Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information for: Versatile Magnetic Resonance Singlet Tags Compatible with Biological Conditions

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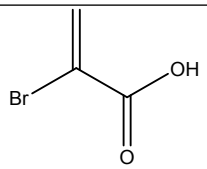
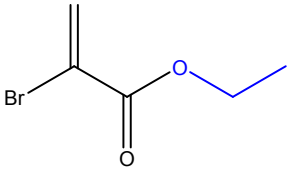
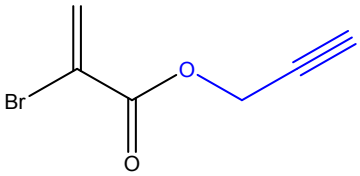
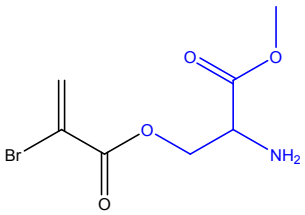
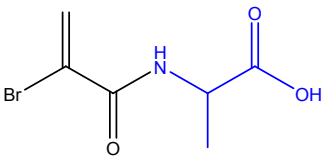
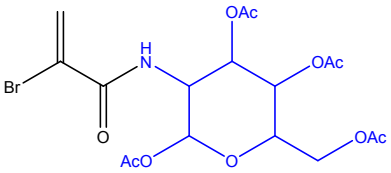
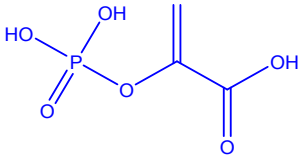
1. NMR experiments

Table 1. *J*-couplings, chemical shift differences and pulse sequence timings. Experiments were performed at 500 MHz.

Compound	Chemical structure	*J* / Hz	*Δν* / Hz	τ_1 / ms	τ_2 / ms
1		1.9	278	131.6	1.8
2		1.7	288	147.1	1.7
3		2.1	281	119.0	1.8
4		2.5	260	104.0	1.9
5		2.7	243	92.6	2.1
6		1.7	256	147.1	2.0
7		1.7	172	147.1	2.8

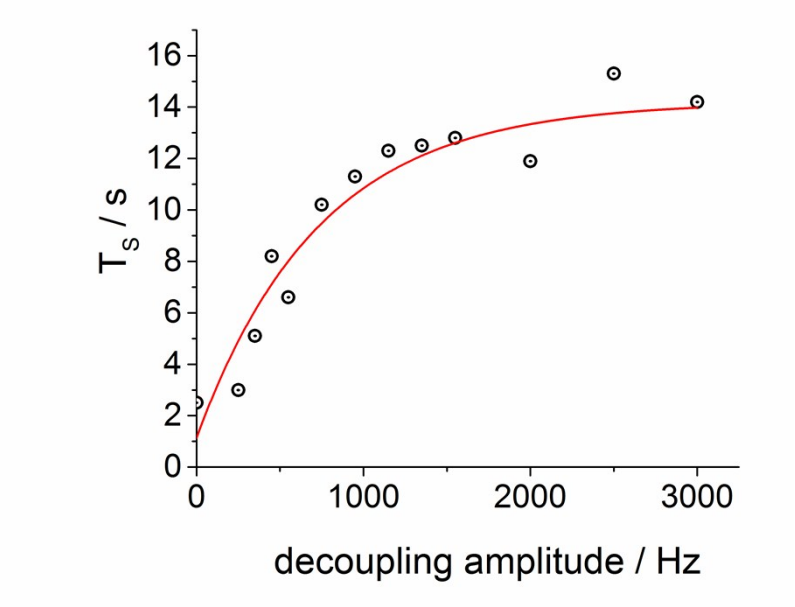


Figure S1. Singlet lifetime of bromoacrylate as a function of decoupling amplitude during spin-lock period (without degassing). With increasing decoupling power, up to 3 kHz, the singlet lifetime of bromoacrylate was found to increase. This demonstrates that singlet-triplet leakage is one of the dominating relaxation processes reducing the singlet lifetime of these systems. The red line was inserted to guide the eye.

2. Relaxation mechanisms

The constituent protons of the singlet state are immune to the motional modulation of the in pair dipole-dipole interaction, but other relaxation mechanisms persist.

Singlet-triplet leakage induced by the large proton chemical shift difference can be a significant contribution to the singlet relaxation rate constant T_S^{-1} [1]. The following expression describes the singlet relaxation rate constant under the singlet-triplet leakage mechanism:

$$R_{STL} = \frac{4B_0^2\gamma_{12}^2\Delta\delta_{12}^2 b_{12}^2\tau_C}{48\pi J_{12}^2 + 3b_{12}^4\tau_C^2}, \qquad (1)$$

where B_0 is the static magnetic field, γ_{12} is the magnetogryric ratio of spins 1 and 2, $\Delta\delta_{12}$ is the chemical shift difference between spins 1 and 2, b_{12} is the direct dipole-dipole coupling constant between spins 1 and 2, τ_C is the overall rotational correlation time and J_{12} is the scalar coupling between spins 1 and 2.

