CHEMBIOCHEM

Supporting Information

Bioconjugation of Green Fluorescent Protein via an Unexpectedly Stable Cyclic Sulfonium Intermediate

Ramiz Nathani, Paul Moody, Mark E. B. Smith, Richard J. Fitzmaurice, and Stephen Caddick*[a]

cbic_201200231_sm_miscellaneous_information.pdf

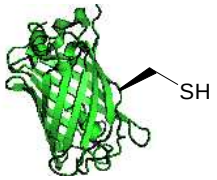
General Procedure

All reagents were purchased from Sigma-Aldrich and used without any purification. Solvents were purchased from Fisher and used without purification. 2,5-Dibromohexanediamide (**2**) was synthesized as reported by Davis.[1]

Protein Mass Spectroscopy

LC-MS was performed on protein samples using a Thermo Scientific uPLC connected to MSQ Plus Single Quad Detector (SQD). Column: Hypersil Gold C4 1.9µm 2.1 x 50 mm. Wavelength: 254 nm. Mobile Phase: 99:1 Water (0.1% formic acid): MeCN (0.1% formic acid) to 1:9 Water (0.1% formic acid): MeCN (0.1% formic acid) gradient over 4 min. Flow Rate: 0.3 mL/min. MS Mode: ES+. Scan Range: m/z = 500-2000. Scan time: 1.5 sec. Data obtained in continuum mode. The electrospray source of the MS was operated with a capillary voltage of 3.5 kV and a cone voltage of 50 V. Nitrogen was used as the nebulizer and desolvation gas at a total flow of 600 L/h. Total mass spectra for protein samples were reconstructed from the ion series using the pre-installed ProMass software using default settings for large proteins in m/z range 500-1500.

Cloning and expression of GFP(S147C) 6



6

Calculated mass = 29343

GFP(S147C) **6** was cloned and expressed as reported by Caddick and analysed by LCMS.[2]

Sequence:

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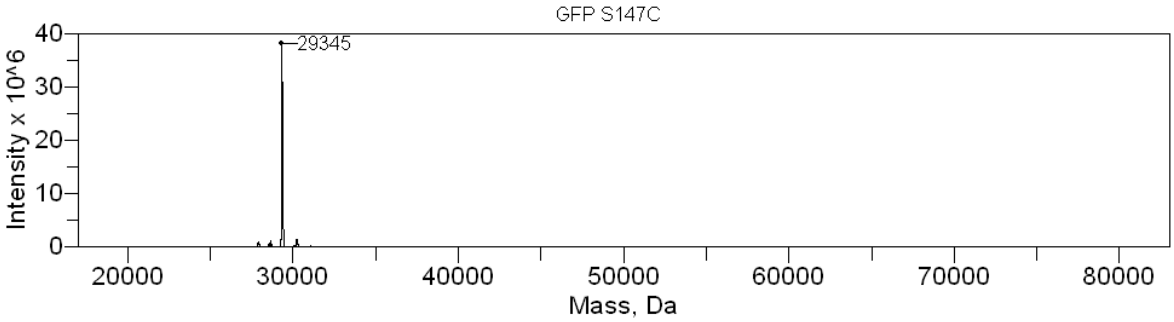
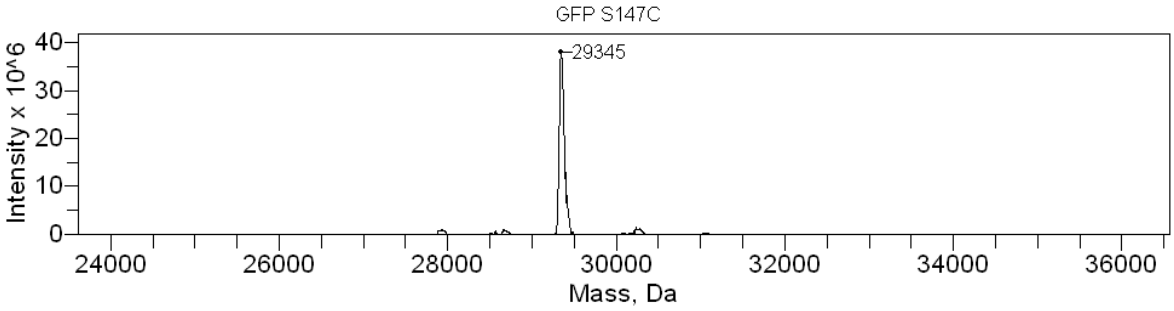
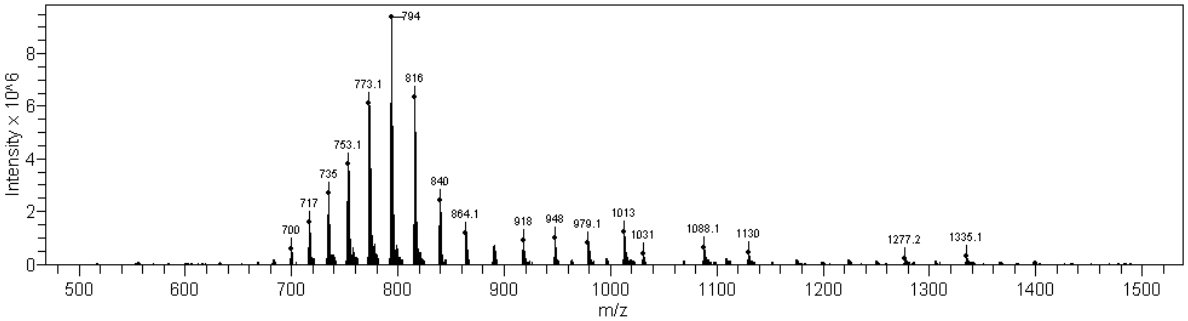
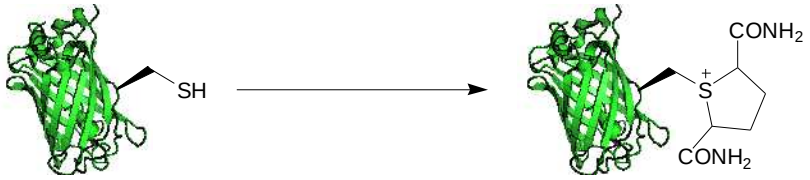






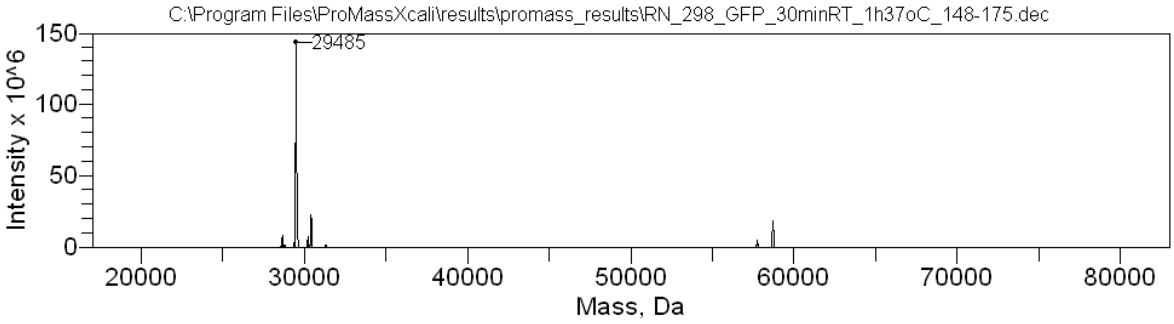
Reaction of GFP(S147C) with 2,5-dibromohexanediamide (2)

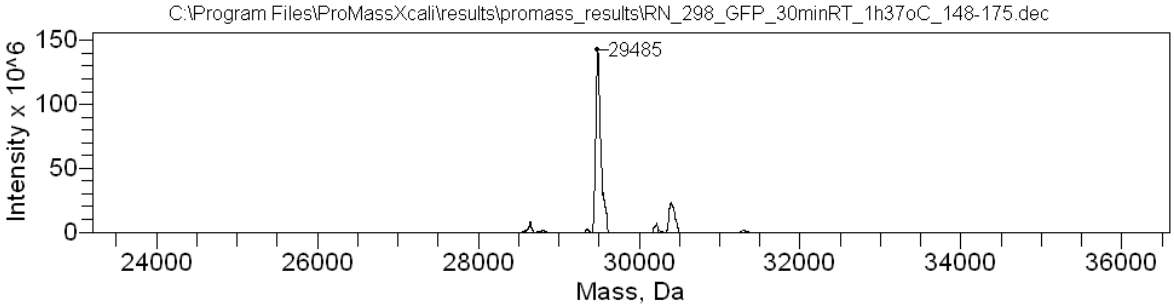


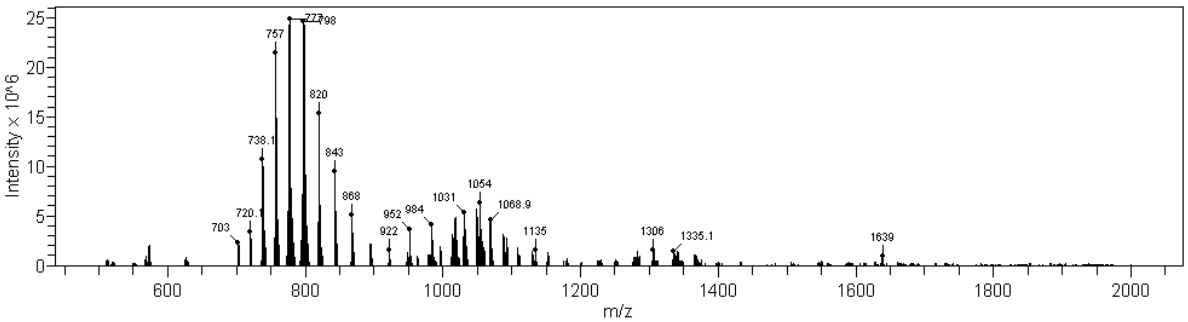
6 **8**

Calculated mass = 29343 Calculated mass = 29486

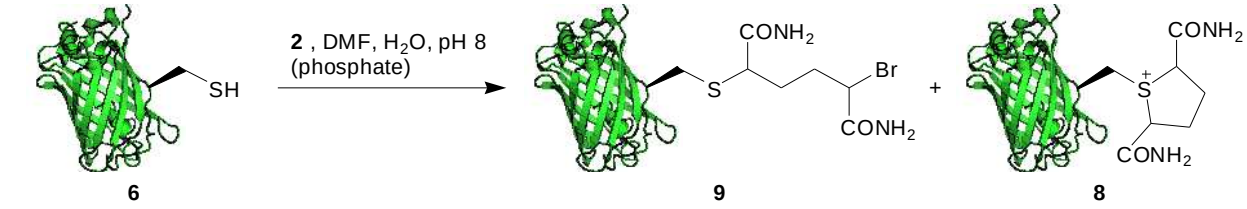
2,5-Dibromohexanediamide (**2**) (10 µL, 340 mM solution in DMF, 1000 equivalents) was added to a solution of GFP(S147C) (100 µL, 1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1 s and then maintained at 21 °C for 30 min. The solid precipitates were removed by centrifugation (1 min, 12K g) and the supernatant was heated to 37 °C for 1 h. The reaction was analyzed by LCMS. Sulphonium **8** formed in >95% conversion.







Optimization of formation of sulphonium 8



6 Calculated mass = 29343

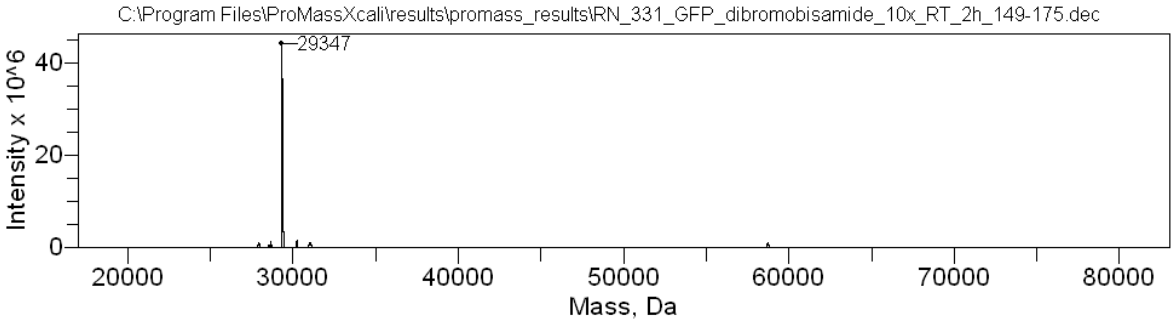
9 Calculated mass = 29566

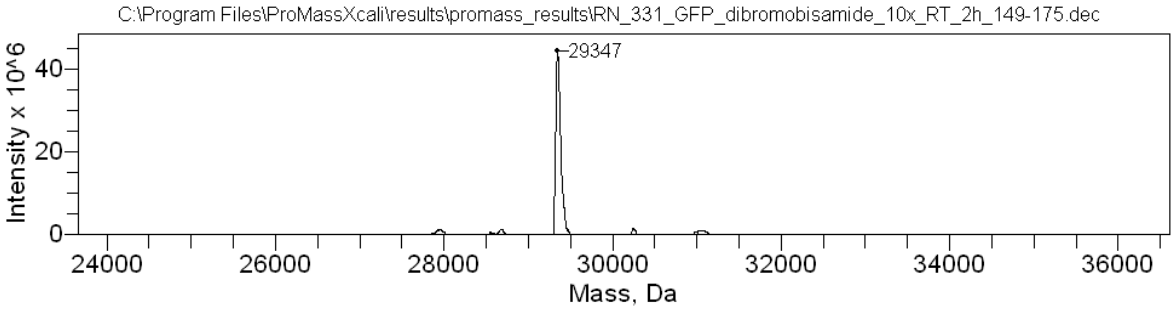
8 Calculated mass = 29486

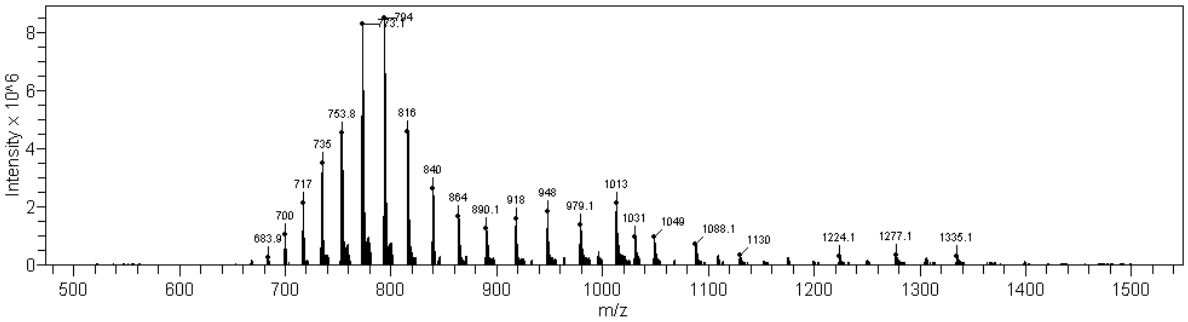
2,5-Dibromohexanediamide (**2**) (10, 25 or 50 equivalents), as a solution in DMF (** mL), was added to a solution of GFP(S147C) (100 µL, 1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1 s, maintained at the required temperature (4 °C, 21 °C or 37 °C) for the prescribed time (2 h, 5 h or 20 h) and analyzed by LCMS.

