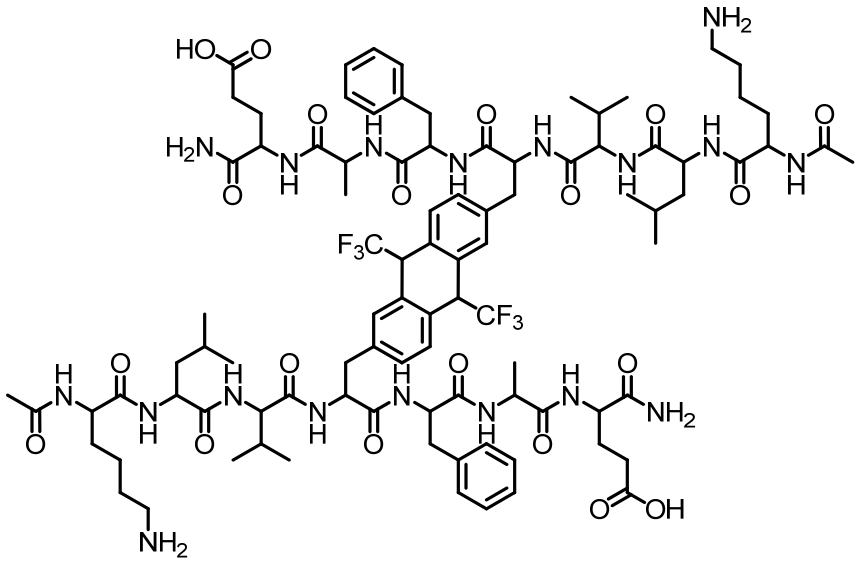
The ion mobility device in the Synapt HDMS[1,2] consists of three travelling wave (T-wave) ion guides: the first is used to store ions before mobility separation (trap T-Wave); the second provides ion mobility separation using a travelling voltage wave (IMS T-Wave); and the third transfers the mobility-separated ions to the time-of-flight analyzer for *m/z* analysis (transfer T-Wave). In these experiments, the IMS T-Wave was operated at a nitrogen pressure of $1.53 \times 10^{-1}$ mbar and the trap/transfer T-Waves at a nominal pressure of $9.47 \times 10^{-3}$ mbar (argon). The ion accelerating voltages into the trap and transfer T-wave devices were set at 5 and 2 V, respectively. MS/MS experiments where appropriate were conducted within the transfer region with a collision energy of 40 eV. Each individual mobility experiment was 18 ms with the 300 $ms^{-1}$ IMS T-Wave pulse amplitude being ramped from 2.9 to 8.6 V during this time; this ensured that the entire range of components was detected within a single experiment. During each mobility separation, ions were accumulated in the trap T-Wave and then released over a period of 90 μs into the IMS T-Wave to start the next mobility separation. The transfer T-Wave had a 300 $ms^{-1}$ 3 V pulse running continually to transfer the mobility separated ions to the oa-TOF whilst maintaining the temporal separation. Ion arrival time (mobility) spectra were recorded through synchronization of the gated release of ions for mobility separation and the oa-TOF mass spectral acquisitions. An individual mobility experiment was made up of 200 sequential oa-TOF mass spectra (90 μs each) giving an overall time of 18 ms (200 × 90 μs). Mass spectra were acquired over a *m/z* range of 950 to 1050 or 100 to 2000 and with repeat 2 s scan time per point (ca. 111 summed individual mobility experiments).

Instrument control and data analysis were performed using MassLynx v. 4.1 software (Waters UK Ltd, Manchester, UK). Mobility data were visualized and processed using the Driftscope module within MassLynx. Mass accuracy was ensured by calibration on a separate introduction of sodium iodide (2 mg $mL^{-1}$ in 1:1 v/v aqueous methanol).

