

ChemMedChem

Supporting Information

Prodrugs of the Archetypal Dynamain Inhibitor Bis-T-22

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Phillip J. Robinson, and Adam McCluskey*

Supplementary Data:

¹H and ¹³C Spectra for Compounds 1-10;

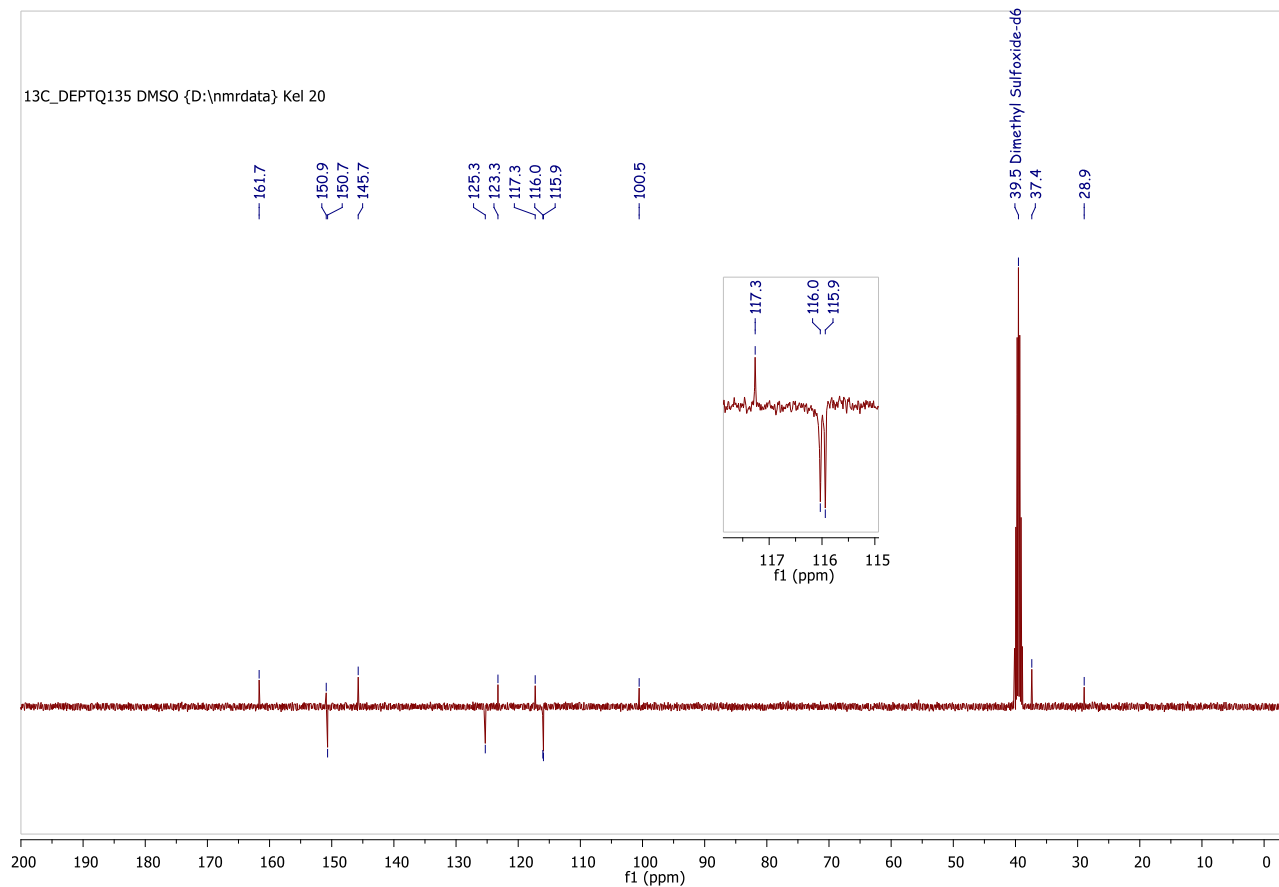
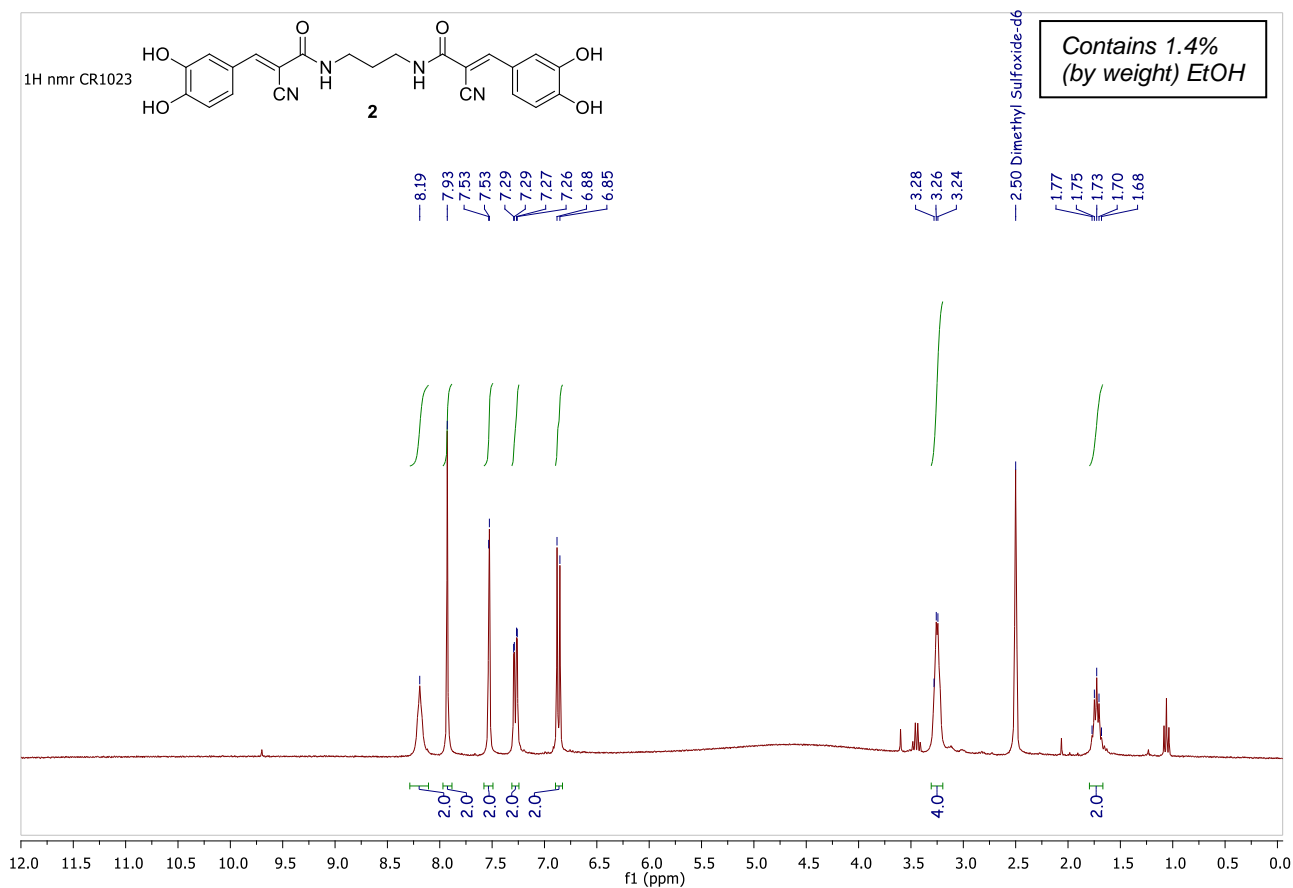
Contents

- *2-Cyano-N-[3-(2-cyanoacetyl-amino)propyl]acetamide (1)*
- *2-Cyano-N-[3-[2-cyano-3-(3,4-dihydroxyphenyl)acryloylamino]propyl]-3-(3,4-dihydroxyphenyl)acrylamide (2)*
- *Acetic acid 2-acetoxy-5-(2-cyano-2-[3-[2-cyano-3-(3,4-diacetoxyphenyl)acryloylamino]propylcarbamoyl]vinyl)phenyl ester (3)*
- *Propionic acid 5-(2-[3-[3-(3,4-bis-propionyloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl]-2-cyanovinyl)-2-propionyloxyphenyl ester (4)*
- *Butyric acid 5-(2-[3-[3-(3,4-bis-butyryloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl]-2-cyanovinyl)-2-butyryloxyphenyl ester (5)*
- *Isobutyric acid 5-(2-[3-[3-(3,4-bis-isobutyryloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl]-2-cyanovinyl)-2-isobutyryloxyphenyl ester (6)*
- *Isobutyryloxy-carbonyl acid 5-(2-[3-[3-(3,4-bis-isobutyryloxy-carbonylphenyl)-2-cyanoacryloylamino]propylcarbamoyl]-2-cyanovinyl)-2-isobutyryloxy-carbonylphenyl ester (7)*
- *Hexanoic acid 5-(2-[3-[3-(3,4-bis-hexanoyloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl]-2-cyanovinyl)-2-hexanoyloxyphenyl ester (8)*
- *2,2-Dimethyl-propionic acid 5-[2-[3-[3-(3,4-bis-(2,2-dimethylpropionyloxy)phenyl)-2-cyanoacryloylamino]propylcarbamoyl]-2-cyanovinyl]-2-(2,2-dimethylpropionyloxy)phenyl ester (9)*
- *Dimethylcarbamic acid 5-(2-[3-[3-(3,4-bis-dimethylcarbamoyloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl]-2-cyanovinyl)-2-dimethylcarbamoyloxy phenyl ester (10)*