<u>10 equivalents, 21 °C</u>

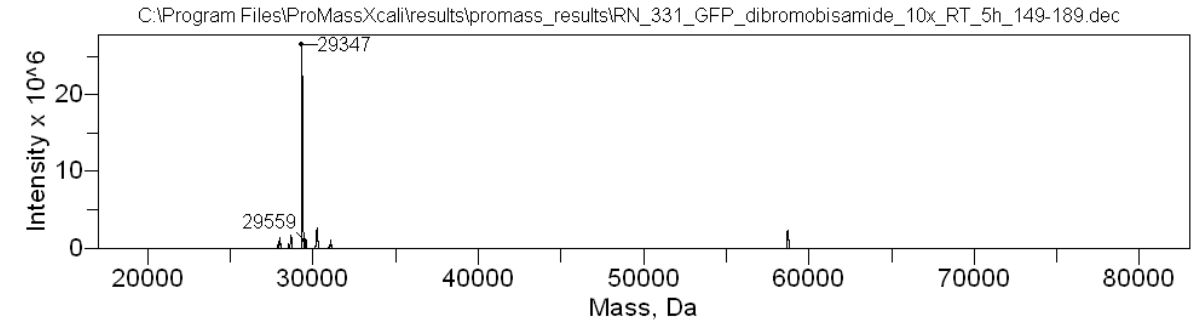
2 h







5 h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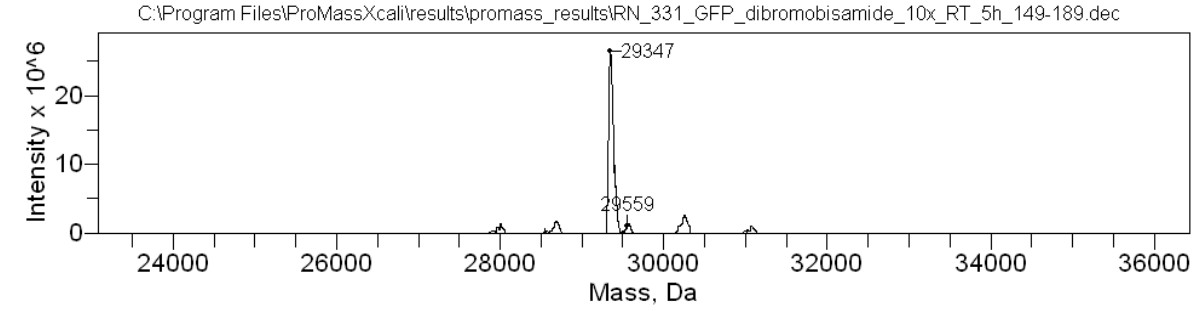
Mass, Da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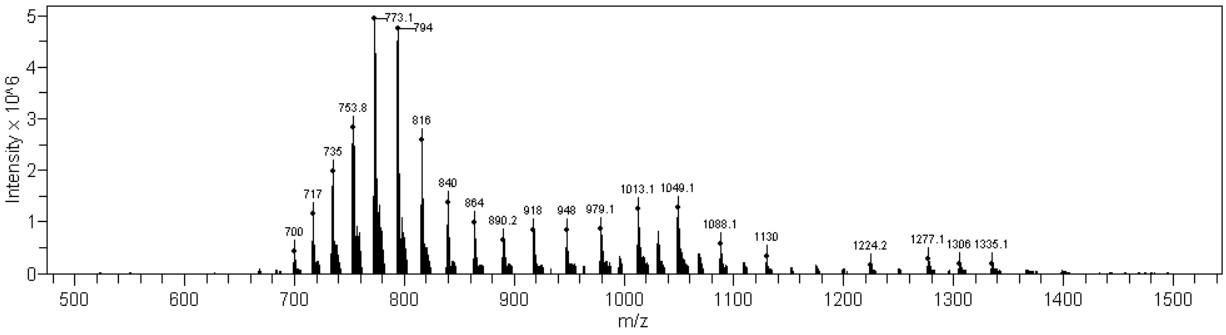
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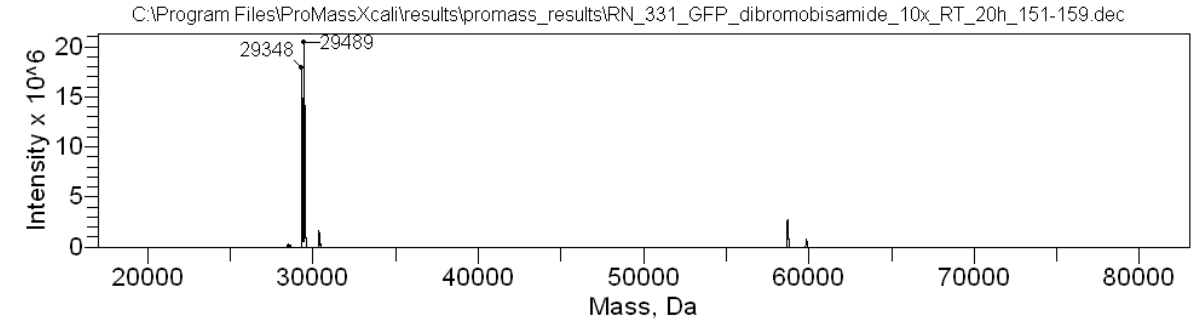
Intensity x 10^6
m/z

20 h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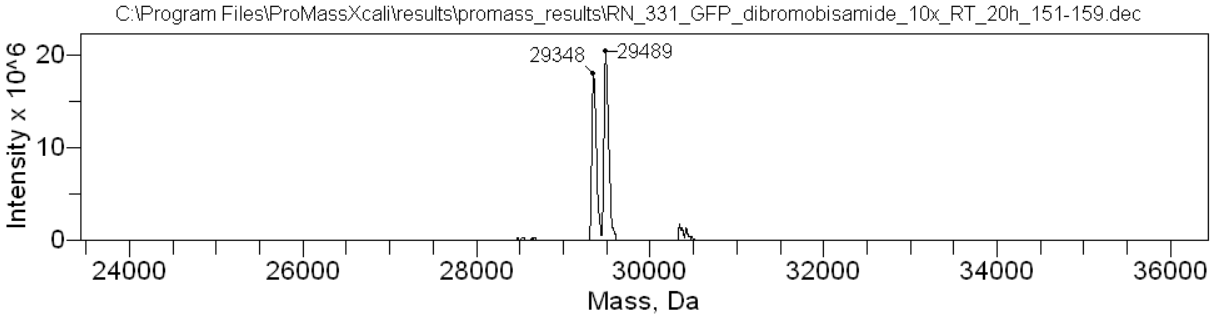
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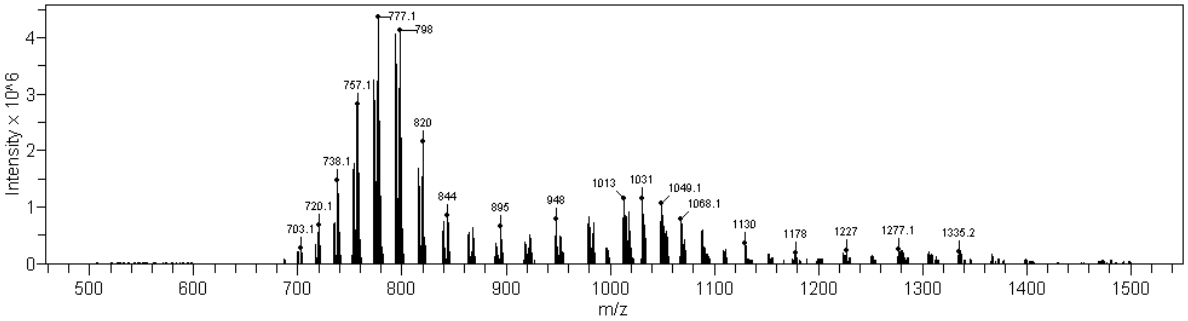
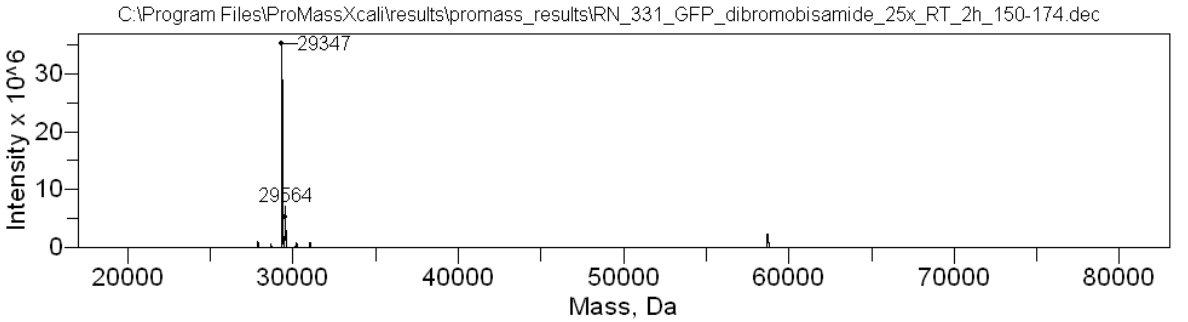
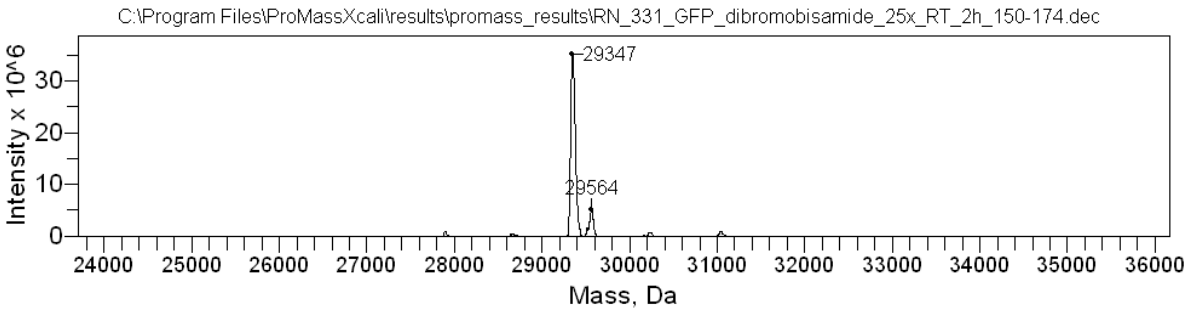
Mass, Da