**Analysis of ESI-IMS-MS spectrum**

Species A to E in Figure 2 were identified by their *m/z* values and isotope distributions. Species A displayed a *m/z* of 974.4 and an inter-peak spacing of 1 Da, indicating singly-charged ions, corresponding to a monomeric peptide of mass 973.4 Da (intra-peptide cross-link). Species B displayed a *m/z* of 974.4 and an inter-peak spacing of 0.5 Da, indicating doubly-charged ions of mass 1946.8 Da, corresponding to a dimeric peptide (two inter-peptide cross-links, or one inter- and one intra-peptide cross-link). Species C displayed a *m/z* of 988.2 and an inter-peak spacing of 0.5 Da, indicating doubly-charged ions of mass 1974.4 Da, corresponding to a second dimeric peptide (one inter-peptide cross-link to a peptide with a linear diazo isomer). Species C' displayed a *m/z* of 983.2 and an inter-peak spacing of 0.5 Da, indicating doubly-charged ions of mass 1964.4 Da (one inter-peptide cross-link to a peptide where carbene has formed a water adduct). Species D and E displayed *m/z* 992.4 and *m/z* 1002.4, respectively, and an inter-peak spacing of 1 Da, indicating singly-charged peptides of masses 991.4 and 1001.4 Da, respectively (D, water adduct; E, linear diazo isomer). The IMS T-Wave device was able to separate species A and B based on their cross-sectional areas and charge densities, further confirming the presence of two unique species with the same *m/z* ratio. Fig. S2 shows a theoretical structure for species B. Note that the C-terminal residue is amidated ( $^{-N}$ ) and the N-terminal residue is acetylated. During fragmentation a loss of 17 Da is often observed and is attributed to the loss of $NH_3$ from the C-terminal residues.

A



B

Ac-KLVF*FAE-N

Ac-KLVF*FAE-N

**Supplementary Figure 3.** A: Theoretical structure consistent with species B, showing an inter-peptide cross-link between the (tfmd)-phe of one peptide and the (tfmd)-phe of a second peptide. B: short-hand notation of species B (F* in this instance refers to cross-linked (tfmd)-phe, Ac refers to the N-terminal acetylation, and $^{-N}$ refers to the C-terminal amidation; the cross-link position between the two is shown as a vertical thick black line).

***From here on, F* refers to the modified phe residue 19 in $A\beta_{16-22}$: unreacted, isomerised or cross-linked, as appropriate to the text.***

**ESI-IMS-MS/MS of Species B (Fig. S4)**

Species B (*m/z* 974.4(2+)) is consistent with a dimer resulting from two inter-peptide cross-links, or one inter- and one intra-peptide cross-link (see Fig. S3). The parent ion with a *m/z* of 974.4 (2+) was selected with a window of 4 mass units using the quadrupole mass analyser before separation *via* use of the IMS-Twave device.[1,2] Collision induced fragmentation occurred in the transfer region

of the T-wave device. This approach allows the selection of the *m/z* 974.4 ions and the separation of the 1+ and 2+ charged species originating from monomeric and dimeric peptides, respectively, prior to fragmentation. The parent ion is shown in Fig. S4 A and E.

Collision induced dissociation resulted in both singly- and doubly-charged fragment ions being produced (Fig. S4 A, B and C). Fragmentation resulting from the loss of the C-terminal F, A and E residues and the N-terminal K, L and V residues can be observed. Table 1 shows the expected *m/z* values resulting from the loss of N- or C-terminal residues. Additionally loss of: 17 Da can be observed due to the loss of the $NH_3$ C-terminal; 18 Da from the loss of $H_2O$ from E; 28 Da from the loss of CO from the *a b* ion pair; and 42 Da from the loss of the N-terminal $CH_3CO$.

Specific ions of interest arise at *m/z* values of 1565.8 (1+) and 783.4 (2+) (F*FAE/KLVF*FAE) resulting from the loss of KLV, and *m/z* values of 1566.8 (1+) and 784.4 (2+) (KLVF*/KLVF*FAE) resulting from the loss of $FAE^{-N}$ + $^{-N}$ (Fig. S4 D, left and S4 E, right). A weak ion at a *m/z* value of 1219.7, corresponding to a cross-linked peptide fragment KLVF*/KLVF*, is observed (Fig. S4 D, right). The fragmentation of resulting singly-charged peptide fragments KLV (Fig. S4 F, left) and FAE can also be observed (Fig. S4 C). The majority of the peptide fragments correspond to either the loss of N-terminal K, L, V or C-terminal F, A, E residues, indicating that F* in one peptide has formed a cross-link with F* in the other peptide.