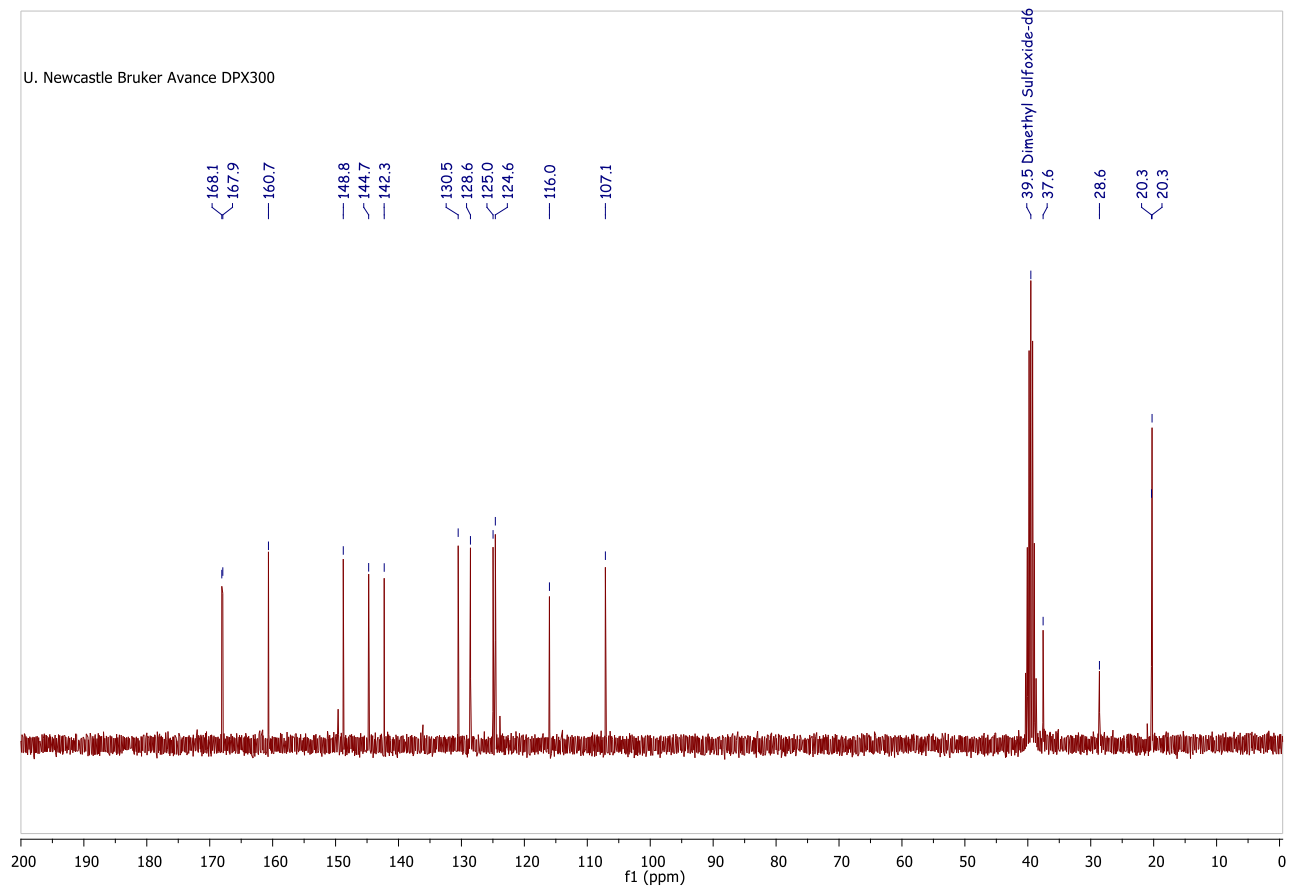
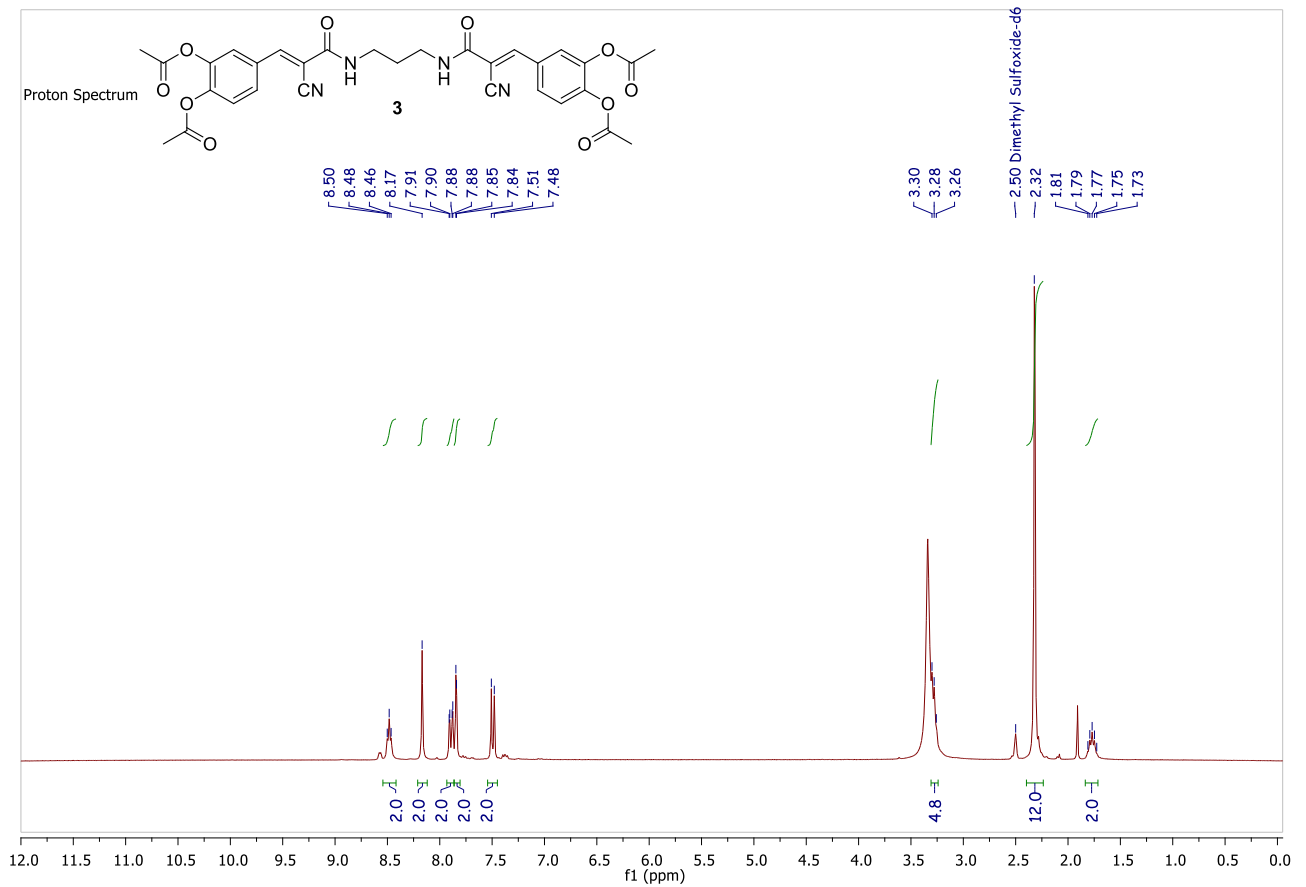
The effect of Bis-T-22 and Pro-Bis-T-22 (4) on the internalisation of EGF-TxR in HeLa cells;