The internuclear distance between the singlet protons is 171.2 pm [2], which leads to a direct dipole-dipole coupling constant of -23.5 kHz. Assuming an overall rotational correlation time of 30 ps, we obtain the following estimate of the singlet relaxation rate constant from singlet-triplet leakage using equation (1): R_{STL} = 0.3 s^{-1}; $\Delta\delta_{12}$ = 556 ppb, J_{12} = 1.9 Hz. This estimate is in reasonable agreement with the experimental singlet relaxation rate constant obtained without an on resonant radiofrequency field during the evolution delay (0.40 ± 0.03 s^{-1}). Discrepancies are attributed to errors in the estimate of the overall rotational correlation time.

In the current set of experiments, the contribution to the singlet relaxation rate constant from singlet-triplet leakage is suppressed very efficiently by the application of an on resonant radiofrequency field during the evolution delay, see figure S2.

After degassing by freeze-pump-thaw cycling, the singlet lifetime in compound 1 (BrAc) increases by a factor of approximately 3. The presence of paramagnetic oxygen dissolved in solution is therefore likely to be an important contribution to the singlet relaxation rate constant in the presence of a 3 kHz on resonant radiofrequency field during the evolution delay. This singlet relaxation rate contribution was estimated from the experimental data to be: 0.0456 ± 0.0005 s^{-1}.

When switching to D_2O the singlet lifetime in compound 1 (BrAc) increases by a factor of approximately 1.5. Dipole-dipole interactions with the solvent are also therefore likely to contribute to the singlet relaxation rate constant in the presence of a 3 kHz on resonant radiofrequency field during the evolution delay. This singlet relaxation rate contribution was estimated from the experimental data to be: 0.024 ± 0.004 s^{-1}.

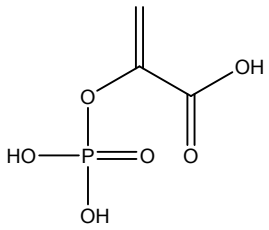
Additional relaxation mechanisms could also be involved, such as scalar relaxation of the second kind with the bromine nucleus, intermolecular dipole-dipole interactions with other spins in solution, and spin rotation. This is plausible since small additional contributions can have a large proportionate effect on the small value of T_S^{-1}. We have not investigated this issue further. The weak dependence of T_S on magnetic field indicates a relatively small relaxation contribution from CSA, as discussed in the main text.

3. Synthetic procedures

Chemicals

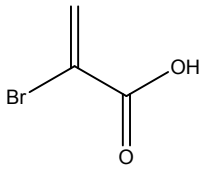
α-bromoacrylic acid, phosphoenol pyruvate, 2,3-dibromopropionic acid, 2,3-dibromopropionic acid, 2,3-dibromopropionyl chloride, ethyl 2,3-dibromopropionate, dimethylaminopyridine (DMAP), *N*-Ethyldiisopropylamine (DIPEA), L-alanine tert-butyl ester hydrochloride, Boc-L-serine methyl ester, 1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride, dimethylformamide, trifluoroacetic acid, propargyl alcohol, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and T3P (50% in ethyl acetate) were purchased from Sigma Aldrich. Ethylacetate (EoAc), dichloromethane (DCM), methanol, NaH_2PO_4, Na_2HPO_4, MgSO4, $NaHCO_3$ and trimethylamine (TEA) were purchased from Fisher Scientific. All chemicals were used without further purification.

phosphenol pyruvate



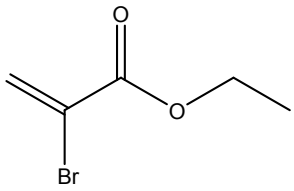
Phosphoenol pyruvate was used as received for all experiments.

α-bromoacrylic acid



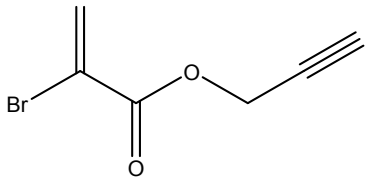
Bromoacylic acid was used as received for all experiments.

ethyl α-bromoacrylate



Ethyl bromoacrylate was synthesized according to literature procedures from ethyl 2,3-dibromopropionate by adding 1 equivalent of TEA in a 1:1 mixture of pentane:diethylether [3]. Filtrations after 3 hours of stirring and removal of the solvent yields the ethyl bromoacrylate.