A second minor cross-linked product can also be observed resulting from one inter and one intra cross linking event. This results in a minor fragment ion at *m/z* 592.2 (F*FAE) (Fig. S4 F, right). This fragment pattern would arise if one of the F* forms a cross-link with K, L or V on the adjacent strand (KLVF*FAE/KLV) forming a 2+ dimer. However, the resulting fragments from this peptide are to weak to be observed.

**ESI-IMS-MS/MS of Species C.**
Species C (*m/z* 988.2(2+)) is consistent with a dimer resulting from one inter-peptide cross-link (other tfmd isomerised to unreactive linear diazo) (Figure 2). The parent ion with a *m/z* value of 988.2 (2+) was selected using the quadrupole mass analyser before separation *via* use of the IMS-Twave device. The parent ion is shown in Fig. S4 A and E.

As with species B, the main peptide fragmentation results from the loss of either N-terminal K, L and V or the C-terminal F, A and E (Fig. S5 A, B and C). Table 2 shows the expected *m/z* values resulting from the loss of N- or C-terminal residues.

The majority of the peptide fragments correspond to either the loss of the N-terminal K, L and V, or C-terminal F, A and E residues (Fig. S5 D, left). Specific ions of interest arise at *m/z* values of 1247.3 (+1) (KLVF*/KLVF*) and 1230.2 (1+) (KLVF*/F*FAE-$^{N}$) (Fig. S5 D, left), showing the loss of the N- and C-terminal fragments. The fragmentation of resulting singly-charged peptide fragments KLV (*m/z* 383.1 (1+)) and FAE (*m/z* 365.0 (1+)) is again observed in these spectra (Fig. S5 C and F). The loss of residues from both C-termini in a cross-linked peptide is observed and highlighted at a *m/z* value of 806.6 (2+), where the loss of AE-$^{N}$ and E-$^{N}$ can be observed (Fig. S5 E, right). As with species B, the majority of the peptide fragments correspond to either the loss of N-terminal K, L, V or C-terminal F, A, E residues. Interestingly, the minor component observed with species B is not present in this spectrum, indicating that only one cross-linking event has taken place.