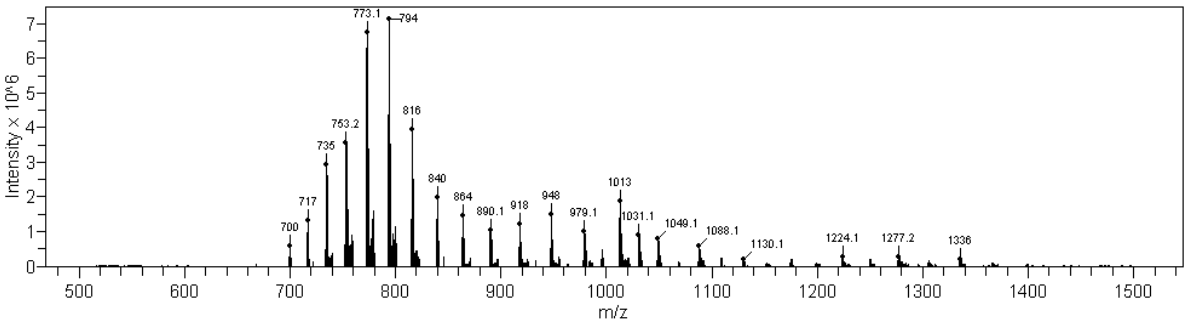


25 equivalents, 21 °C

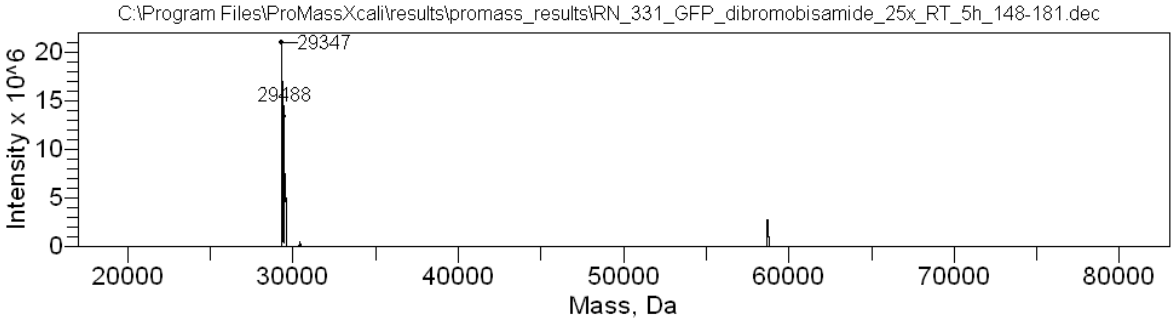
2 h







5 h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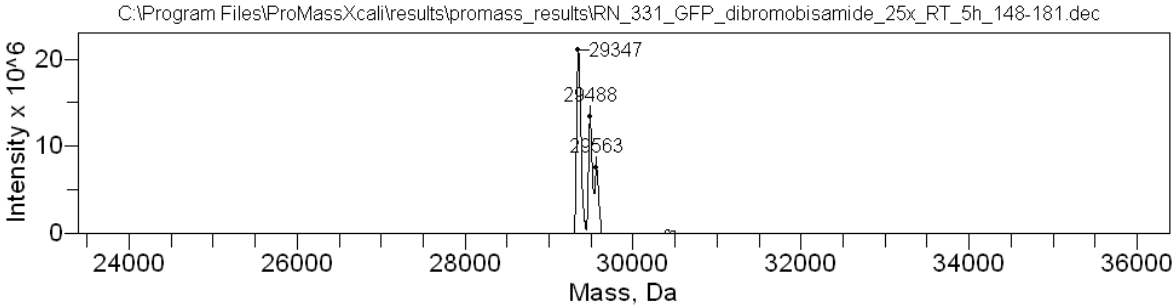
Mass, Da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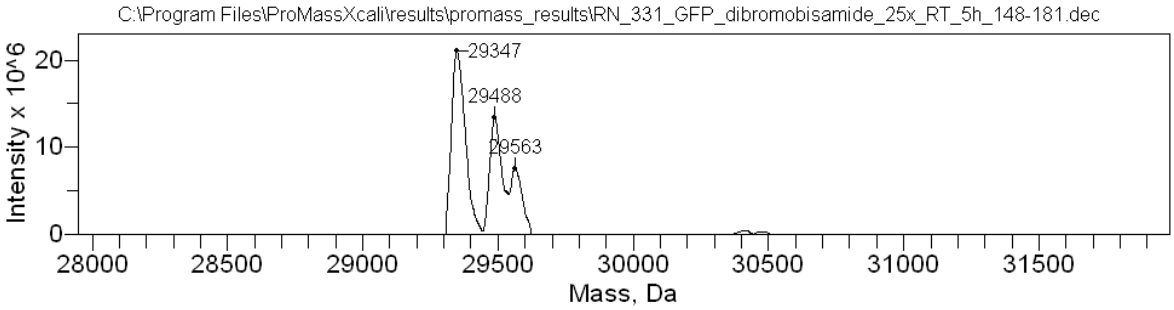
Intensity x 10^6
Mass, Da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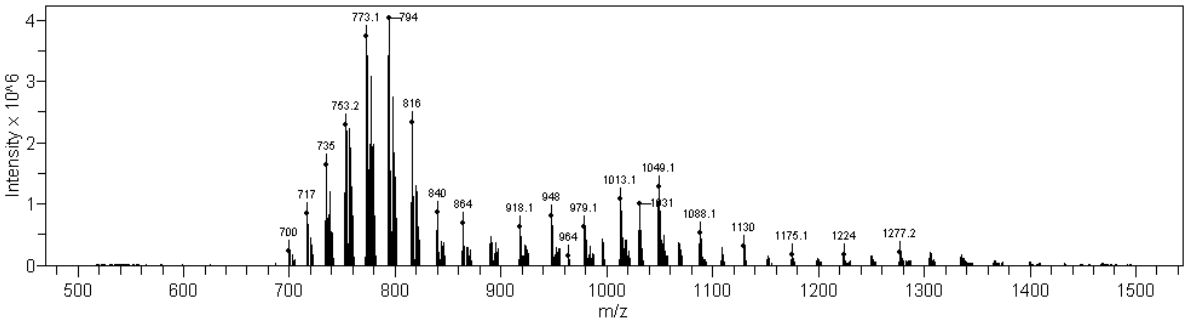
Intensity x 10^6
Mass, Da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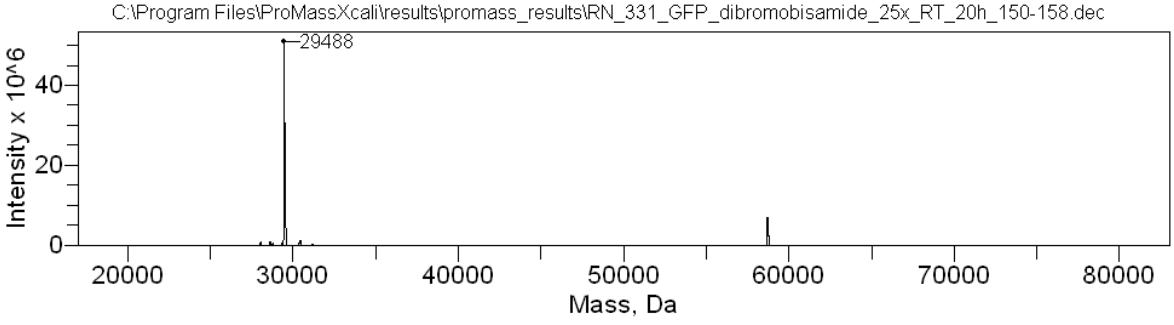
Intensity x 10^6
m/z

20 h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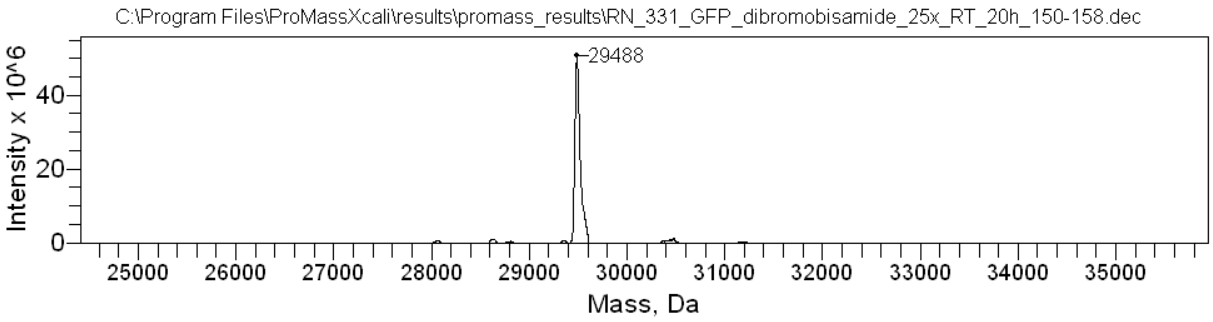
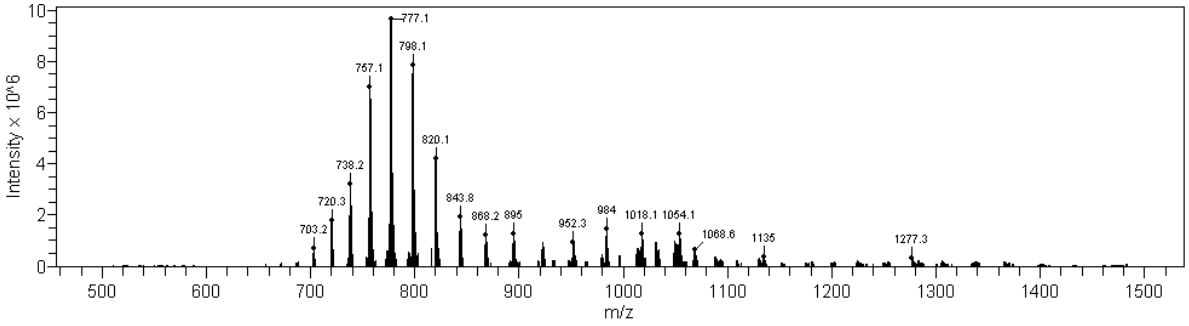
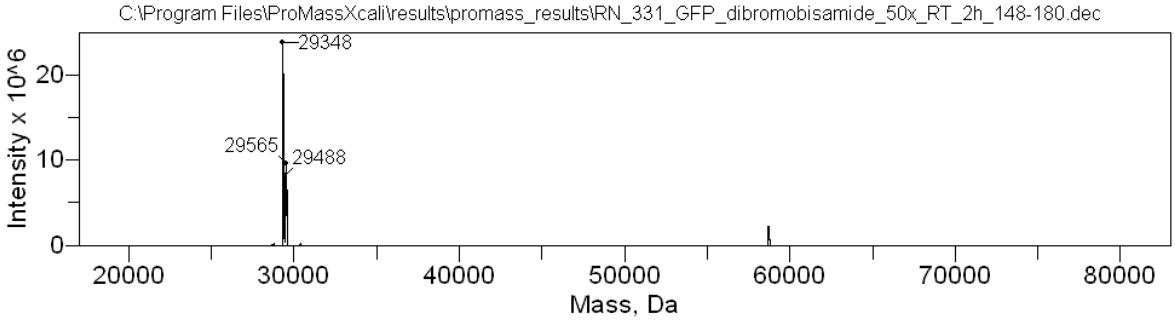
Mass, Da

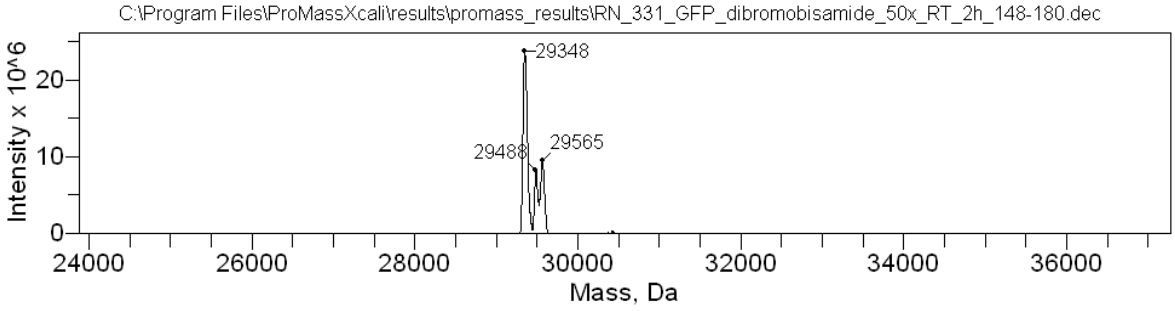


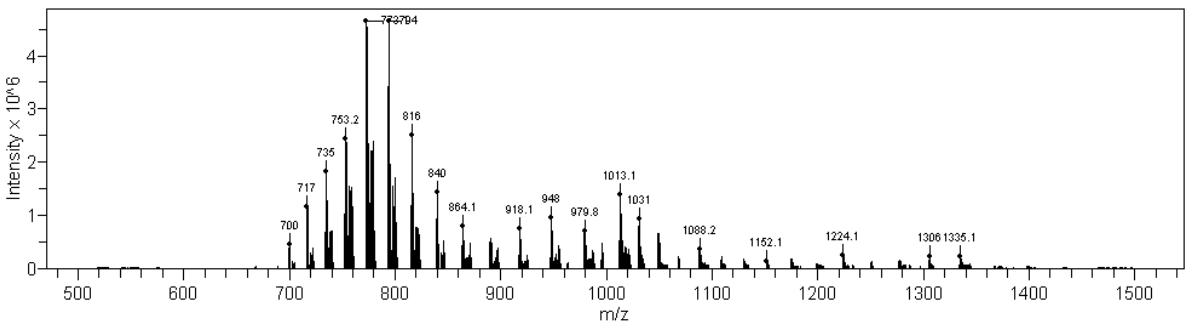


50 equivalents, 21 °C

2 h







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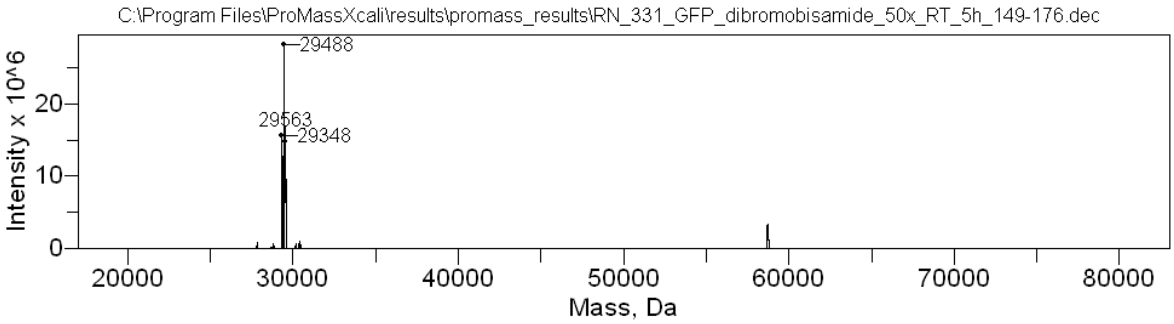
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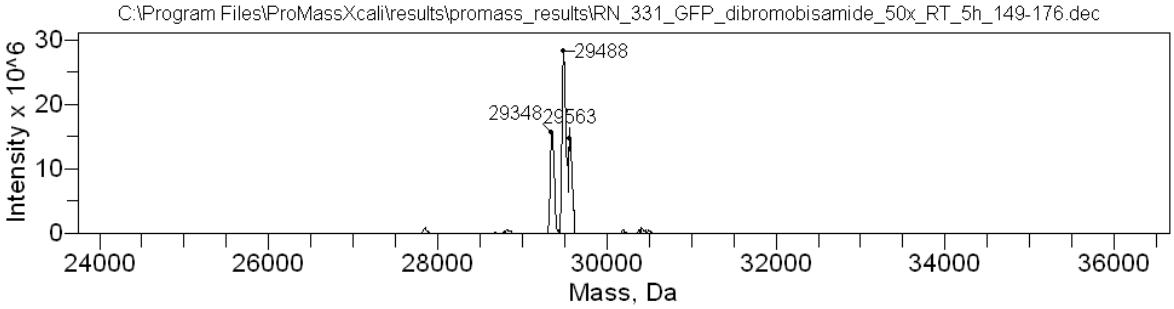
Mass, Da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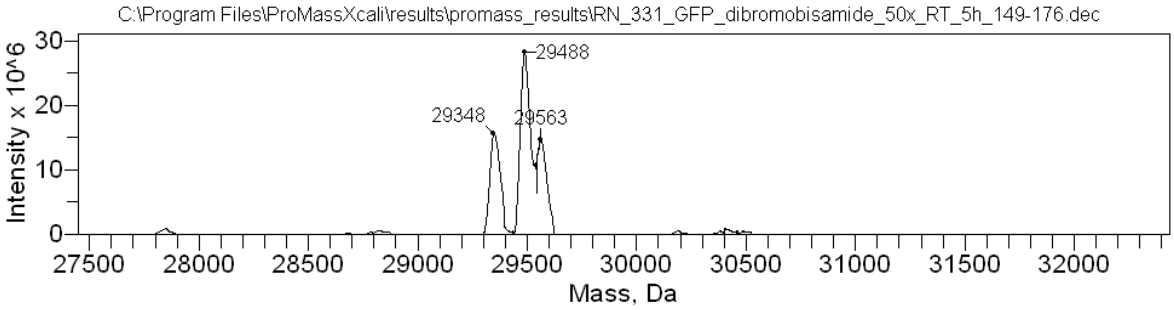
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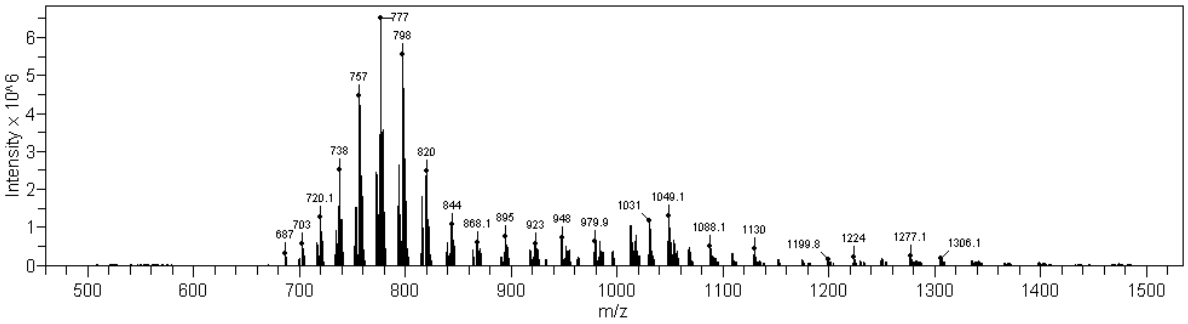
Mass, Da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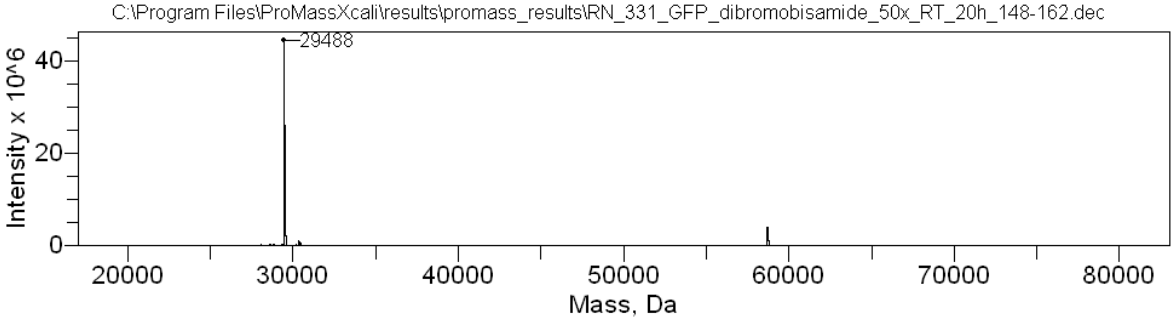
Intensity x 10^6
m/z