The effect of 100 mM Bis-T-22 on TxR-Tfn endocytosis in seven cell lines

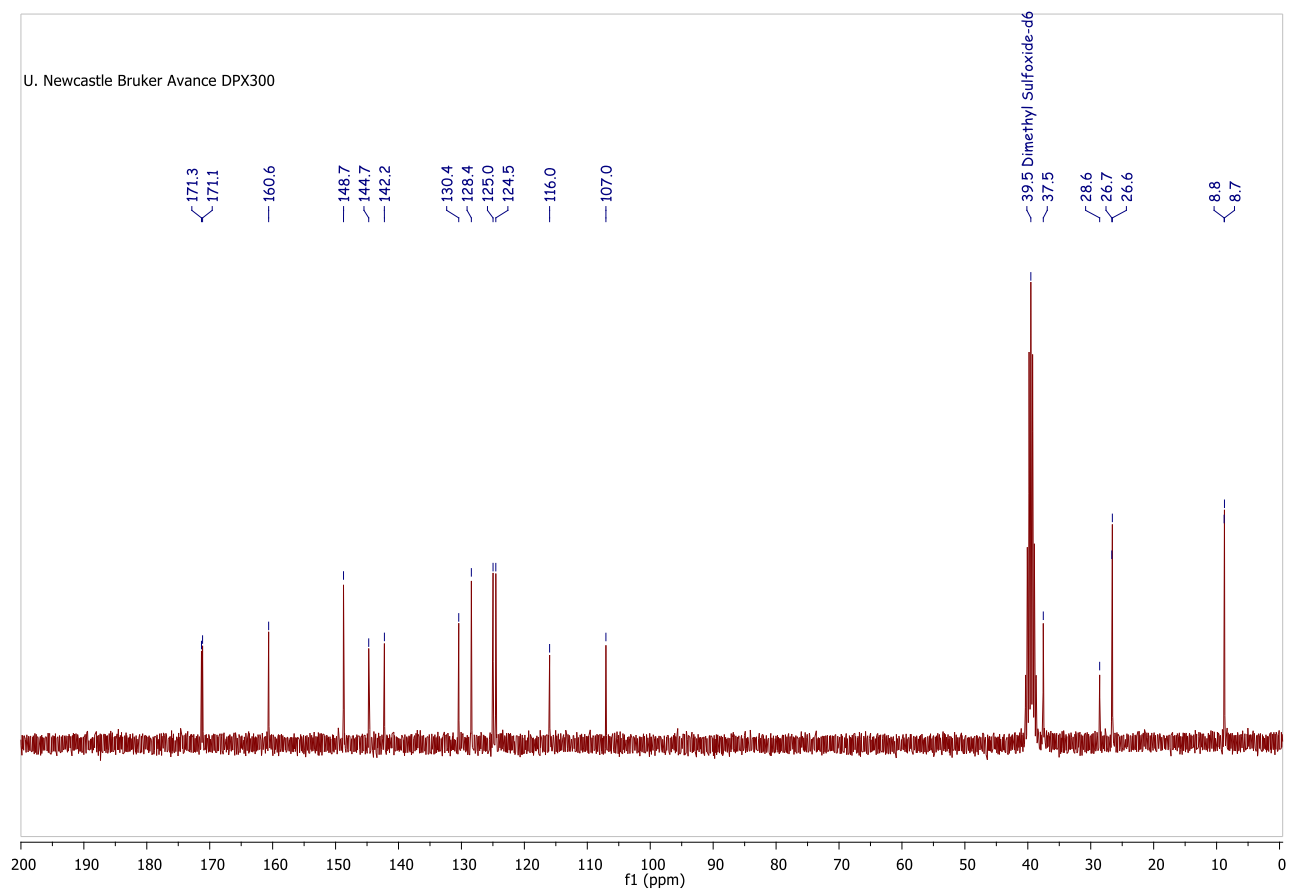
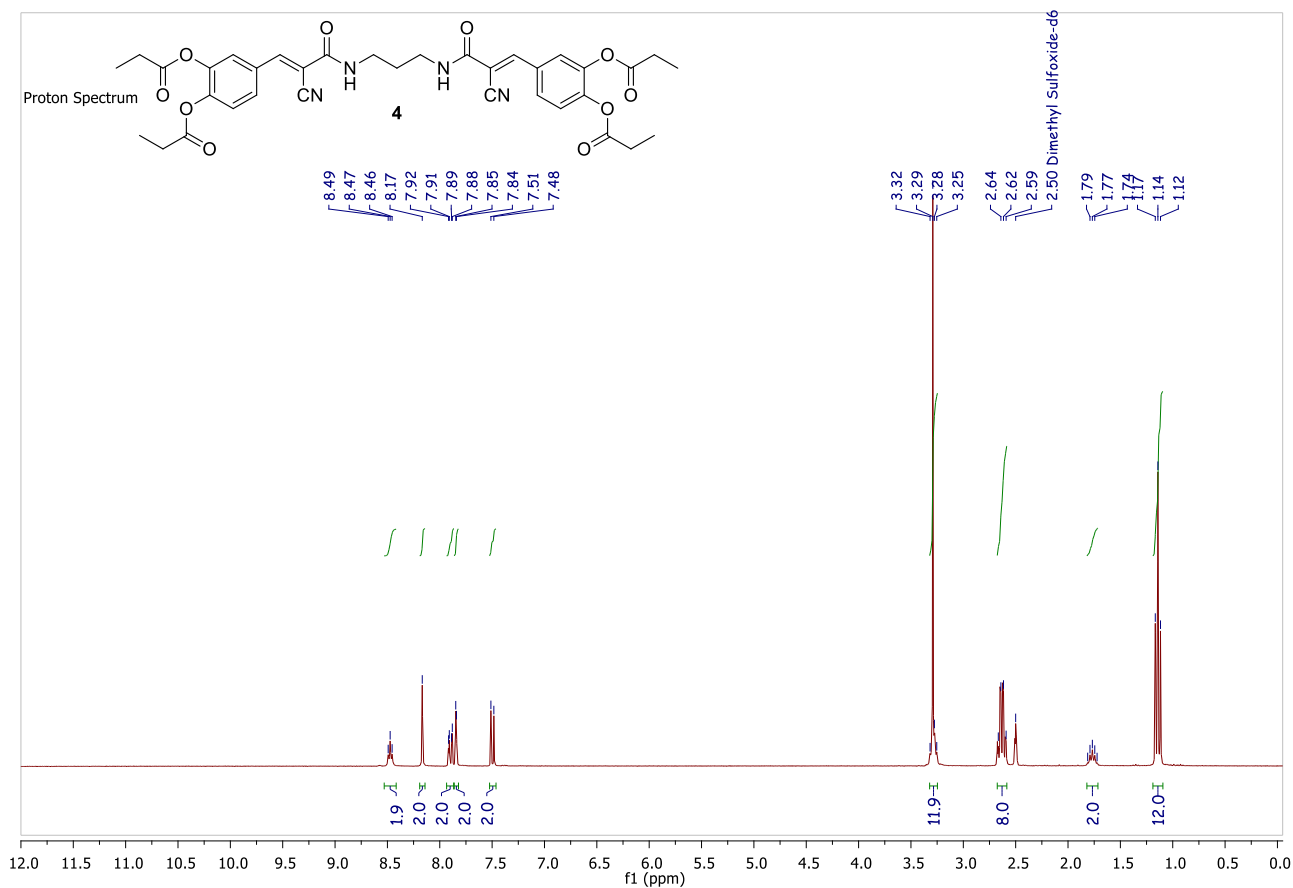
2-Cyano-N-{3-[2-cyano-3-(3,4-dihydroxyphenyl)acryloylamino]propyl}-3-(3,4-dihydroxyphenyl)acrylamide (**2**)