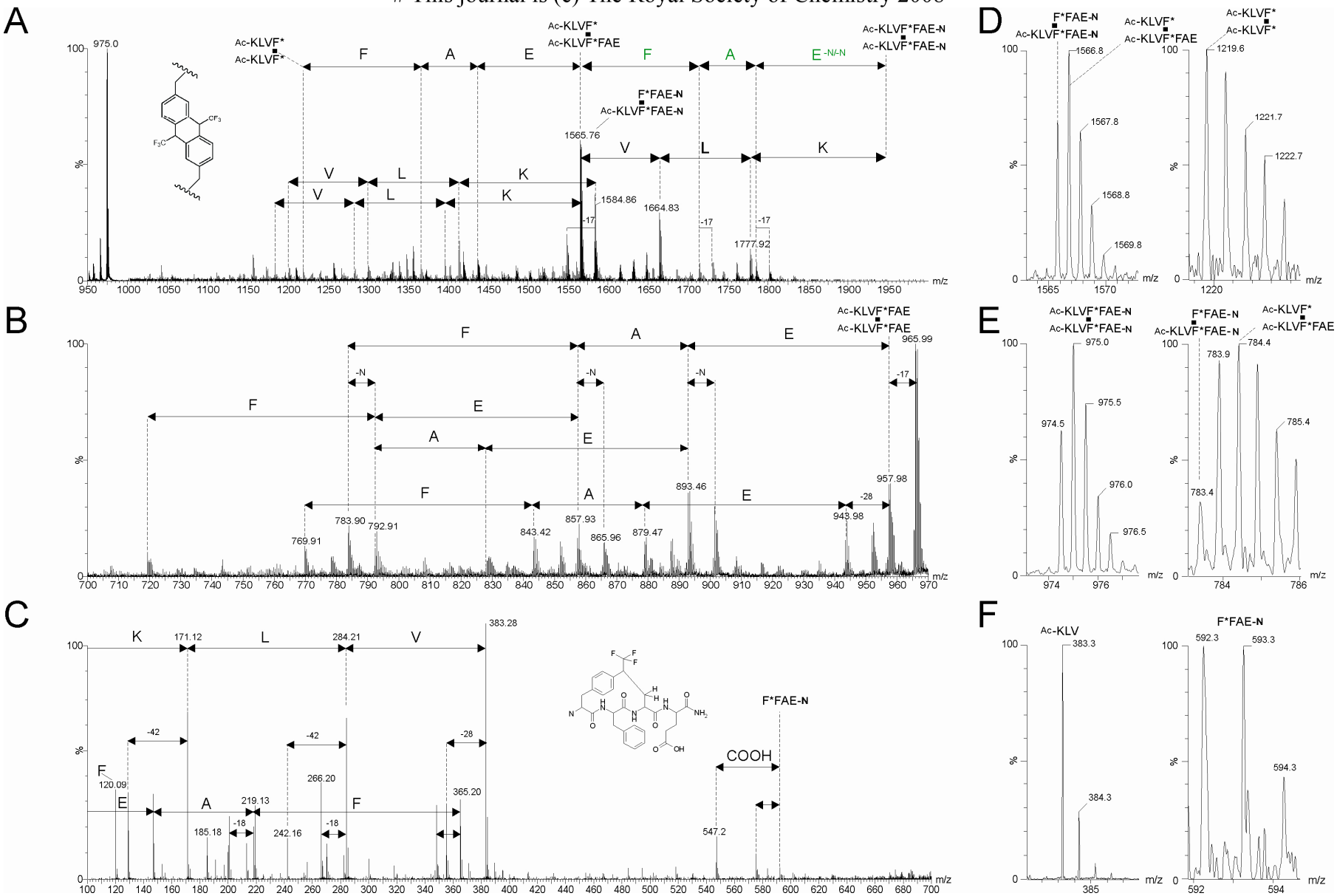
Species C contains a single inter-peptide cross-link formed by one of the F*; the other F* is a linear diazo isomer. The fragment ion for this latter species alone (i.e. not cross-linked to anything) would have a *m/z* value of 255.1 (1+) (i.e., phe - $H_1$ + $C_2F_3N_2$). Hence, if the inter-peptide cross-link was not between the two F* residues, mass differences of *m/z* 255.1 (1+) would be observed (Table 2),

and fragments such as KLVF* (*m/z* 619.2 (1+)) would be present. Mass differences corresponding to the diazo isomer are not observed within the fragmentation pattern (Fig. S5). The observation of fragment ions resulting from the loss of K, L, V, F, A, E and $^{-N}$ with both species C and D (Fig. S4 and S5) and the identification of the resulting KLV and FAE$^{-N}$ fragments (Fig. S4 C and S5 C), along with the observation of key fragments such as KLVF*/KLVF* (Fig. S4 D), and KLVF*/F*FAE-$^{N}$ (Fig. S5 D) show that F* in one peptide has formed a cross-link with F* in the other peptide.

1. Pringle, S. D.; Giles, K.; Wildgoose, J. L.; Williams, J. P.; Slade, S. E.; Thalassinos, K.; Bateman, R. H.; Bowers, M. T.; Scrivens, J. H. An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/travelling wave IMS/oa-ToF instrument. *Int. J. Mass Spectrom.* 2007, 261, 1-12.
2. Giles, K.; Pringle, S. D.; Worthington, K. R.; Little, D. R.; Wildgoose, J. L.; Bateman, R. H. Applications of a travelling wave-based radio-frequency-only stacked ring ion guide. *Rapid Commun. Mass Spectrom.* 2004, 18, 2401-2414.

# Supplementary Material (ESI) for Chemical Communications


**Supplementary Figure 4.** Collision induced fragmentation of the doubly-charged Species B. *m/z* range 1000 – 2000 is magnified 7 times. The loss of the C-terminal $FAE^{-N}$ residues from one peptide chain is highlighted in green and the *m/z* values for the resulting ions are shown in Table 1.