20 h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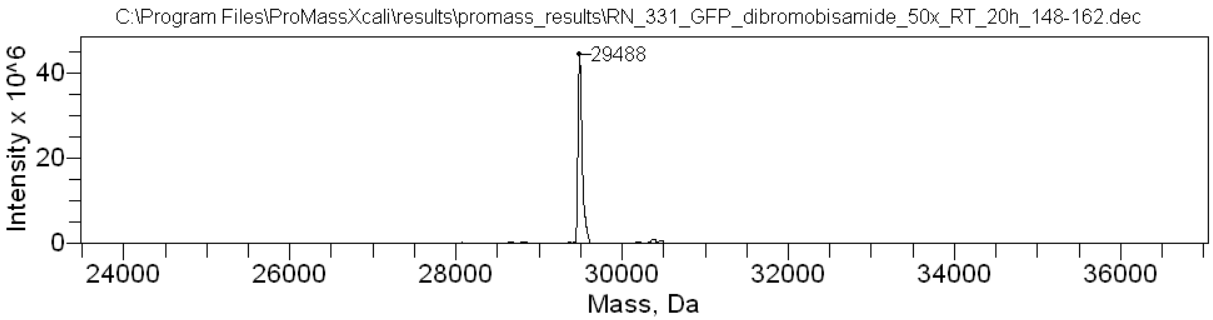
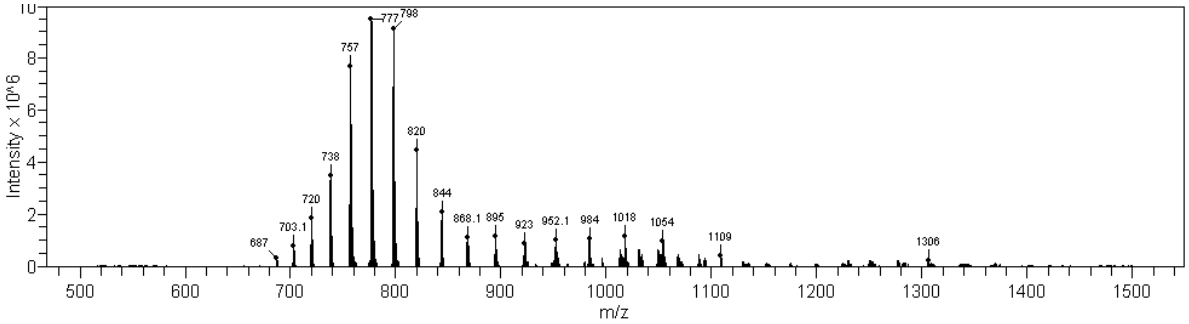
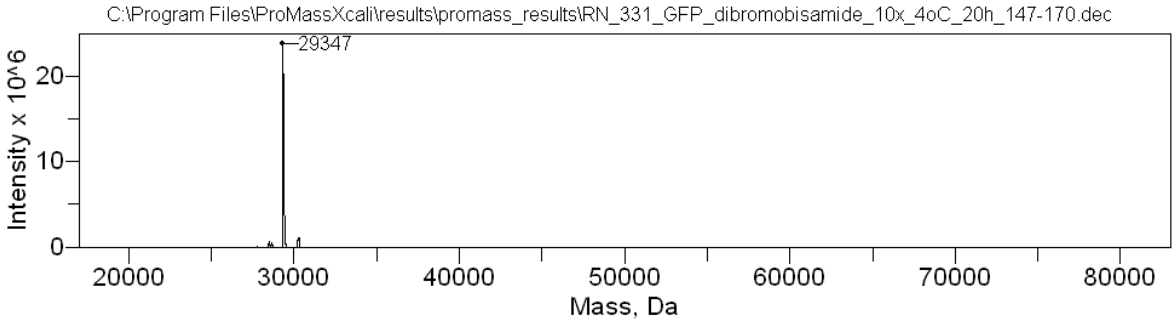
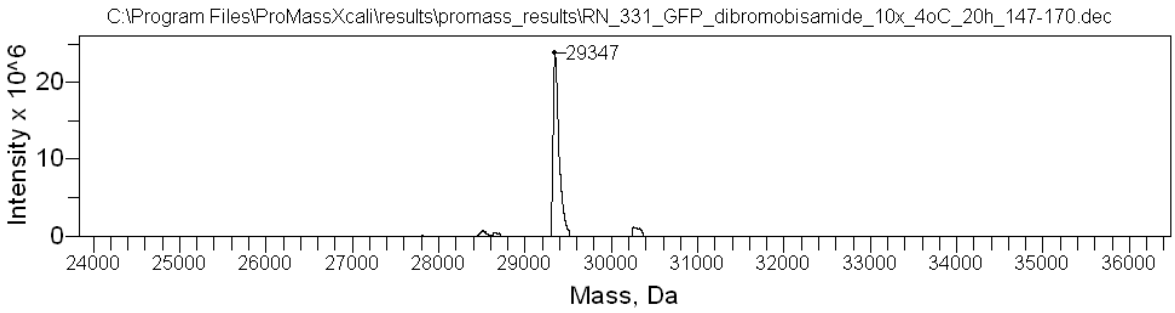
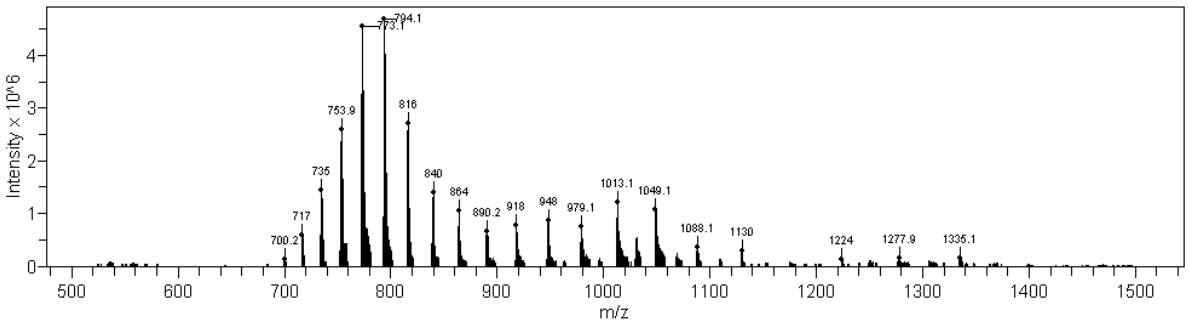