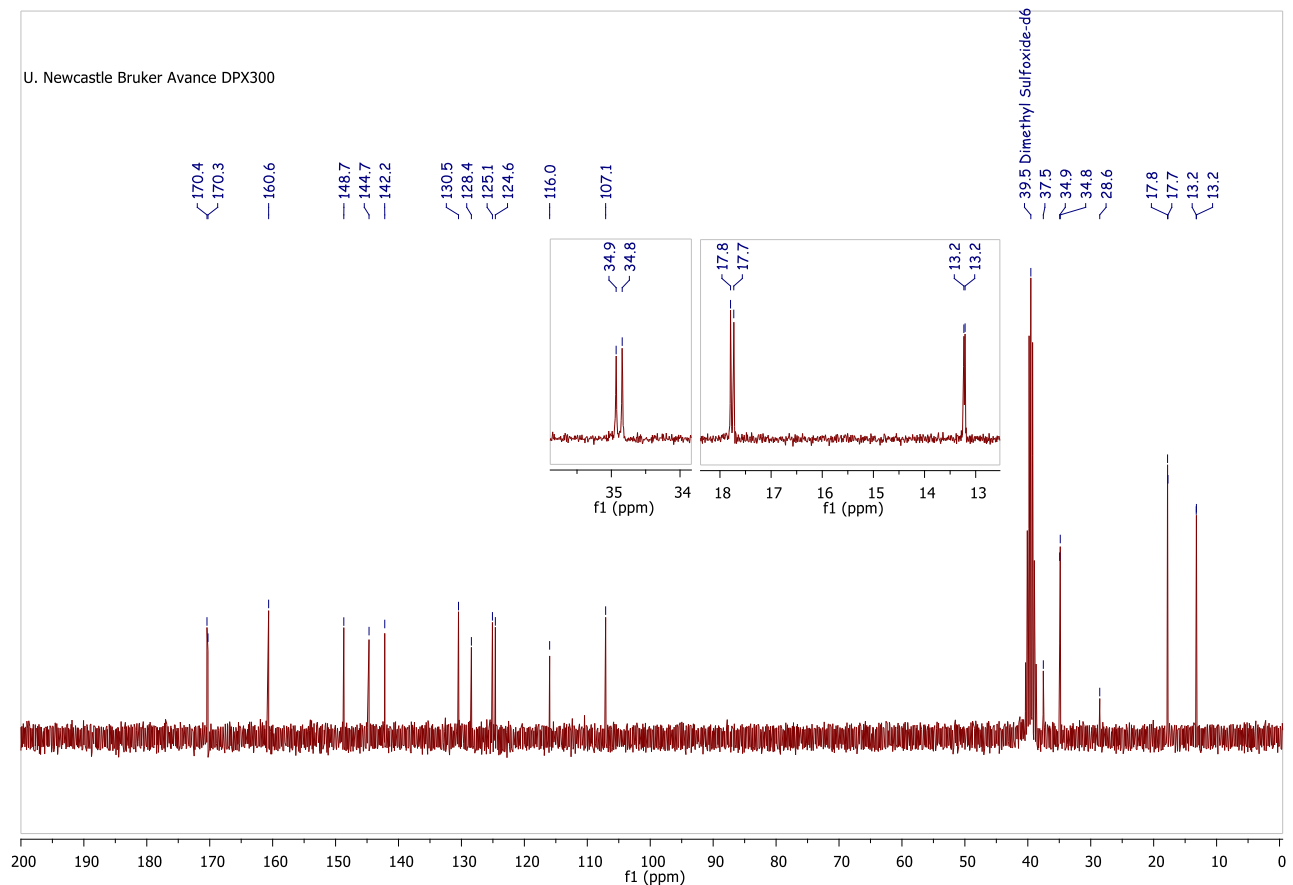
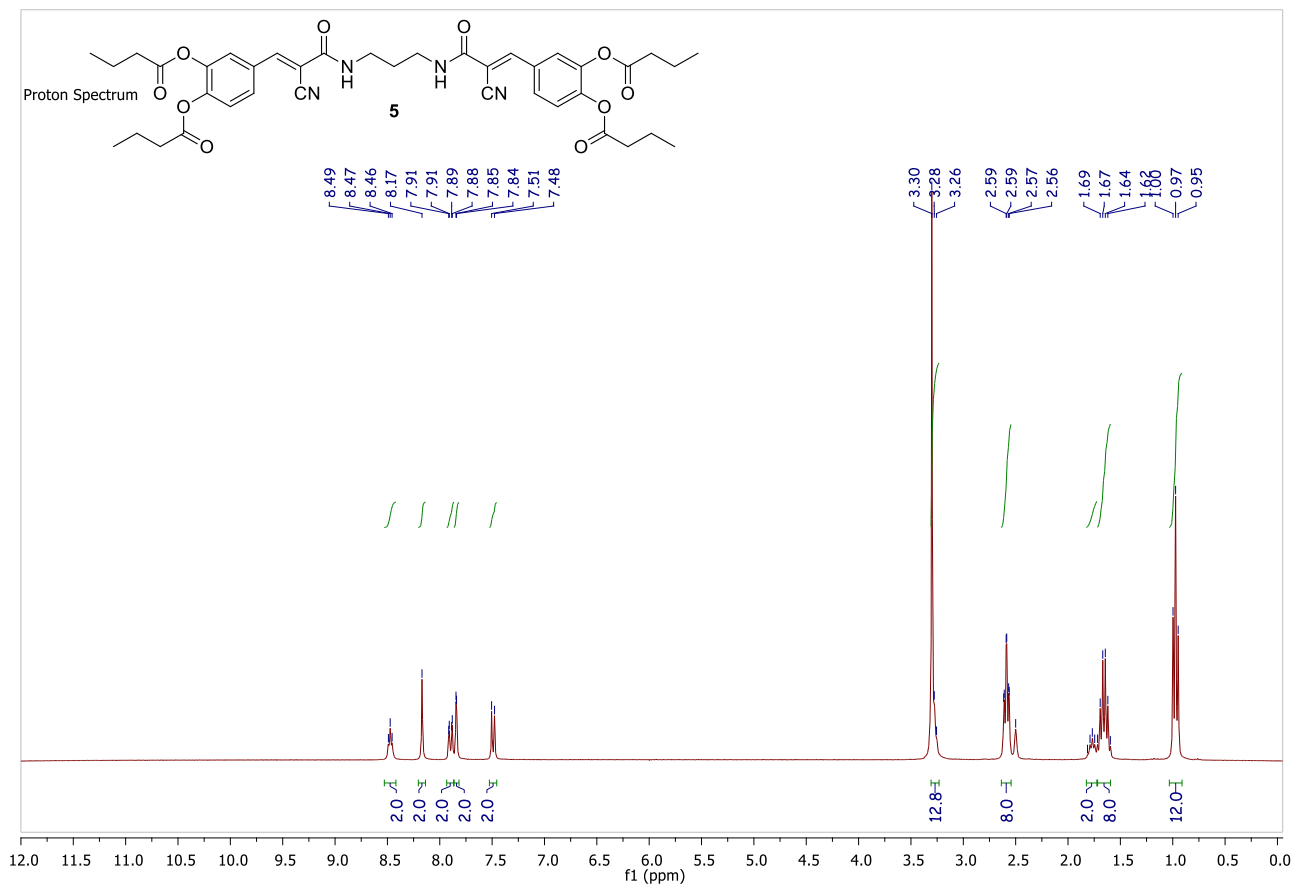
Acetic acid 2-acetoxy-5-(2-cyano-2-{3-[2-cyano-3-(3,4-diacetoxyphenyl)acryloylamino]propylcarbamoyl}vinyl)phenyl ester (**3**)



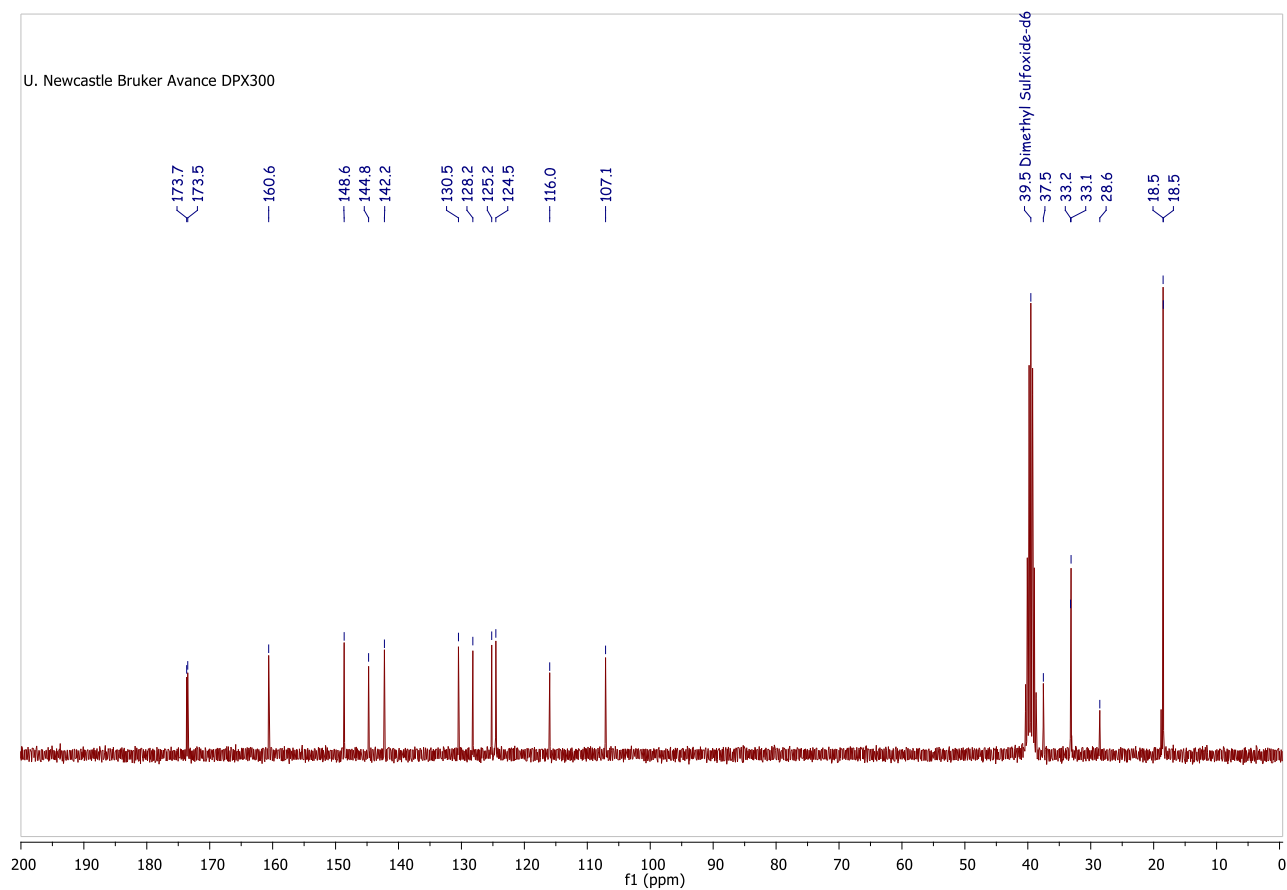
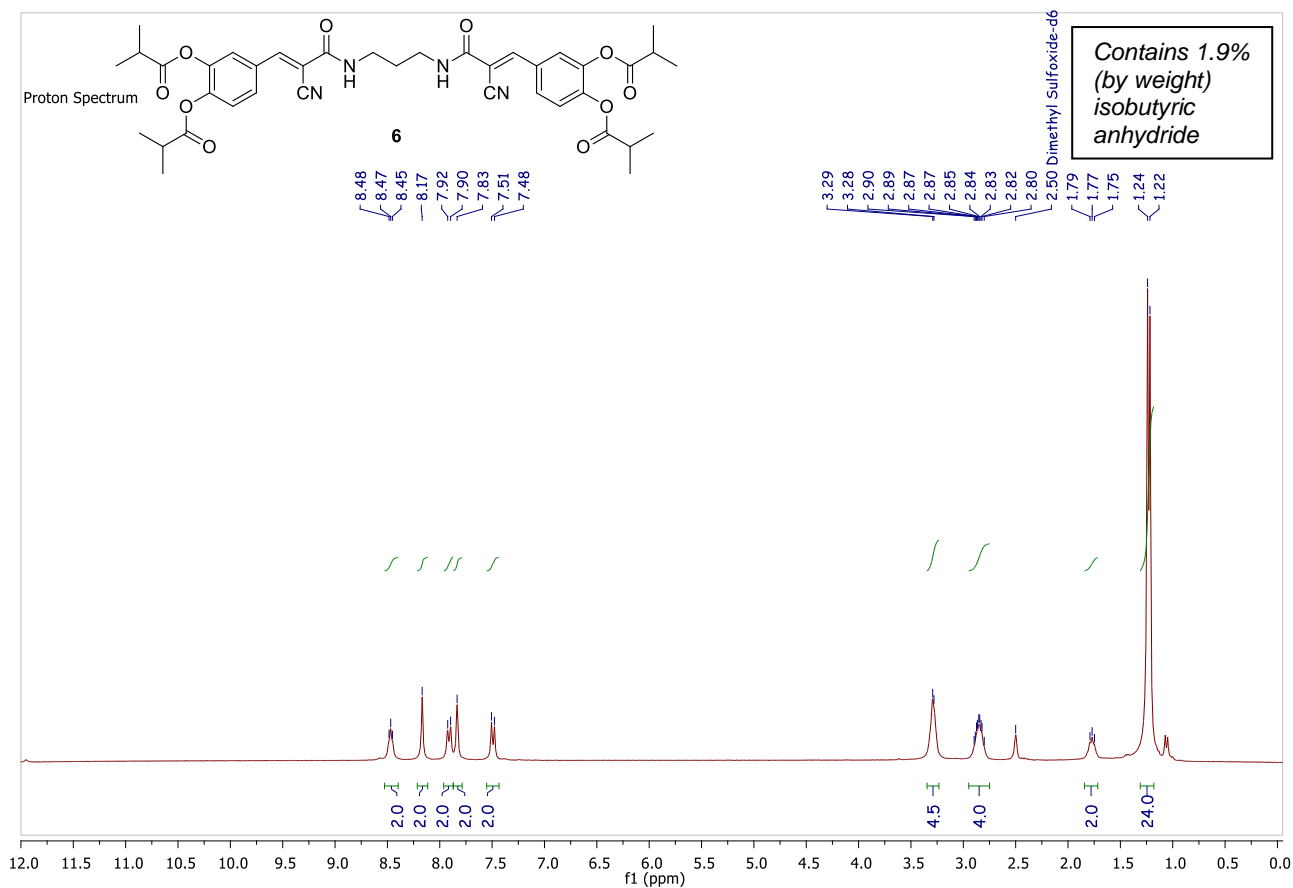
Propionic acid 5-(2-(3-(3,4-bis-propionyloxyphenyl)-2-cyanoacryloylamino)propylcarbamoyl)-2-cyanovinyl)-2-propionyloxyphenyl ester (**4**)