A
B
C
D
E
F
Ac-KLVF*
Ac-KLVF*FAE
Ac-KLVF*FAE-N
F*FAE-N
975.0
1565.76
1584.86
1664.83
1777.92
965.99
957.98
893.46
783.90
792.91
769.91
843.42
857.93
865.96
879.47
943.98
383.28
171.12
284.21
120.09
219.13
266.20
365.20
185.18
242.16
547.2
COOH
1566.8
1567.8
1568.8
1569.8
1219.6
1221.7
1222.7
975.5
974.5
976.0
976.5
783.9
784.4
785.4
783.4
Ac-KLV
383.3
384.3
592.3
593.3
594.3
m/z

# Supplementary Material (ESI) for Chemical Communications


**Supplementary Figure 5.** Collision induced fragmentation of the doubly-charged Species C. *m/z* range 1000 – 2000 is magnified 7 times. Loss of FAE from KLVF*FAE/KLVF* *m/z* 1594.2 is highlighted in green and the respective *m/z* values for the resulting ions are shown in Table 2.

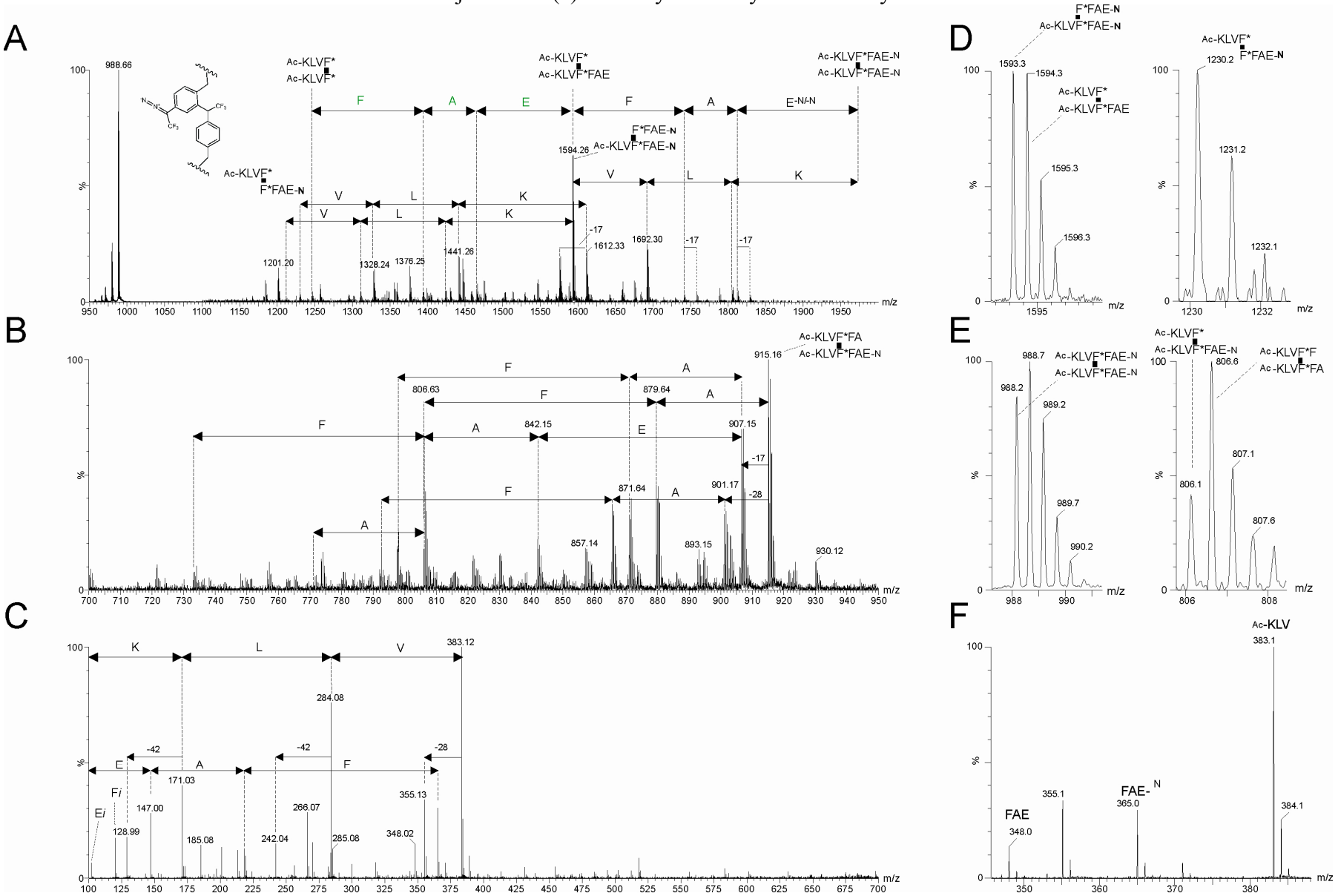