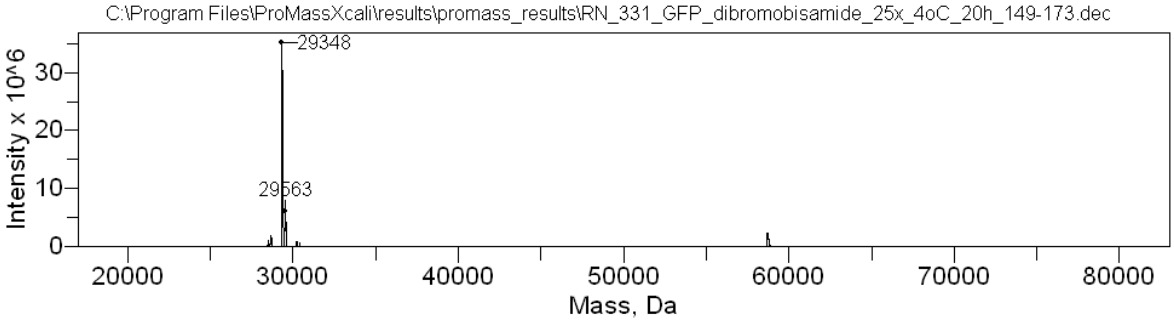
10 equivalents, 4 °C, 20 h

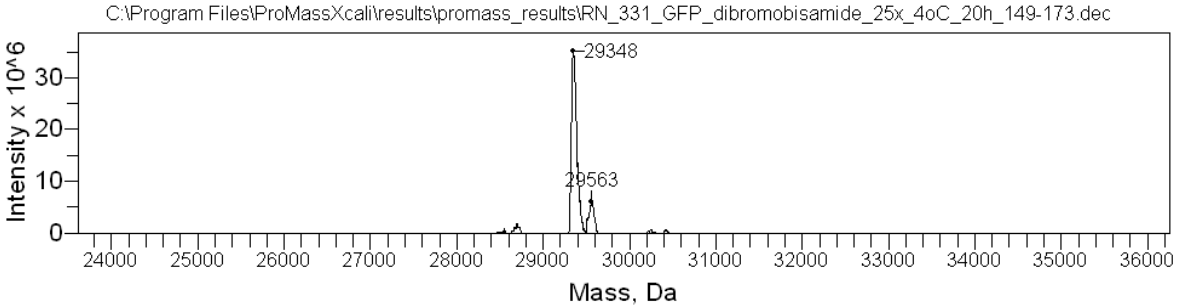


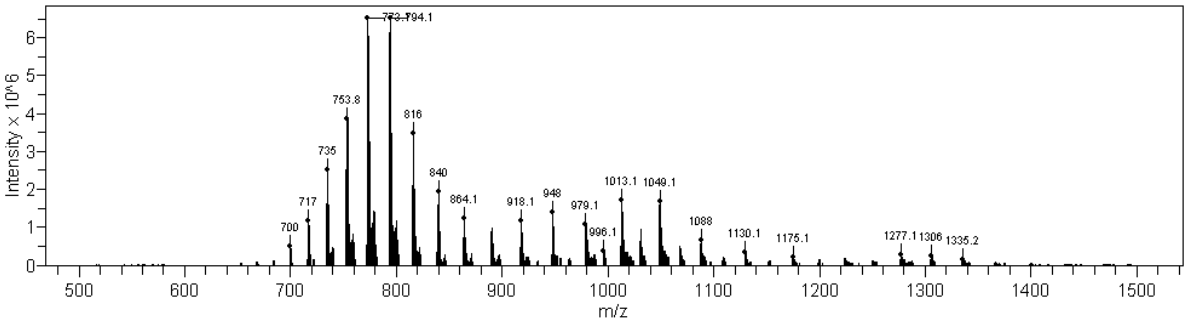




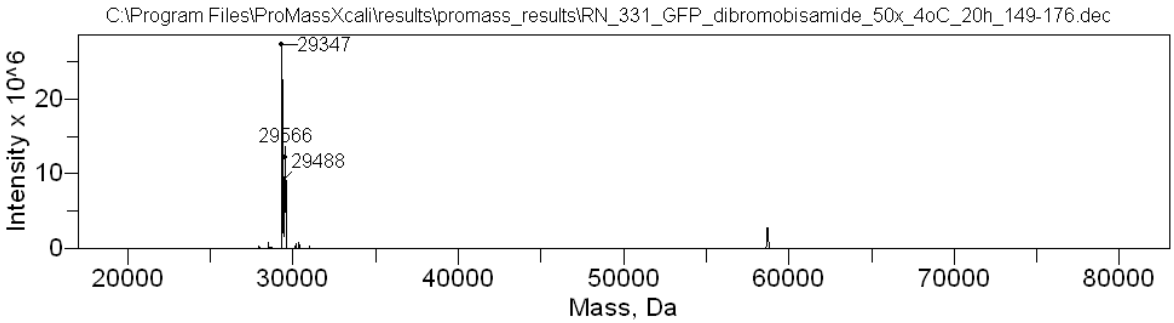
25 equivalents, 4 °C, 20 h

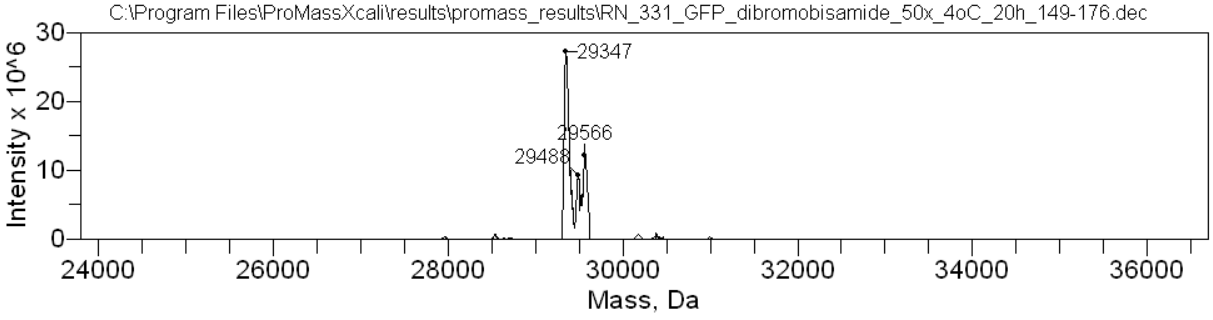


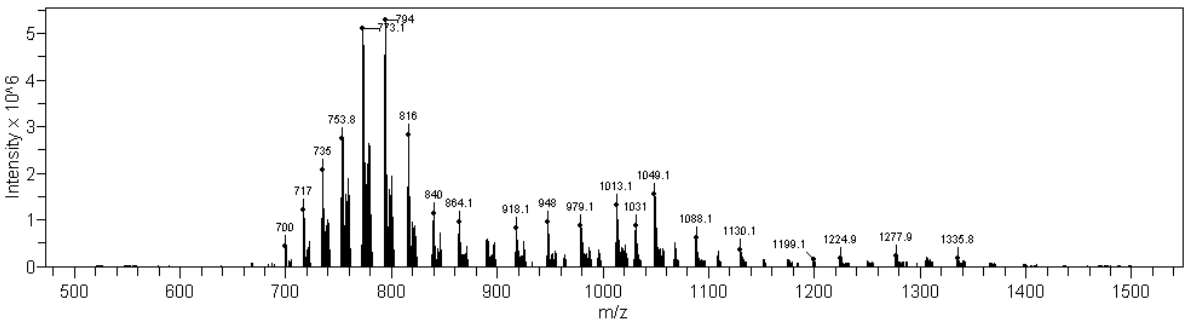




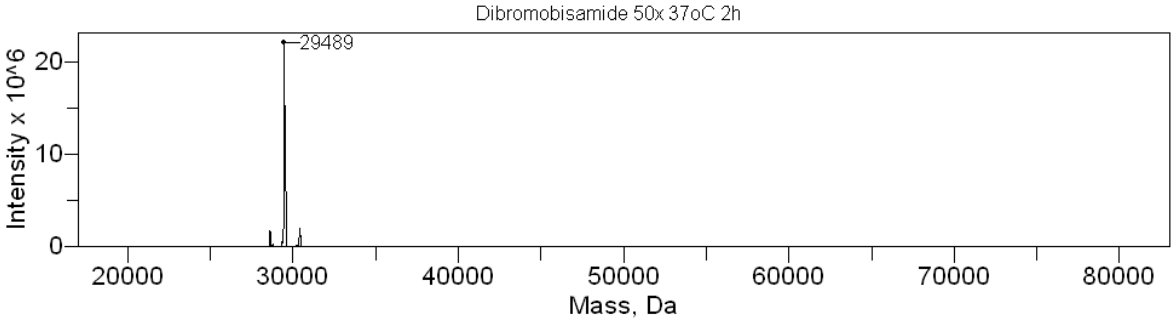
50 equivalents, 4 °C, 20 h

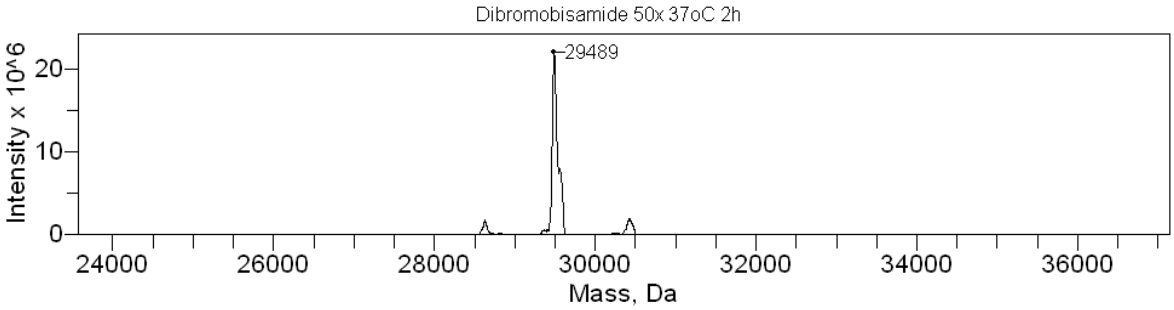


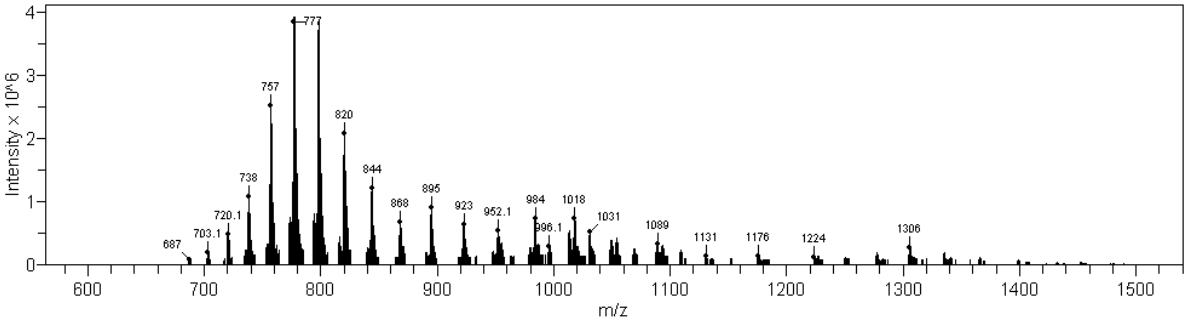




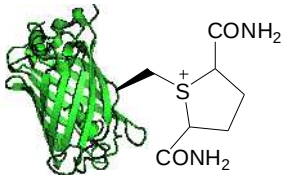
50 equivalents, 37 °C, 2 h







Synthesis of sulphonium 8

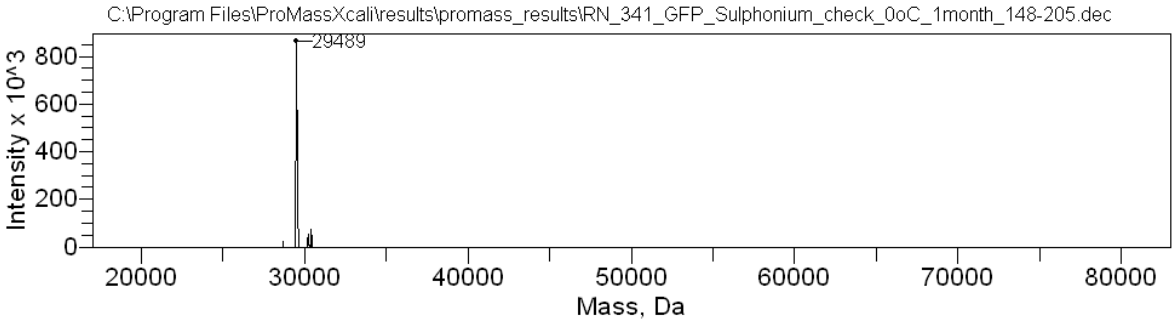


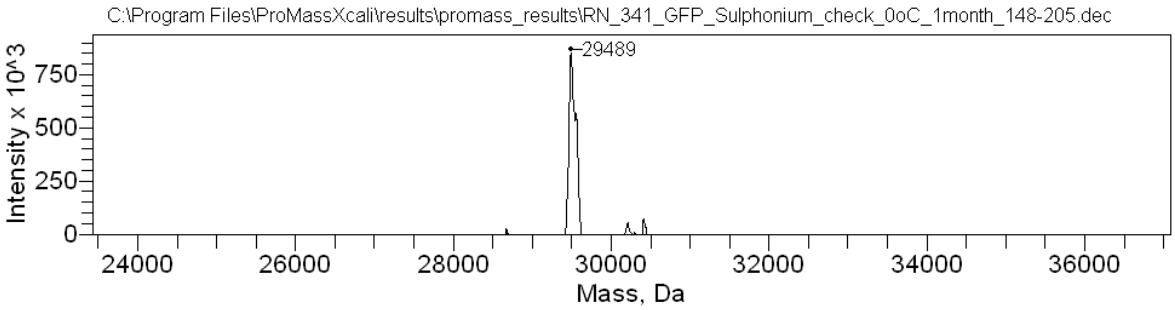
8

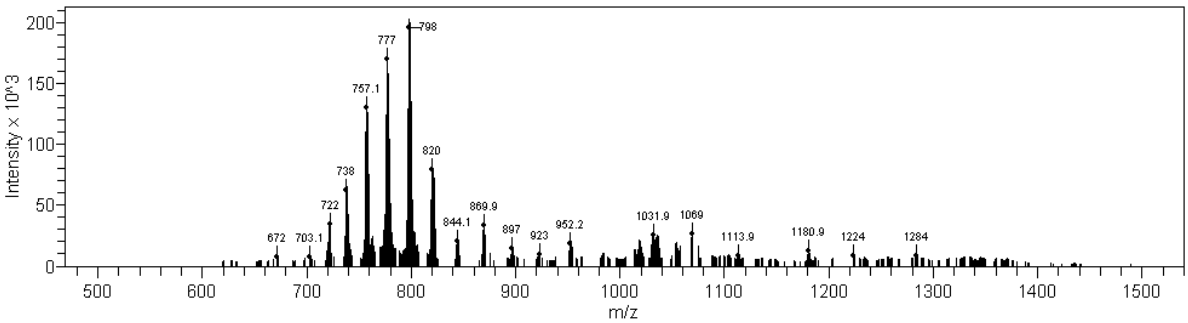
Calculated mass = 29486

2,5-Dibromohexanediamide (**2**) (17 mM solution in DMF, 50 equivalents) was added to a solution of GFP(S147C) (1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1 s and then maintained at 21 °C for 20 h. Analysis by LCMS indicated complete formation of sulphonium **8**. Excess 2,5-dibromohexanediamide (**2**) was removed by repeated diafiltration into fresh buffer (100mM sodium phosphate, pH 8.0) using VivaSpin sample concentrators (GE Healthcare, 10,000 MWCO) and analyzed by LCMS.

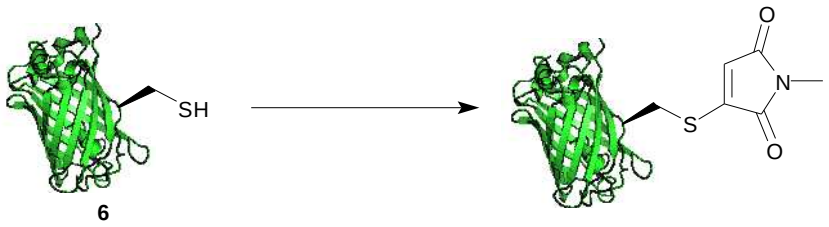
The solution of sulfonium **8** sufficiently stable to be at 4 °C for 1 month







Reaction of GFP(S147C) 6 with N-methylbromomaleimide

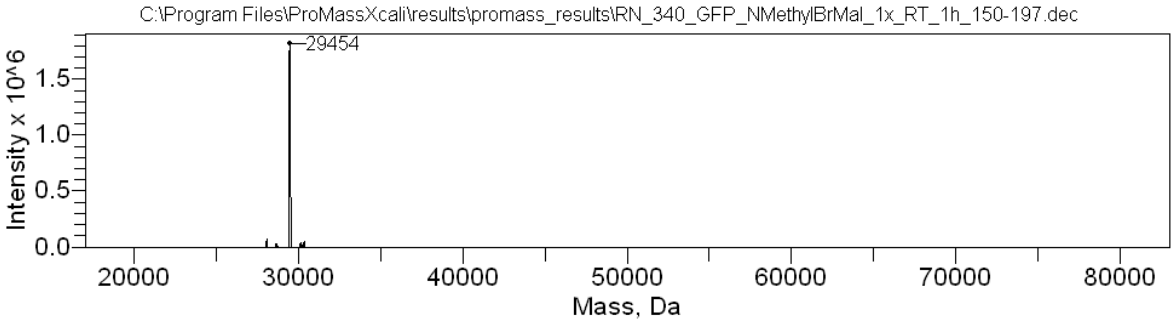


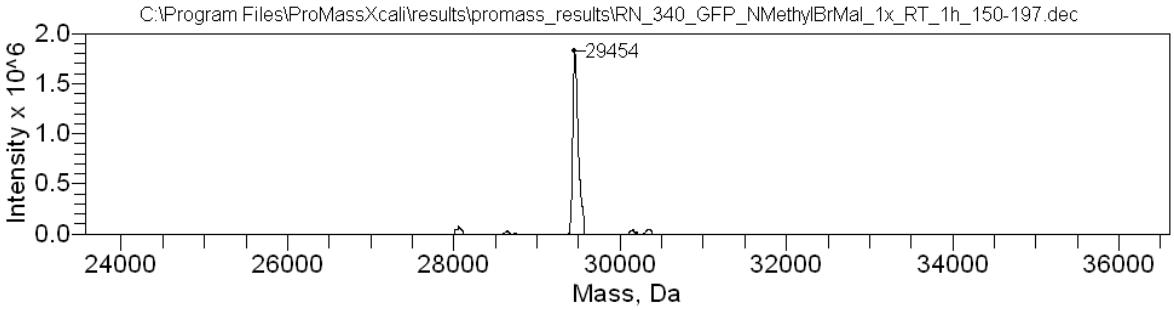
6

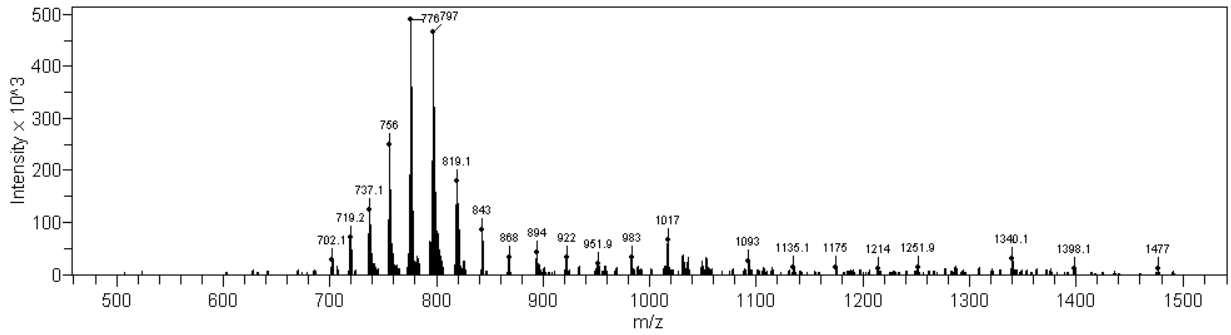
Calculated mass = 29343

Calculated mass = 29454

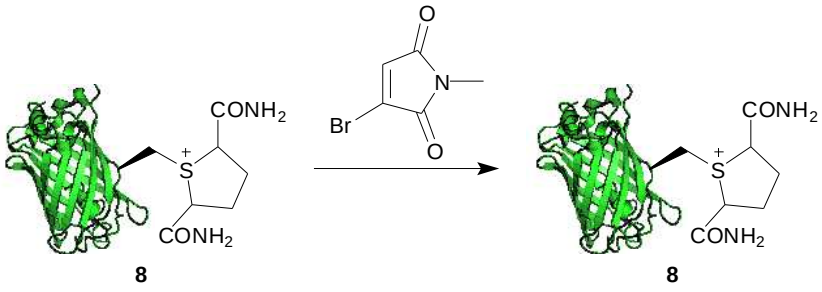
N-Methylbromomaleimide (5 µL, 6.8 mM solution in DMF, 1 equivalent) was added to a solution of GFP(S147C) **6** (100 µL, 1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1 s, maintained at 21 °C for 1 h and analysed by LCMS.