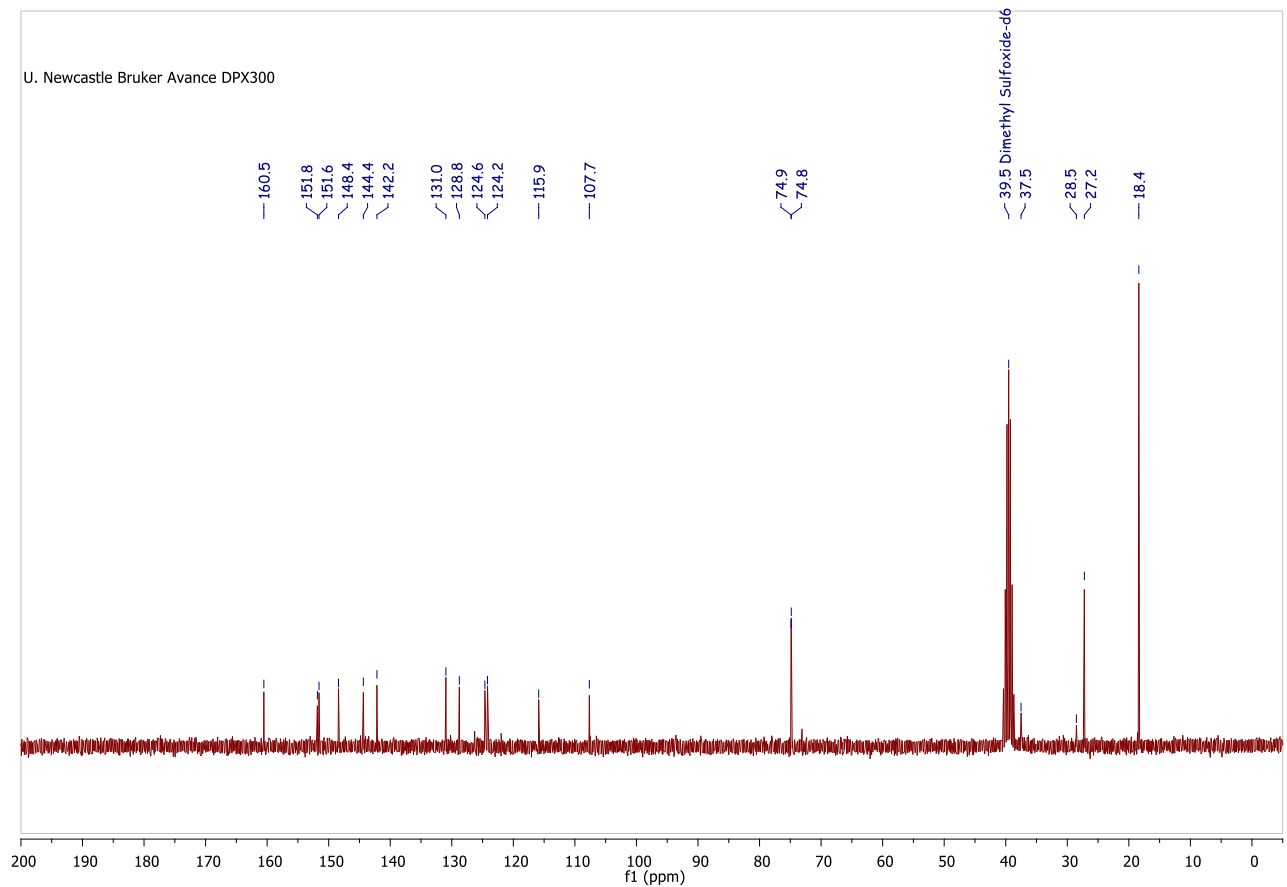
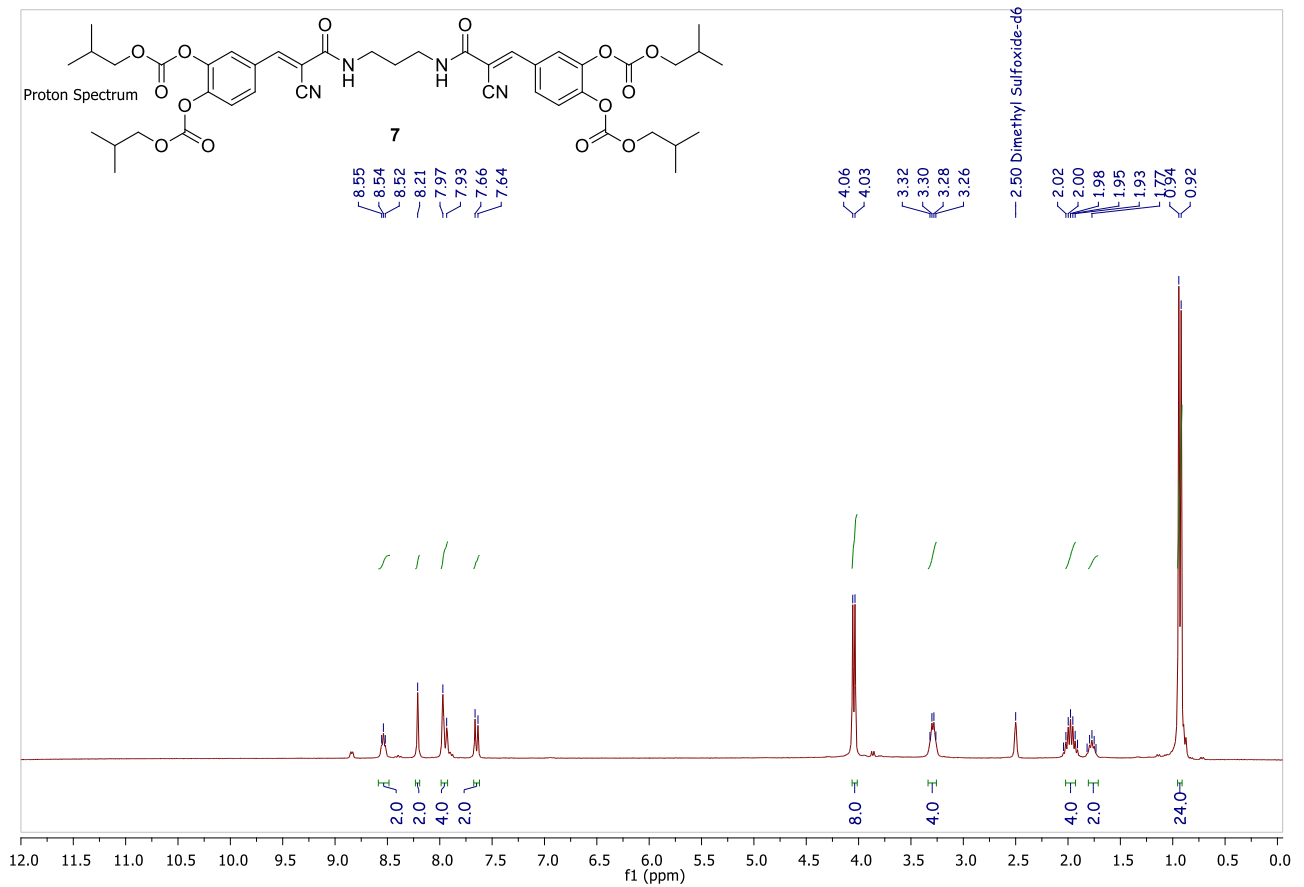
Butyric acid 5-(2-(3-(3,4-bis-butyryloxyphenyl)-2-cyanoacryloylamino)propylcarbamoyl)-2-cyanovinyl)-2-butyryloxyphenyl ester (5)