**Table 1.** Possible fragmentation products resulting from the loss of single residues from either the N- or C- termini of the cross-linked doubly- and singly-charged species B. The mass of the parent ion is shown in red for both the 1+ and 2+ species of this peptide. Loss of $FAE^{-N/-N}$ is shown in green and highlighted in Fig. S4 A.

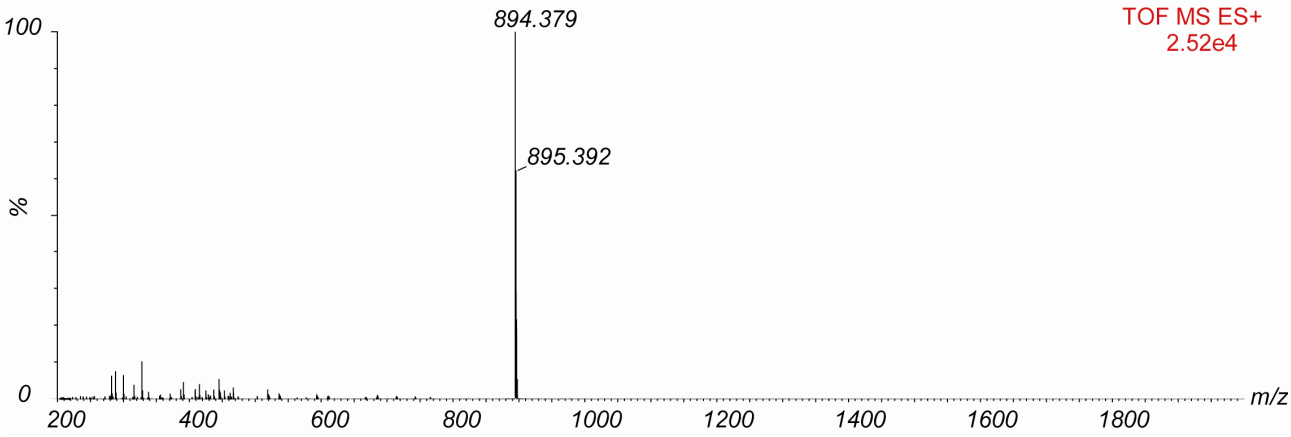
| | Mass +2 | | | | |
|---|---|---|---|---|---|
| **+2** | 974.5 | **-NH3** | **E** | **A** | **F** |
| **-NH3** | 966.0 | 957.5 | 893.0 | 857.5 | 783.9 |
| **E** | 901.5 | 893.0 | 828.5 | 792.9 | 719.4 |
| **A** | 866.0 | 857.5 | 792.9 | 757.4 | 683.9 |
| **F** | 792.4 | 783.9 | 719.4 | 683.9 | 610.3 |
| **F*** | --- | --- | --- | --- | --- |
| **V** | 783.3 | 762.3 | 698.3 | 641.8 | 592.2 |
| **L** | 833.0 | 812.0 | 748.0 | 691.5 | 641.8 |
| **K** | 889.5 | 868.5 | 804.5 | 748.0 | 698.3 |
| **CH2CO** | 953.5 | 932.5 | 868.5 | 812.0 | 762.3 |
| | 974.5 | **CH2CO** | **K** | **L** | **V** |