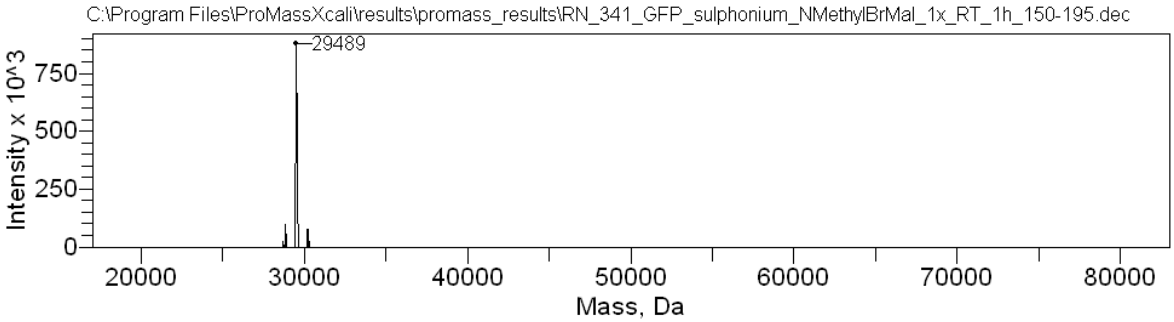
Reaction of sulphonium 8 with N-methylbromomaleimide

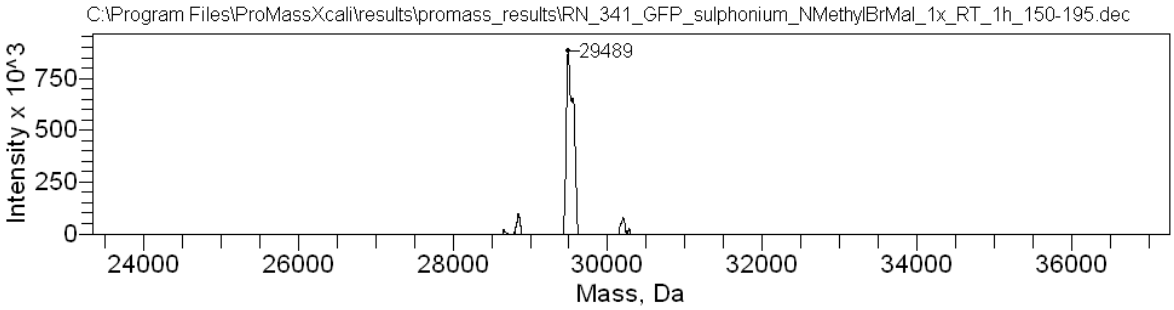


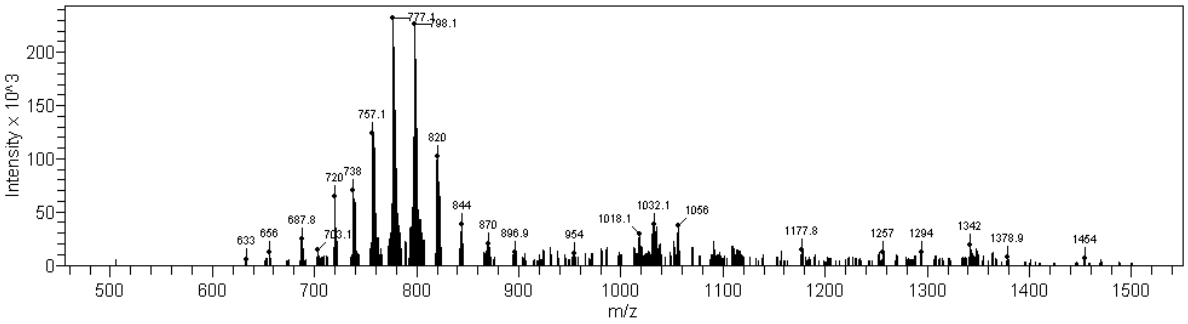
8 **8**

Calculated mass = 29486 Calculated mass = 29486

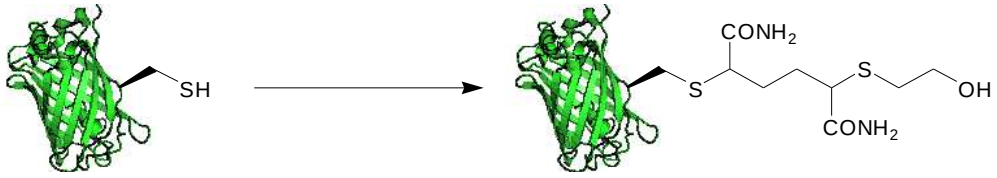
N-methylbromomaleimide (5 µL, 6.8 mM solution in DMF, 1 equivalent) was added to a solution of sulfonium **8** (100 µL, 1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1 s, maintained at 21 °C for 1 h and analysed by LCMS.







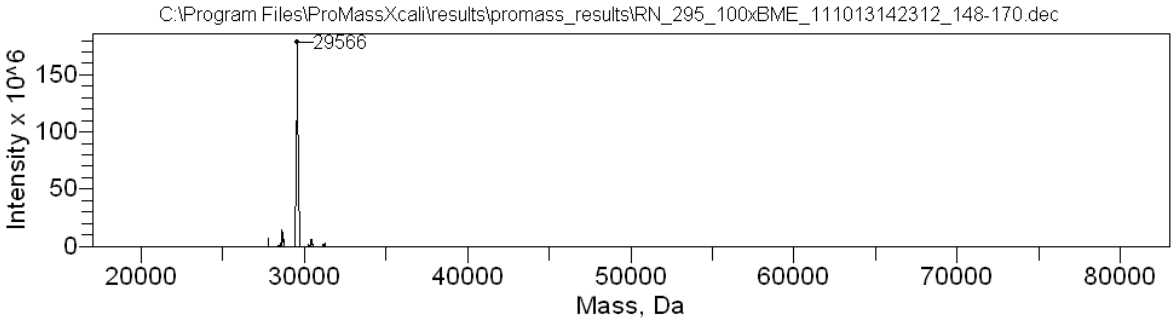
Reaction of sulphonium 8 with β-mercaptoethanol

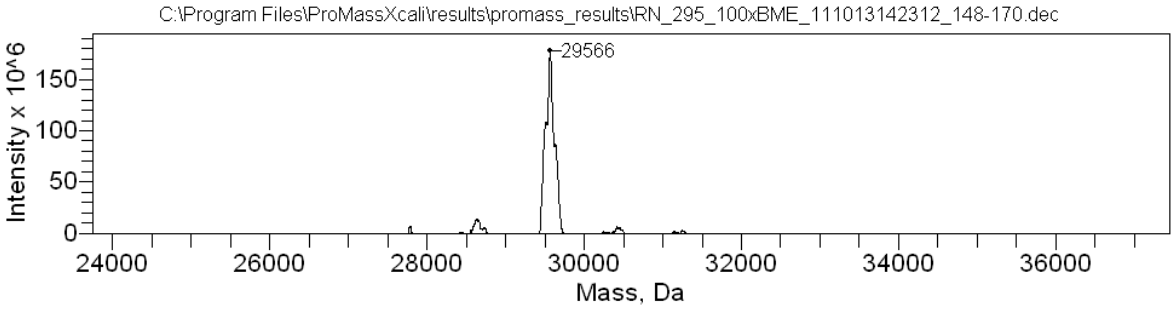


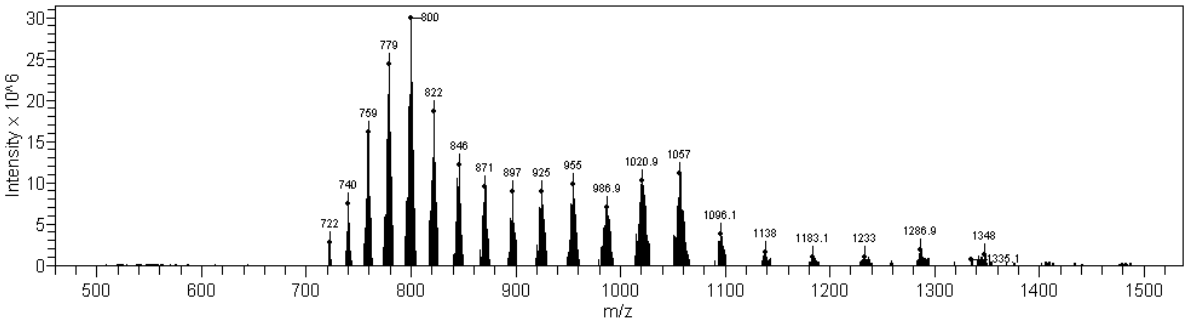
6 Calculated mass = 29343

10a Calculated mass = 29564

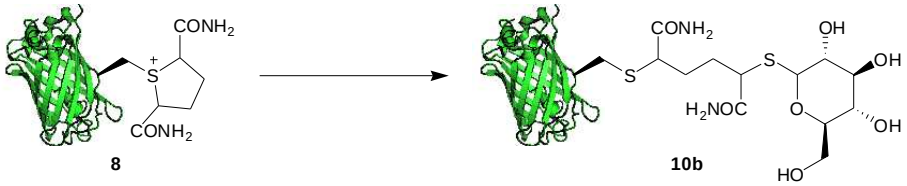
β-Mercaptoethanol (5 μL, 680 mM solution in H_2O, 1000 equivalents) was added to a solution of sulphonium **8** (formed from reaction of GFP(S147C) **6** with 2,5-dibromohexanediamide (**2**) (1000 equivalents)). The mixture was vortexed for 1 s, maintained at 37 °C for 2.5 h and analysed by LCMS





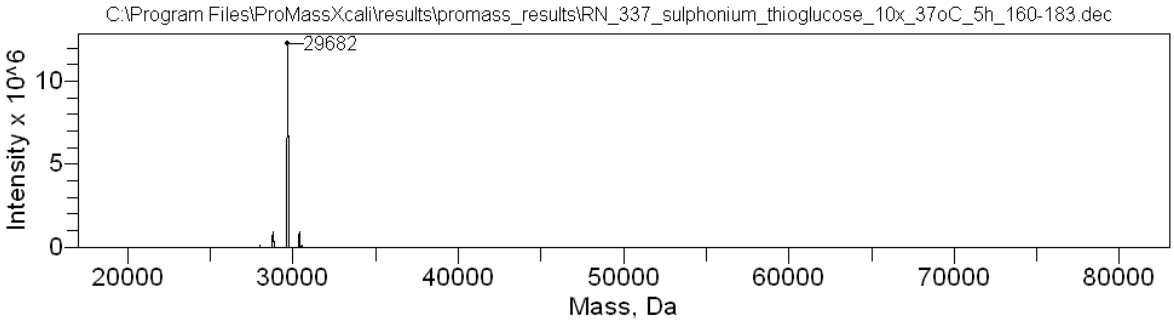


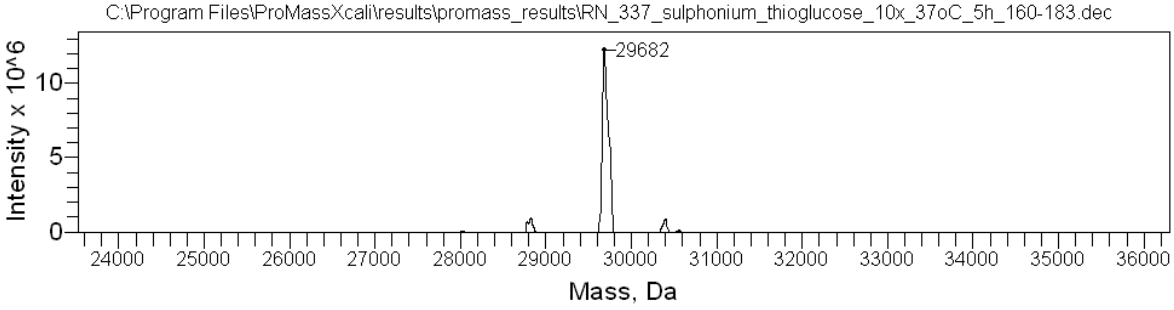
Reaction of sulphonium 8 with thioglusose

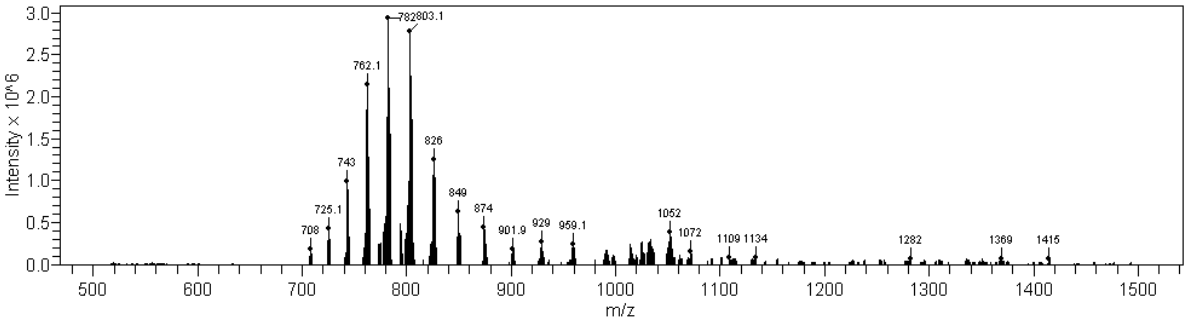


Thioglucose (5 µL in H_2O, 10 or 100 equivalents) was added to a solution of sulfonium **8** (100 µL, 1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1 s, maintained at the required temperature (21 or 37 °C) for 5 h and analysed by LCMS