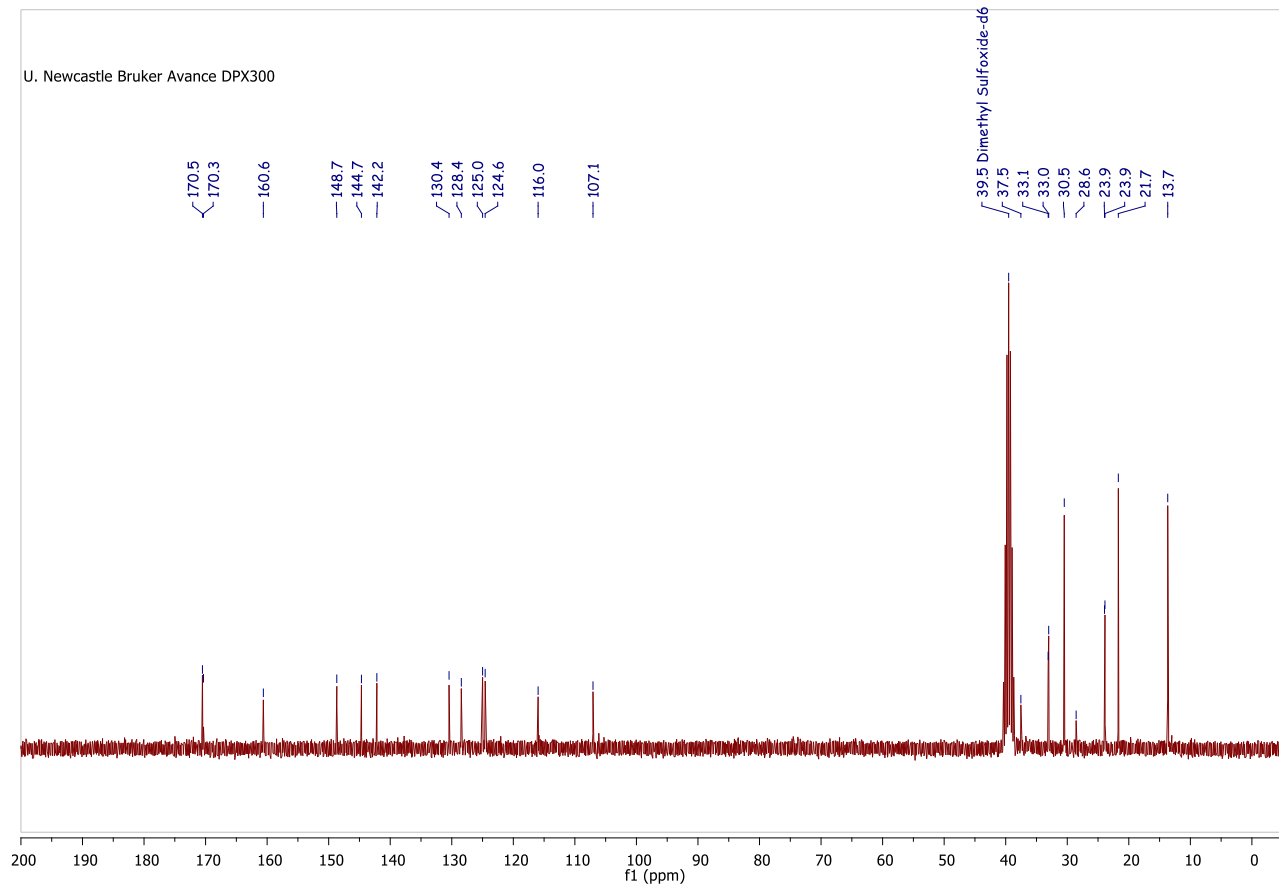
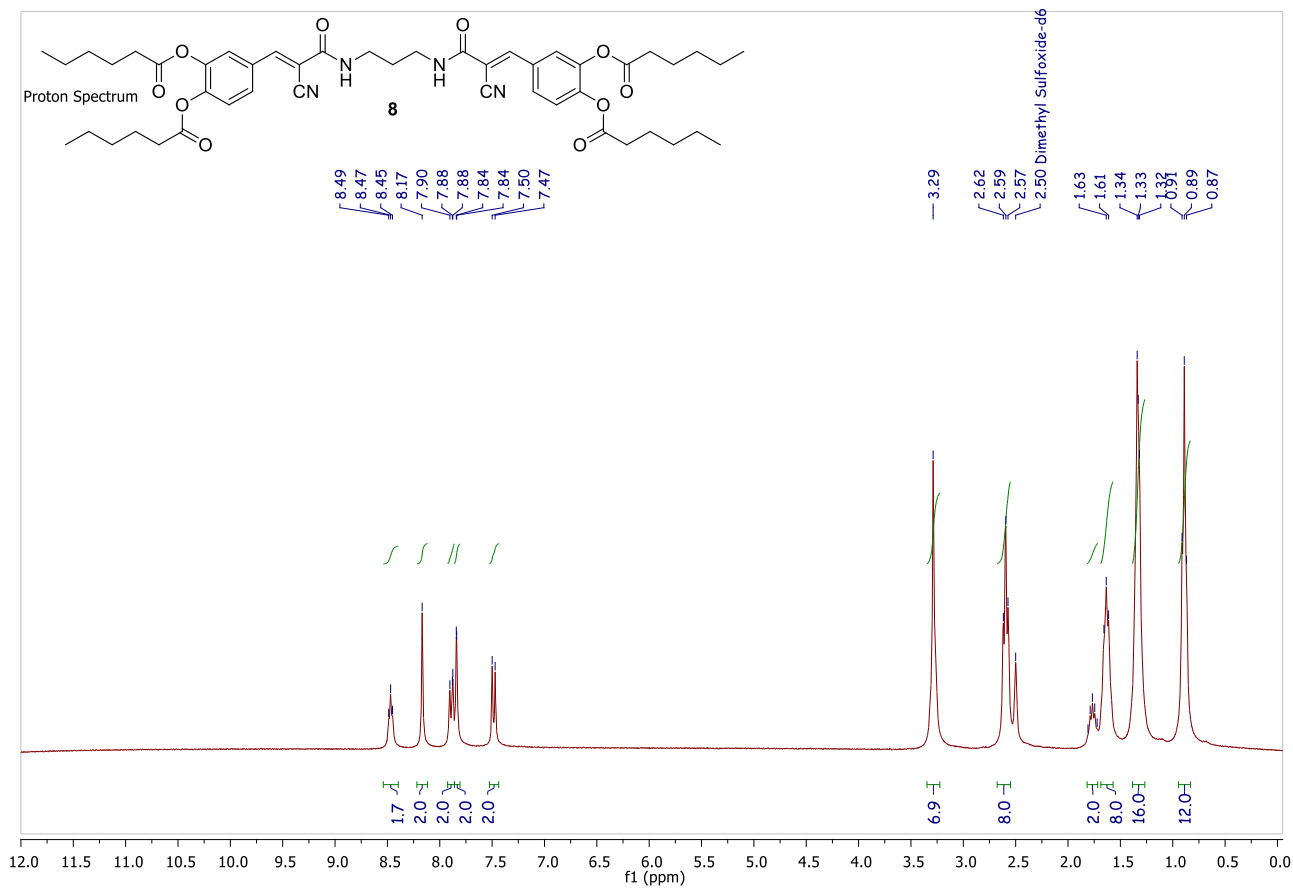
Isobutyric acid 5-(2-{3-[3-(3,4-bis-isobutyryloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl}-2-cyanovinyl)-2-isobutyryloxyphenyl ester (6)