| | Mass +1 | | | | |
|---|---|---|---|---|---|
| **+1** | 1948.0 | **-NH3** | **E** | **A** | **F** |
| **-NH3** | **1931.0** | **1914.0** | **1784.9** | **1713.9** | **1566.8** |
| **E** | 1802.0 | 1784.9 | 1655.9 | 1584.9 | 1437.8 |
| **A** | 1730.9 | 1713.9 | 1584.9 | 1513.8 | 1366.8 |
| **F** | 1583.8 | 1566.8 | 1437.8 | 1366.8 | 1219.7 |
| **F*** | --- | --- | --- | --- | --- |
| **V** | 1565.7 | 1523.7 | 1395.7 | 1282.7 | 1183.4 |
| **L** | 1665.0 | 1623.0 | 1495.0 | 1382.0 | 1282.7 |
| **K** | 1778.0 | 1736.0 | 1608.0 | 1495.0 | 1395.7 |
| **CH2CO** | 1906.0 | 1864.0 | 1736.0 | 1623.0 | 1523.7 |
| | 1948.0 | **CH2CO** | **K** | **L** | **V** |

**Table 2.** Possible fragmentation products resulting from the loss of single residues from either the N- or C- termini of the cross-linked doubly- and singly-charged species C. The mass of the parent ion is shown in red for both the 1+ and 2+ species of this peptide. Loss of FAE from KLVF*FAE/KLVF*, *m/z* 1594.2, is shown in green and highlighted in Fig. S5 A. The grey entries show expected *m/z* values for the loss of a diazo isomer or unreacted tfmd-phe residue. The linear diazo fragment ion would be expected to have a *m/z* value of 255.1 (1+) [phe – $H_1$ + $C_2F_3N_2$].

| | Mass +2 | | | | |
|---|---|---|---|---|---|
| **+2** | 988.2 | **-NH3** | **E** | **A** | **F** |
| **-NH3** | 979.7 | 971.2 | 906.7 | 871.2 | 797.6 |
| **E** | 915.2 | 906.7 | 842.2 | 806.6 | 733.1 |
| **A** | 879.7 | 871.2 | 806.6 | 771.1 | 697.6 |
| **F** | 806.1 | 797.6 | 733.1 | 697.6 | 624.0 |
| **F*** | **678.6** | **670.1** | **605.6** | **570.1** | **496.5** |
| | **669.5** | **648.5** | **584.5** | **528.0** | **478.4** |
| **V** | 797.0 | 776.0 | 712.0 | 655.5 | 605.9 |
| **L** | 846.7 | 825.7 | 761.7 | 705.2 | 655.5 |
| **K** | 903.2 | 882.2 | 818.2 | 761.7 | 712.0 |
| **CH2CO** | 967.2 | 946.2 | 882.2 | 825.7 | 776.0 |
| | 988.2 | **CH2CO** | **K** | **L** | **V** |

| | Mass +1 | | | | |
|---|---|---|---|---|---|
| **+1** | 1975.4 | **-NH3** | **E** | **A** | **F** |
| **-NH3** | 1958.4 | 1941.4 | 1812.3 | 1741.3 | **1594.2** |
| **E** | 1829.4 | 1812.3 | 1683.3 | 1612.3 | **1465.2** |
| **A** | 1758.3 | 1741.3 | 1612.3 | 1541.2 | **1394.2** |
| **F** | 1611.2 | 1594.2 | 1465.2 | 1394.2 | **1247.1** |
| **F*** | **1356.2** | **1339.2** | **1210.2** | **1139.2** | **992.1** |
| | **1338.1** | **1296.1** | **1168.1** | **1055.1** | **955.8** |
| **V** | 1593.1 | 1551.1 | 1423.1 | 1310.1 | 1210.8 |
| **L** | 1692.4 | 1650.4 | 1522.4 | 1409.4 | 1310.1 |
| **K** | 1805.4 | 1763.4 | 1635.4 | 1522.4 | 1423.1 |
| **CH2CO** | 1933.4 | 1891.4 | 1763.4 | 1650.4 | 1551.1 |
| | 1975.4 | **CH2CO** | **K** | **L** | **V** |




**Supplementary Figure 6.** Mass Spectrum of unmodified Ac-KLVFFAE-$NH_2$ following irradiation for 1 hour using a 6W bench-top thin layer chromatography (TLC) visualization lamp. No changes in the MS spectrum of the unmodified peptide were observed after irradiation.