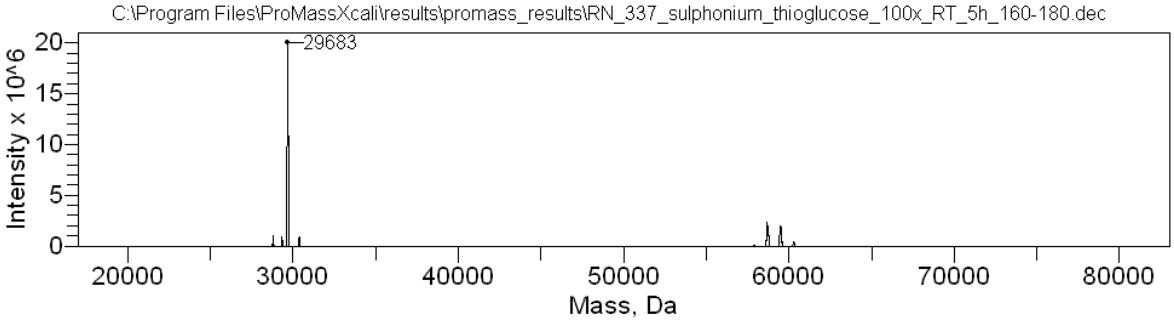
<u>10 equivalents, 37 °C, 5 h</u>

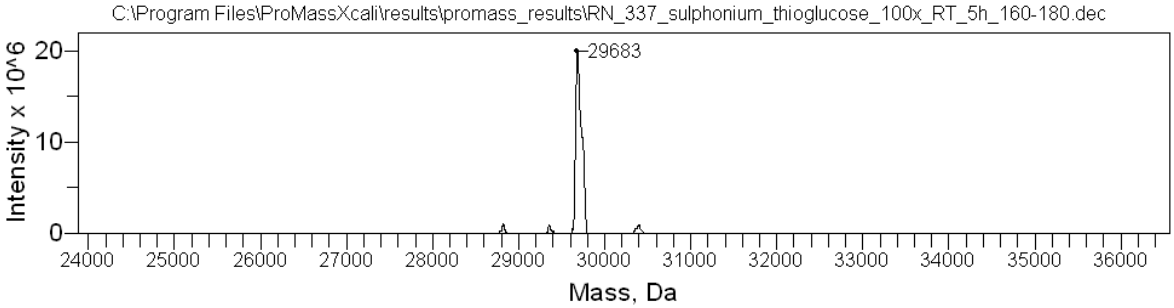


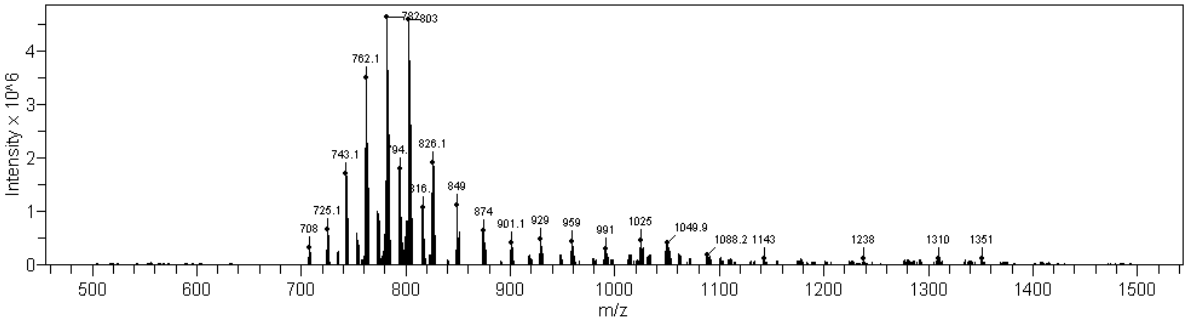




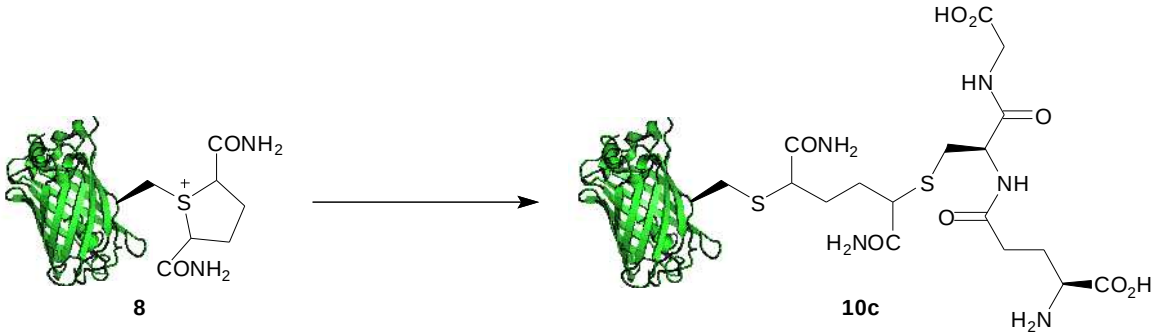
100 equivalents, 21 °C, 5 h





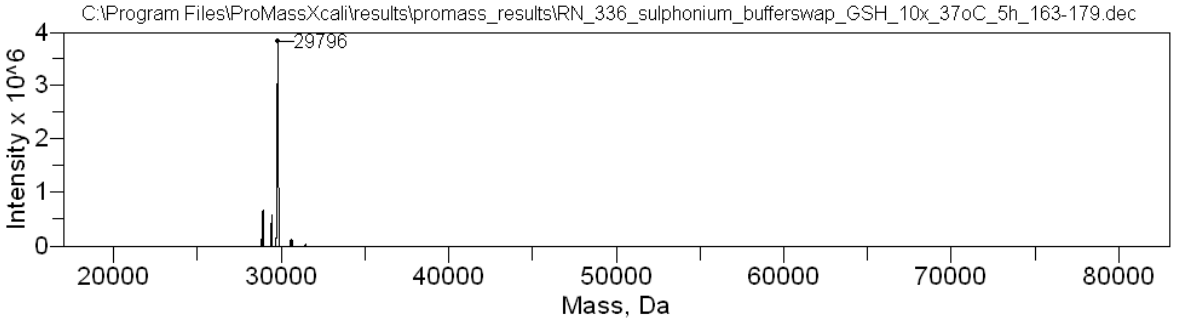


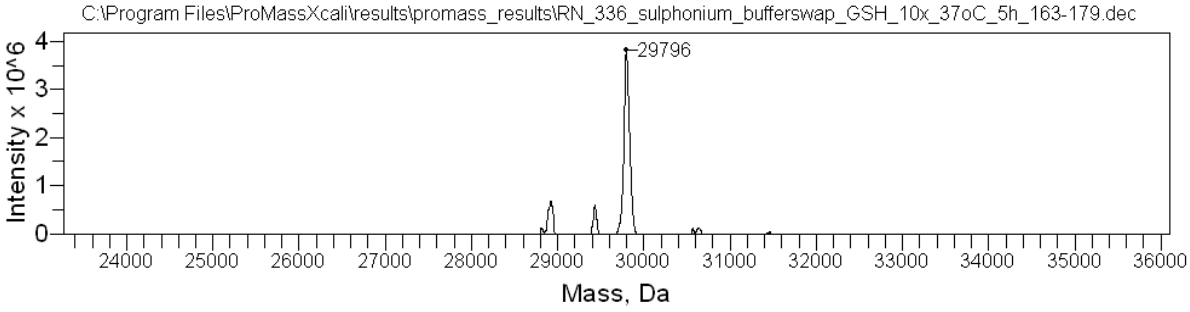
Reaction of sulphonium 8 with glutathione

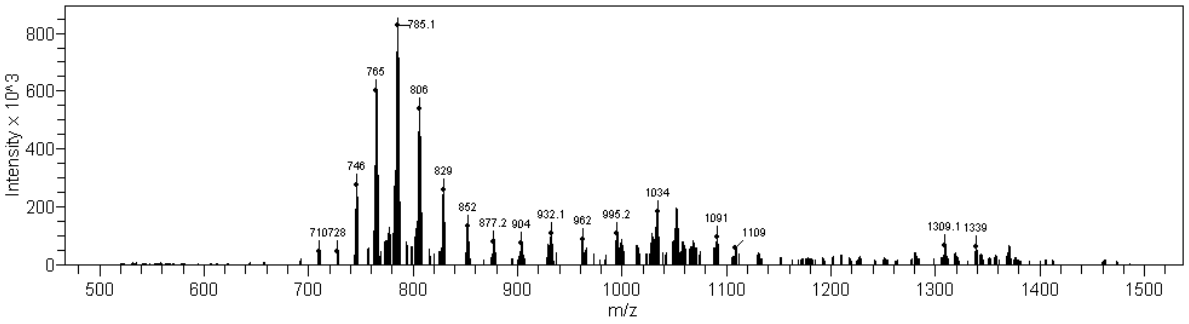


Glutathione (5 µL in H_2O, 10, 100 or 200 equivalents) was added to a solution of sulfonium **8** (100 µL, 1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1 s, maintained at the required temperature (21 or 37 °C) for the prescribed time (2 h or 5 h) and analysed by LCMS.

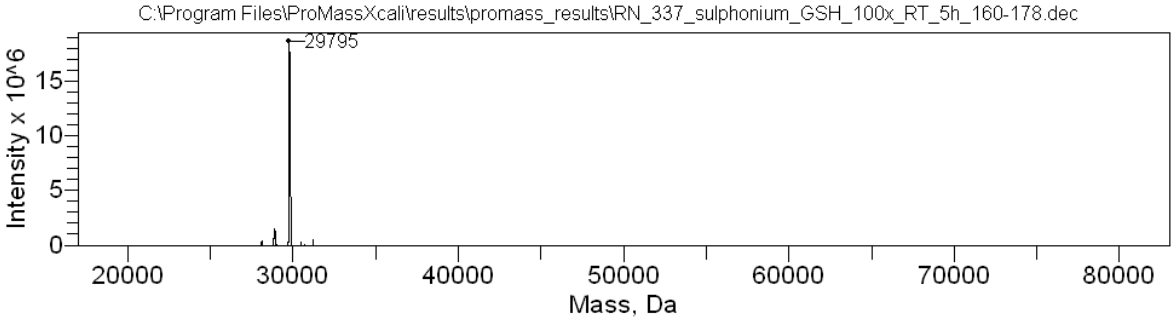
<u>10 equivalents, 37 °C, 5 h</u>

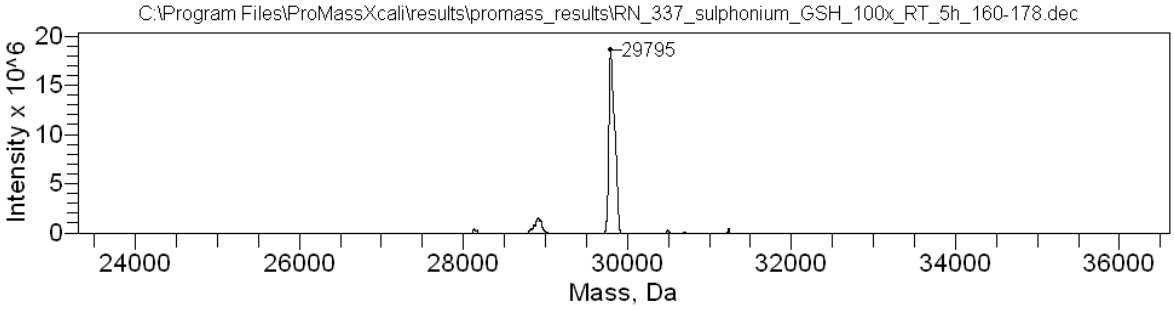


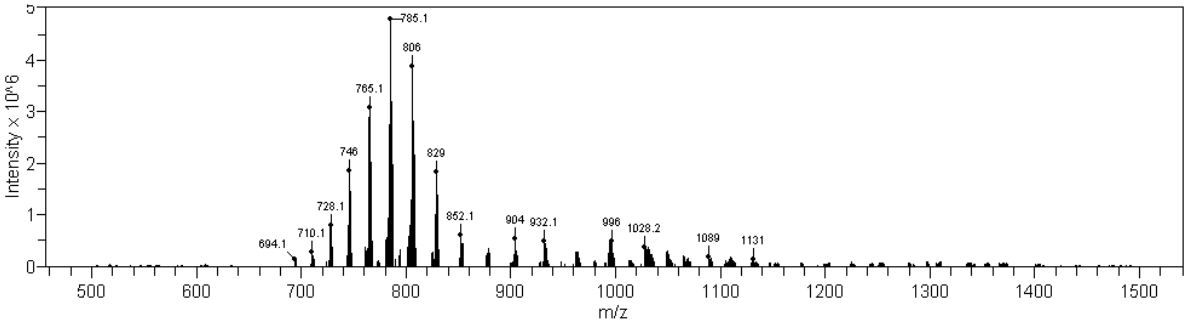




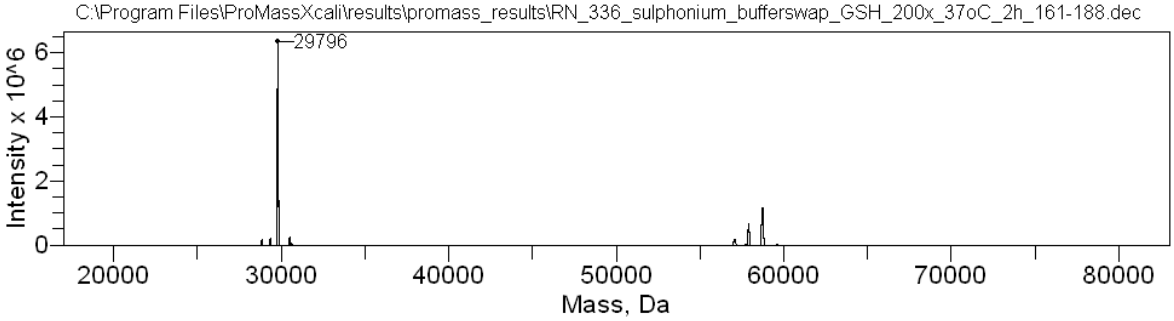
100 equivalents, 21 °C, 5 h

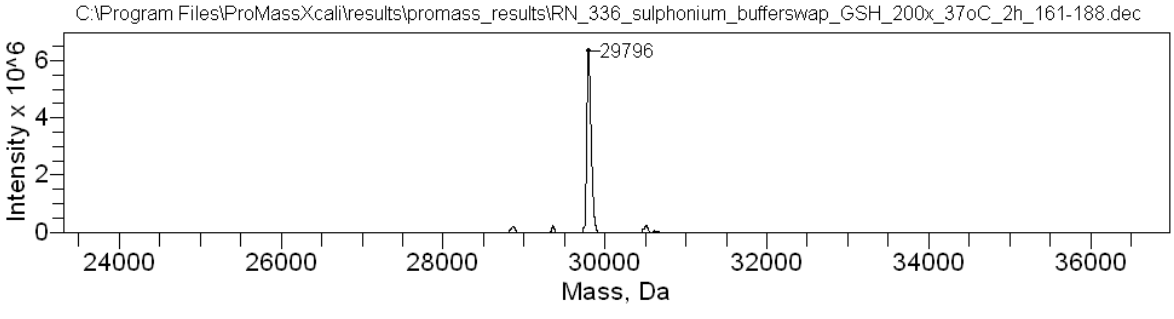


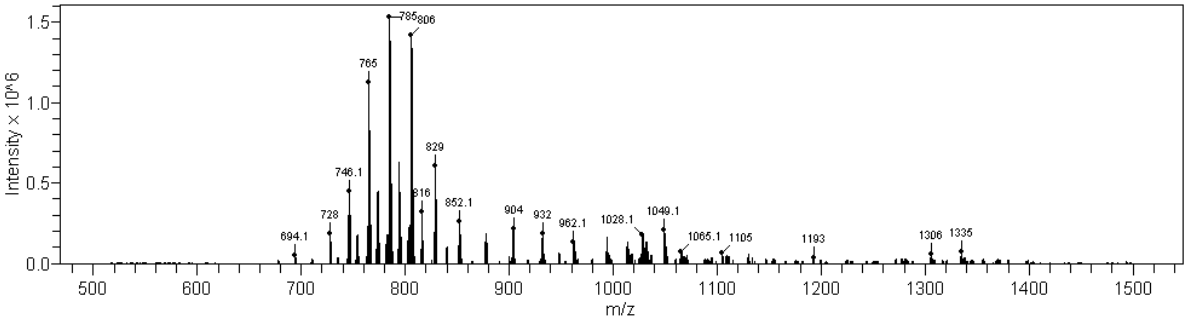




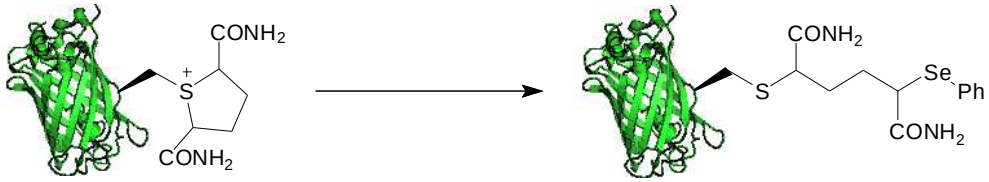
200 equivalents, 37 °C, 2 h






Intensity x 10^6
1.5
1.0
0.5
0.0
785
806
765
829
746.1
816
852.1
728
694.1
904
932
962.1
1028.1
1049.1
1065.1
1105
1193
1306
1335
500
600
700
800
900
1000
1100
1200
1300
1400
1500
m/z