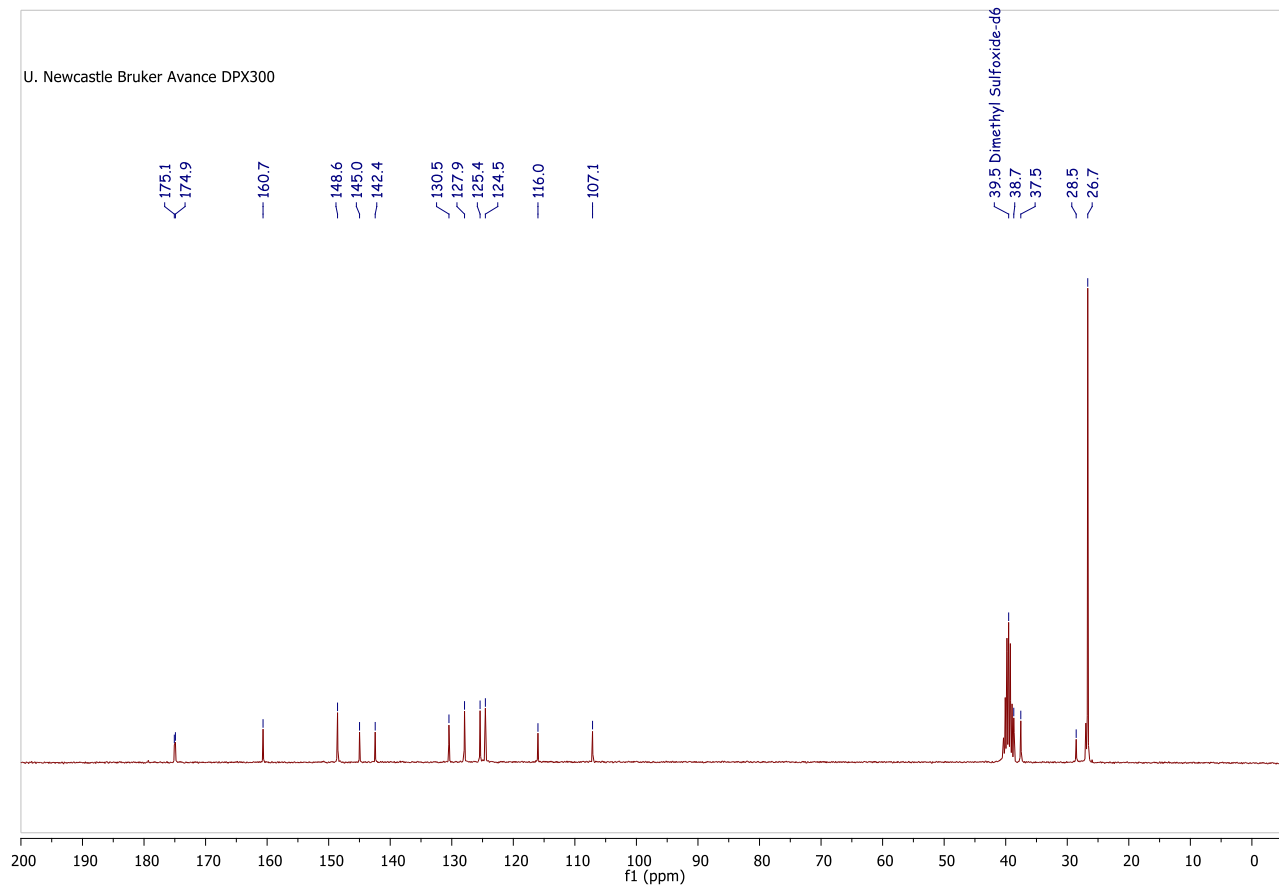
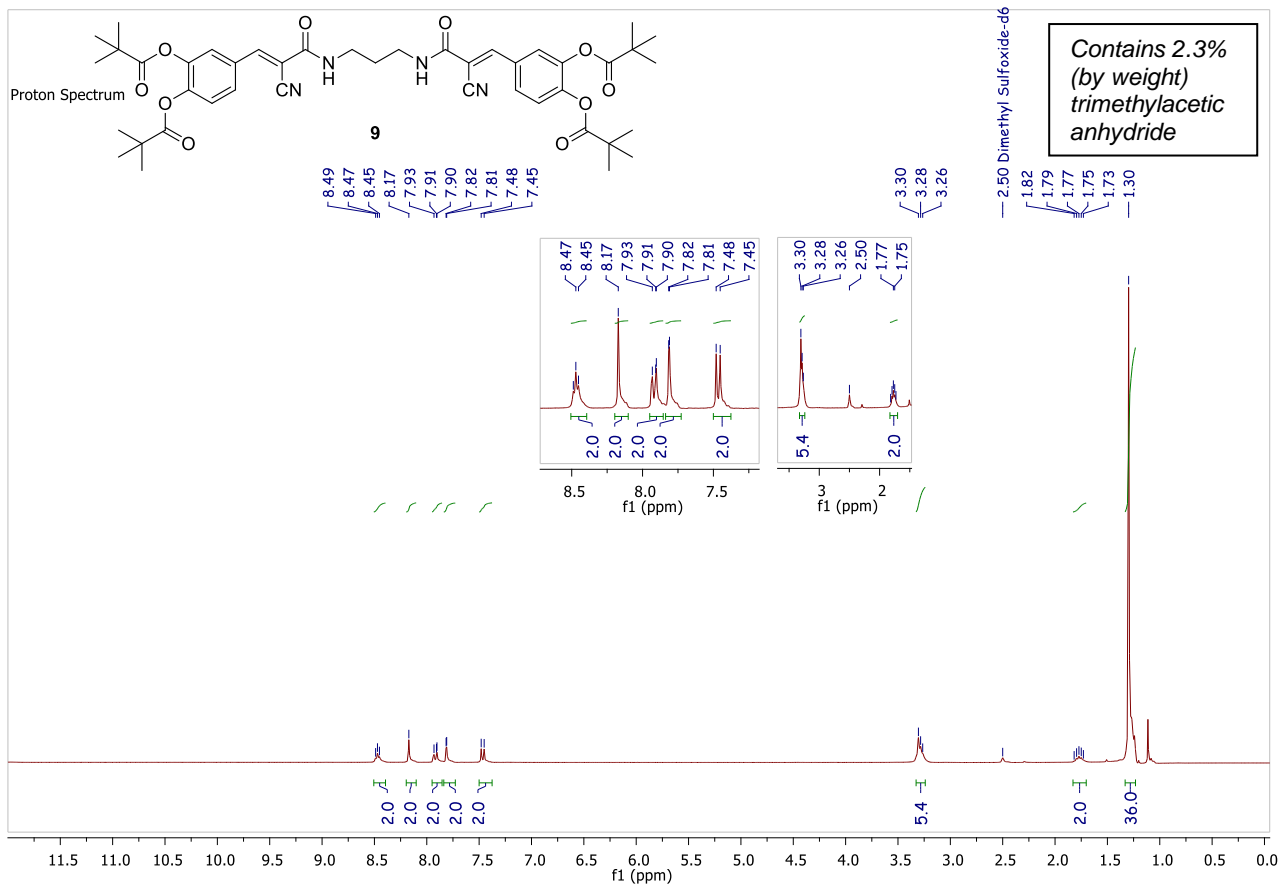
Isobutyloxylcarbonyl acid 5-(2-{3-[3-(3,4-bis-isobutyryloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl}-2-cyanovinyl)-2-isobutyryloxyphenyl ester (7)



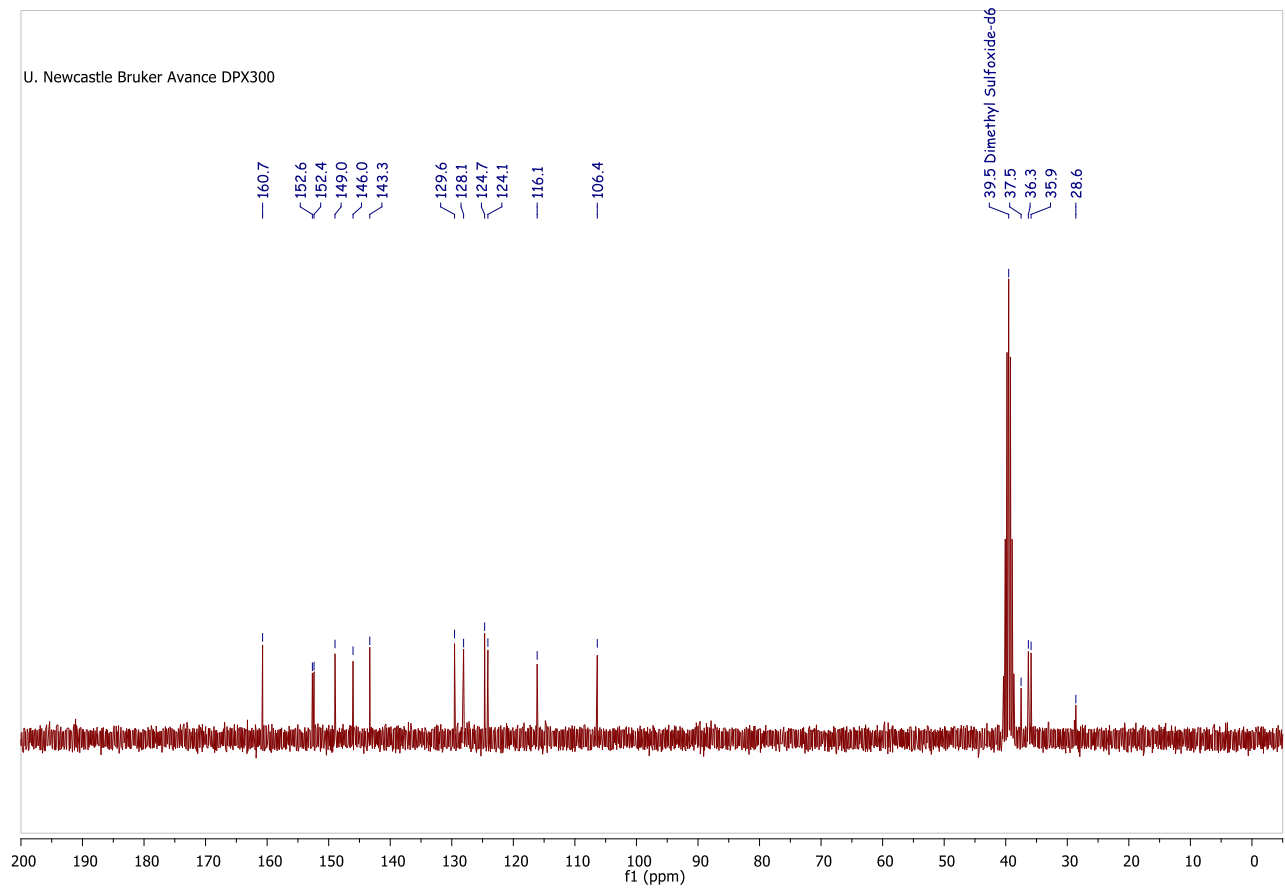
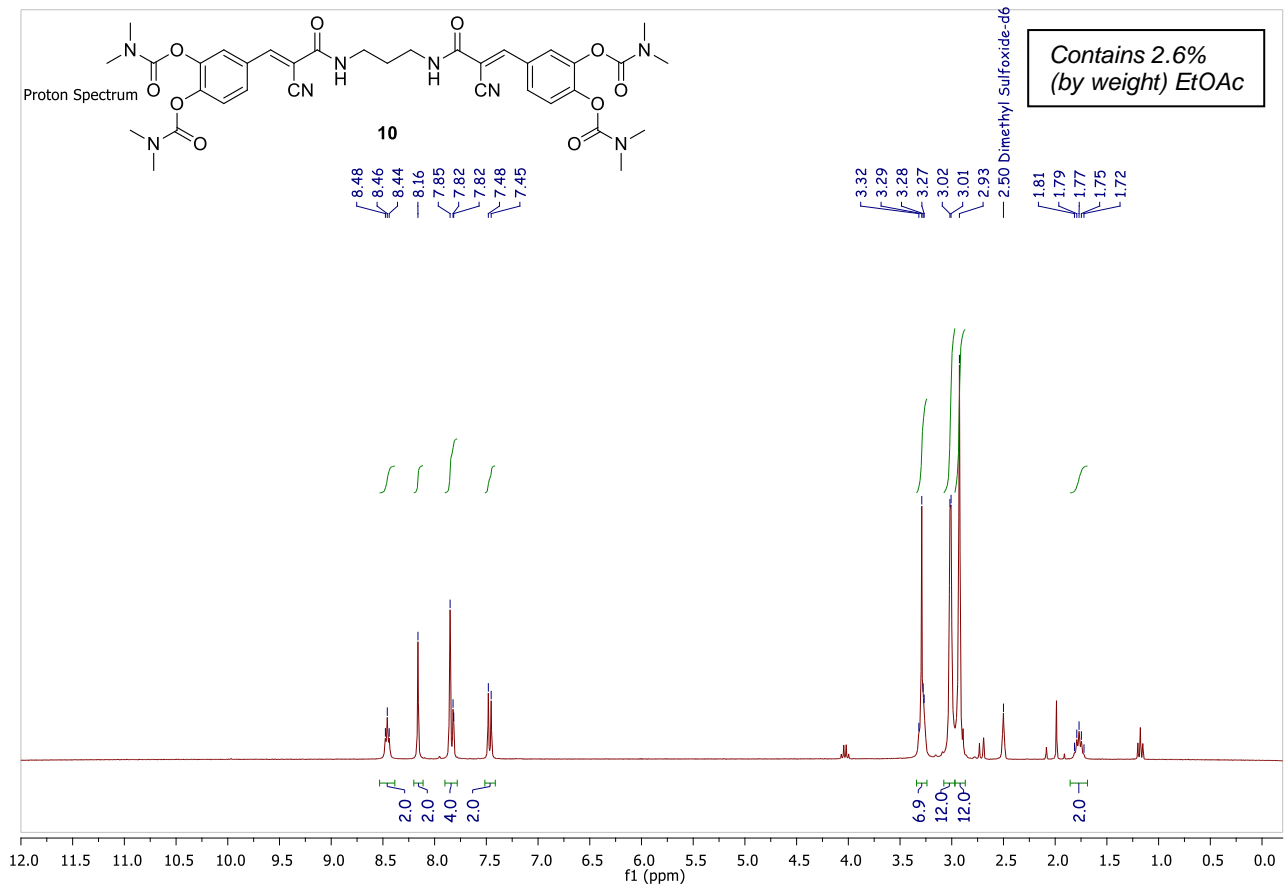
Hexanoic acid 5-(2-(3-(3,4-bis-hexanoyloxyphenyl)-2-cyanoacryloylamino)-propylcarbamoyl)-2-cyanovinyl)-2-hexanoyloxyphenyl ester (**8**)