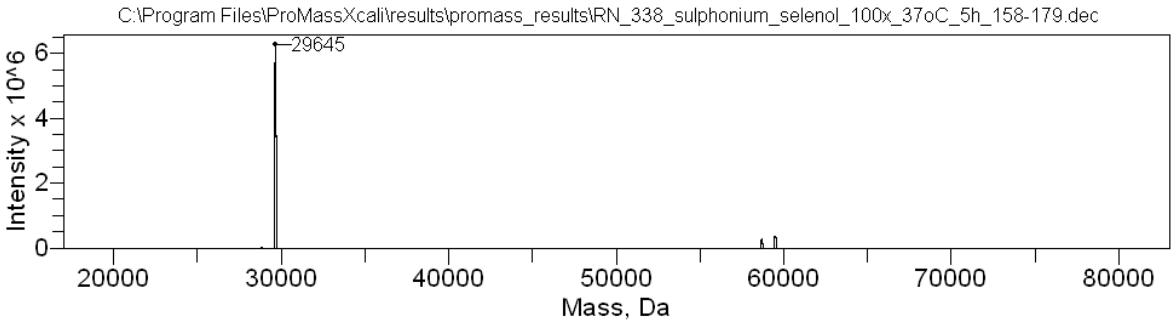
Reaction of sulphonium 8 with benzeneselenol

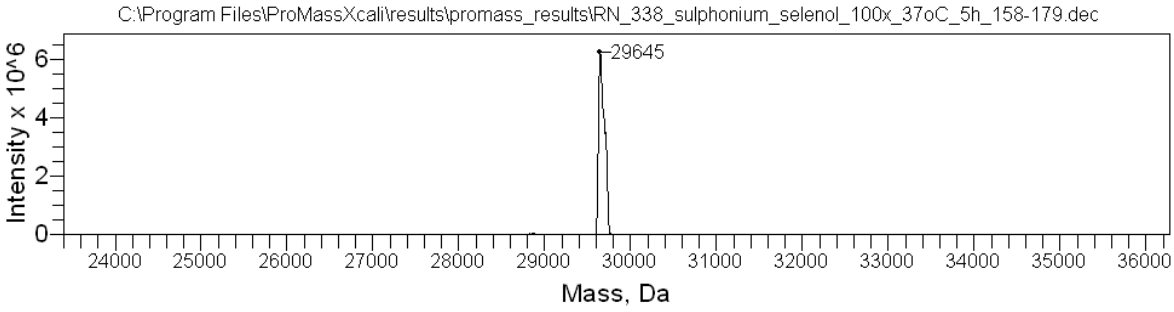


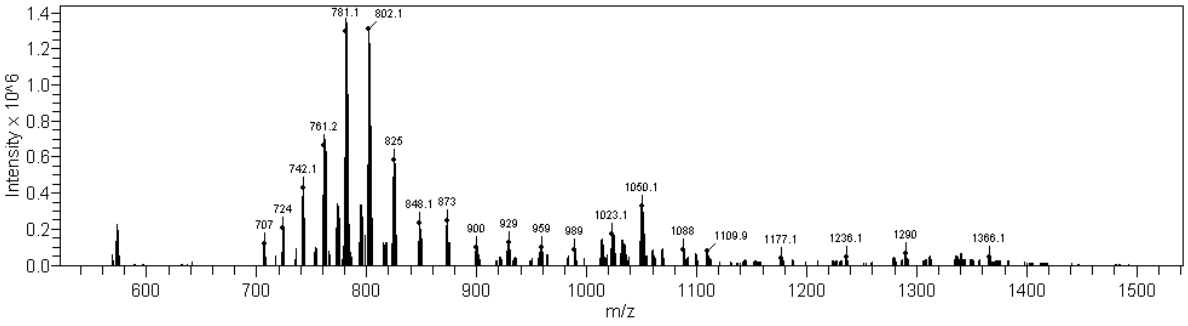
8 **10d**

Calculated mass = 29486 Calculated mass = 29643

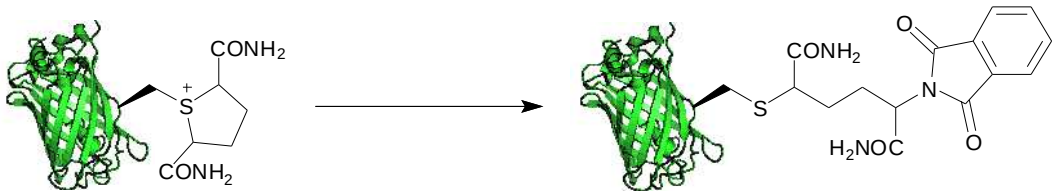
Benzeneselenol (5 µL, 68 mM solution in DMF, 100 equivalents) was added to a solution of sulfonium **8** (100 µL, 1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1s, maintained at the 37 °C for 5 h and analysed by LCMS.







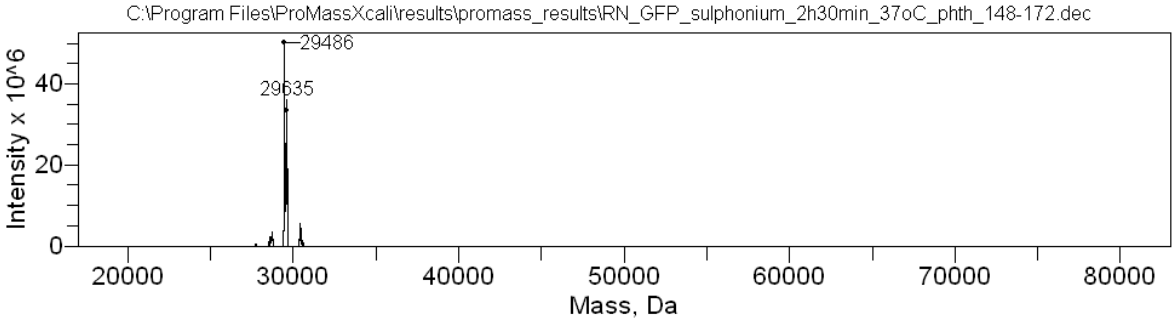
Reaction of sulphonium 8 with phthalimide

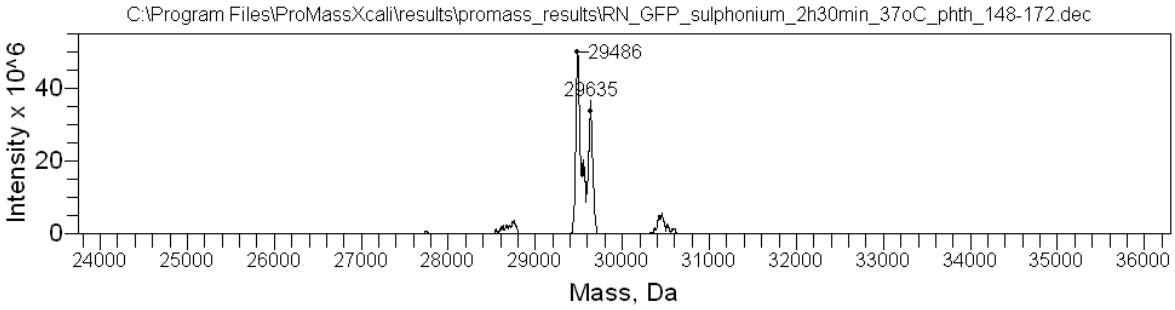


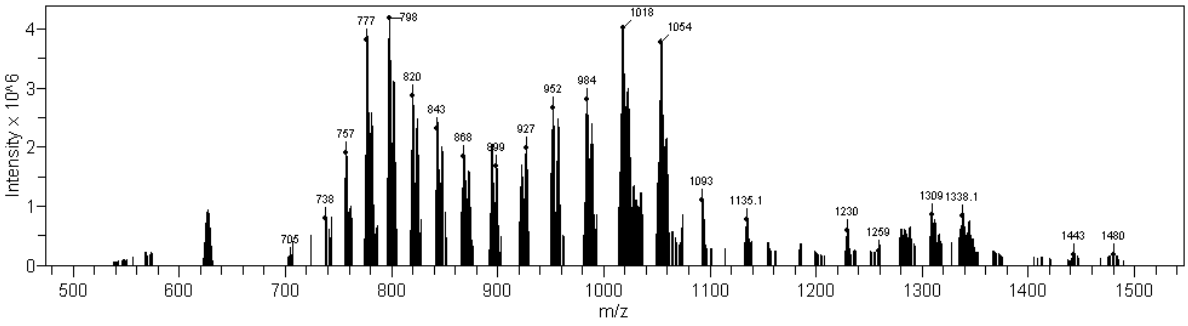
8 Calculated mass = 29486

10e Calculated mass = 29633

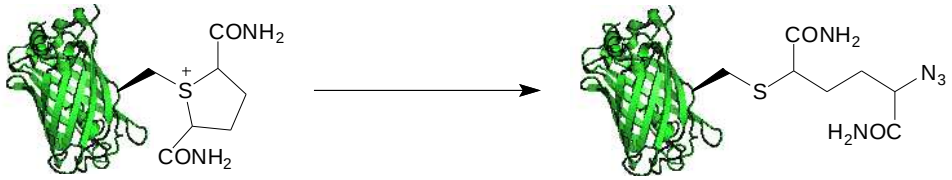
Potassium phthalimide (10 µL, 340 mM solution in DMF, 1000 equivalents) was added to a solution of sulfonium **8** (100 µL, 1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1s, maintained at 37 °C for 2.5 h and analysed by LCMS.







Reaction of sulphonium 8 with azide

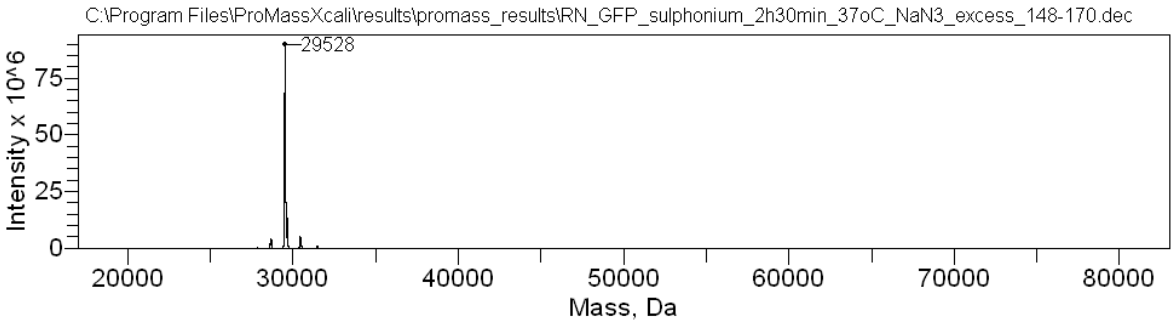


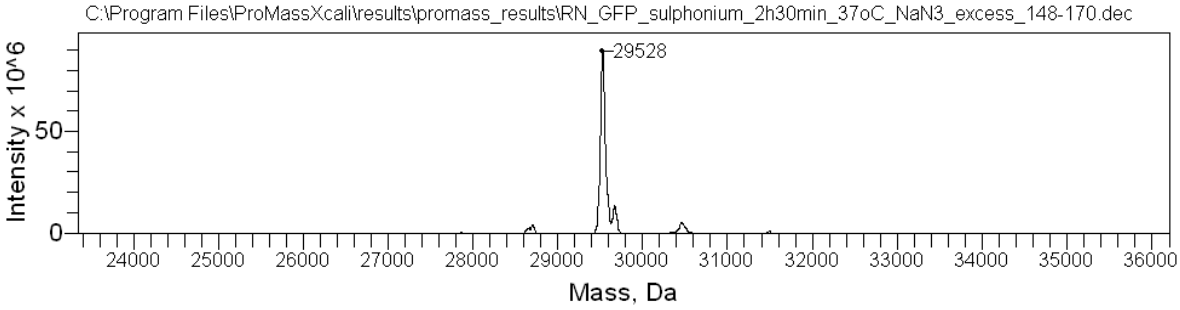
8 Calculated mass = 29486

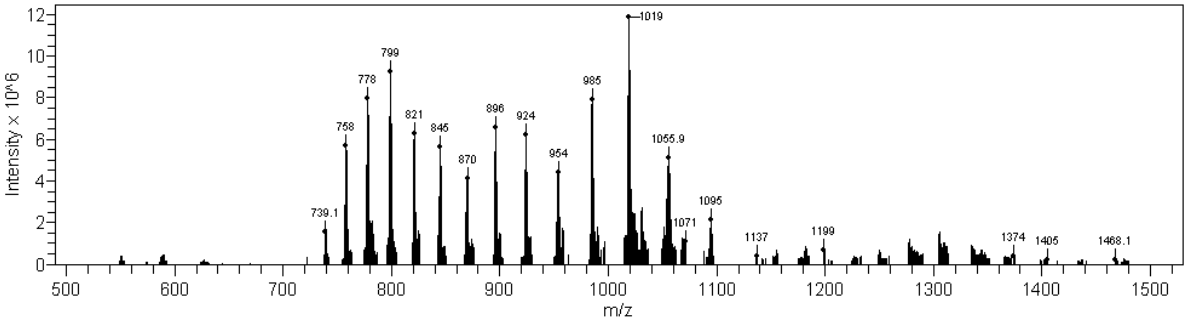
10f Calculated mass = 29528

Sodium azide (10 µL, 340 mM solution in water, 1000 equivalents) was added to a solution of sulfonium **8** (100 µL, 1.0 mg/mL) in sodium phosphate (100 mM, pH 8.0) at 21 °C. The mixture was vortexed for 1s, maintained at 37 °C for 2.5 h and analysed by LCMS.

(Expected Mass : 29528, Observed Mass : 29528)







References

1. J. M. Chalker, S. B. Gunnoo, O. Boutureira, S. C. Gerstberger, M. Fernández-González, G. J. L. Bernardes, L. Griffin, H. Hailu, C. J. Schofield and B. G. Davis, *Chem. Sci.*, 2011, **2**, 1666.
2. P. Moody, M. E. B. Smith, C. P. Ryan, V. Chudasama, J. R. Baker, J. Molloy and S. Caddick, *ChemBioChem*, 2011, 1-3.