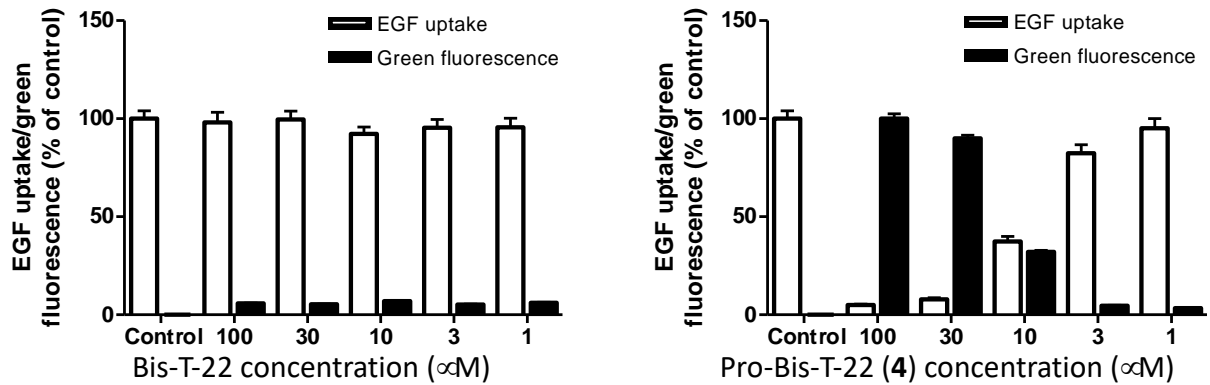
2,2-Dimethyl-propionic acid 5-[2-(3-{3-[3,4-bis-(2,2-dimethylpropionyloxy)phenyl]-2-cyanoacryloyl amino}propylcarbamoyl)-2-cyanovinyl]-2-(2,2-dimethylpropionyloxy)phenyl ester (9)



Dimethylcarbamic acid 5-(2-{3-[3-(3,4-bis-dimethylcarbamoyloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl}-2-cyanovinyl)-2-dimethylcarbamoyloxy phenyl ester (10)



Supplementary Figure S1



The effects of Bis-T-22 and (4) on internalisation of EGF-TxR in HeLa cells. Cultured cells were incubated with 1 μ g/ml EGF-TxR for 10 min at 37°C, acid washed, fixed and internalised Tfn-TxR detected by fluorescence microscopy.

The above figure shows the relationship between green fluorescent intensity and the level of EGF-TxR uptake in COS7 cells.

Left Panel: At all concentrations of Bis-T-22 there was no significant level of green fluorescence detected in cells and no effect of EGF uptake.

Right Panel: The level of green fluorescence within cells was proportional to the concentration of (4) and this was inversely related to the uptake of EGF.

Supplementary Table S1. The effect of 100 μ M Bis-T-22 (**2**) on the uptake of a transferrin red dye (TxR-Tfn) by endocytosis in a panel of 7 cell lines. The compound was present for 15 min (this Table) but the results were the same after 60 min drug exposure (not shown). The amount of TxR-Tfn uptake was visually assessed and score from 1 to 5 "+" symbols for the degree of inhibition.

Cell Line	Effect of Bis-T-22
Swiss 3T3	++++
HER14	++++
B104	+++
A431	++
B35	+
HeLa	+
COS-7	+

++++ = endocytosis fully blocked; + = endocytosis unaffected

Supplementary Table S2

Compound	HRMS Calculated for	Found
<i>Acetic acid 2-acetoxy-5-(2-cyano-2-{3-[2-cyano-3-(3,4-diacetoxyphenyl) acryloylamino]propylcarbamoyl}vinyl)phenyl ester (3)</i>	C ₃₁ H ₂₈ N ₄ O ₁₀ ; 616.1805	616.1823
<i>Propionic acid 5-(2-{3-[3-(3,4-bis-propionyloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl}-2-cyanovinyl)-2-propionyloxyphenyl ester (4)</i>	C ₃₅ H ₃₆ N ₄ O ₁₀ ; 672.2431	672.2444
<i>Butyric acid 5-(2-{3-[3-(3,4-bis-butyryloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl}-2-cyanovinyl)-2-butyryloxyphenyl ester (5)</i>	C ₃₉ H ₄₄ N ₄ O ₁₀ ; 728.3057	728.3071
<i>Isobutyric acid 5-(2-{3-[3-(3,4-bis-isobutyryloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl}-2-cyanovinyl)-2-isobutyryloxyphenyl ester (6)</i>	C ₃₉ H ₄₄ N ₄ O ₁₀ ; 728.3057	728.3062
<i>Isobutyryloxy carbonyl acid 5-(2-{3-[3-(3,4-bis-isobutyryloxy carbonylphenyl)-2-cyanoacryloylamino]propylcarbamoyl}-2-cyanovinyl)-2-isobutyryloxy carbonylphenyl ester (7)</i>	C ₄₇ H ₆₀ N ₄ O ₁₄ ; 904.4106	904.4119
<i>Hexanoic acid 5-(2-{3-[3-(3,4-bis-hexanoyloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl}-2-cyanovinyl)-2-hexanoyloxyphenyl ester (8)</i>	C ₄₇ H ₆₀ N ₄ O ₁₀ ; 840.4309	840.4322
<i>2,2-Dimethyl-propionic acid 5-[2-{3-[3-(3,4-bis-(2,2-dimethylpropionyloxy)phenyl]-2-cyanoacryloyl amino]propylcarbamoyl}-2-cyanovinyl]-2-(2,2-dimethylpropionyloxy)phenyl ester (9)</i>	C ₄₃ H ₅₂ N ₄ O ₁₀ ; 784.3683	784.3690
<i>Dimethylcarbamic acid 5-(2-{3-[3-(3,4-bis-dimethylcarbamoxyloxyphenyl)-2-cyanoacryloylamino]propylcarbamoyl}-2-cyanovinyl)-2-dimethylcarbamoxyloxy phenyl ester (10)</i>	C ₃₅ H ₄₀ N ₈ O ₁₀ ; 732.2867	732.2880