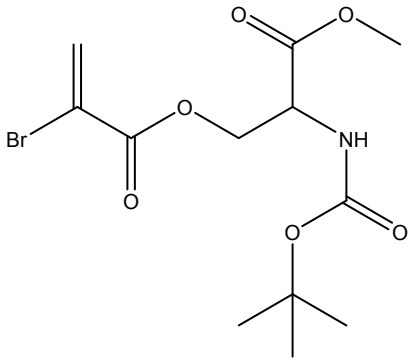
prop-2-yn-1-yl 2-bromoacrylate



2,3-dibromopropionic acid (1 equivalent, 1mmol) and propargyl alcohol (1 equivalent, 1 mmol) were dissolved in 20 ml DCM and 20 mg of DMAP were added. The solution was cooled to 0°C and 1.1 equivalent (1.1 mol) EDC were added. Afterwards the solution was stirred for 1 hour at 0°C and 3 hours at room temperature. The organic phase was washed twice with 10 ml 0.5 M HCl and 10 ml saturated $NaHCO_3$, dried over $MgSO_4$. Removal of the solvent under vacuum yields prop-2-yn-1-yl 2-bromoacrylate which does not require further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (d, *J* = 1.8 Hz, 1H), 6.35 (d, *J* = 1.8 Hz, 1H), 4.83 (d, *J* = 2.5 Hz, 2H), 2.54 (t, *J* = 2.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.2, 131.7, 120.4, 76.8, 75.7, 54.0; HRMS (m/z): $[M]^+$ calcd. for $C_6H_5O_2Br$ 187.9472; found: 187.9471.

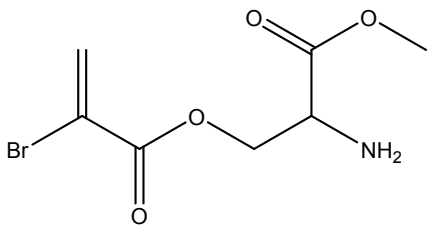
2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl 2-bromoacrylate



2,3-dibromopropionic acid (1 equivalent, 1 mmol) and Boc-serine-OMe (1 equivalent, 1 mmol) were dissolved in 20 ml DCM and 20 mg of DMAP were added. The solution was cooled to 0°C and 1.1 equivalent (1.1 mol) EDC were added. Afterwards the solution was stirred for 1 hour at 0°C and 3 hours at room temperature. The organic phase was washed twice with 10 ml 0.5 M HCl and 10 ml saturated $NaHCO_3$ and dried over $MgSO_4$. Removal of the solvent under vacuum, taking up the residue in petrol ether, filtration and reducing to drieness yields 2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl 2-bromoacrylate.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.95 (d, *J* = 1.7 Hz, 1H), 6.30 (d, *J* = 1.7 Hz, 1H), 5.32 (m, 1H), 4.67 (m, 1H), 4.51 (m, 2H), 3.79 (s, 3H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 161.4, 155.3, 131.5, 120.4, 80.6, 66.5; 52.9, 52.8; HRMS (m/z): $[M+Na]^+$ calcd. For $C_{12}H_{18}O_6NBr$ 374.0215; found: 374.0213.

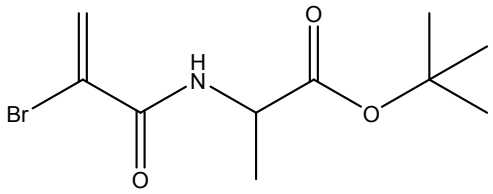
2-amino-3-methoxy-3-oxopropyl 2-bromoacrylate



Removal of the Boc protection group was accomplished by dissolving 1 mmol of 2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl 2-bromoacrylate in 10 ml DCM and 2 ml TFA. Stirring for 2 hours with subsequent removal of solvent and acid under vacuum yielded 2-amino-3-methoxy-3-oxopropyl 2-bromoacrylate which can be used without further purification.

^{1}H NMR (400 MHz, D_2O): δ 6.98 (d, *J* = 2.5Hz, 1H), 6.46 (d, *J* = 2.5 Hz, 1H), 4.70 (m, 2H), 4.57 (m, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, D_2O): 167.7, 162.6, 133.7, 118.7, 63.6, 54.0, 52.0; HRMS (m/z): $[M+Na]^+$ calcd. For $C_8H_{10}O_6NBr$ 317.9589; found: 317.9590.

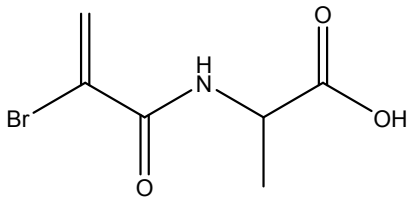
tert-butyl (2-bromoacryloyl)alaninate



Alanine-*tert*-butyl ester (1 equivalent, 1 mmol) and 2,3-dibromopropionic acid (1 equivalent, 1 mmol) were dissolved in 10 ml DMF and cooled to 0°C. Afterwards, 2 equivalents of DIPEA and 2 equivalents of T3P are added subsequently. The reaction was stirred overnight whilst warming_to room temperature. 20 ml of ethyl acetate were added to the solution which was washed twice with 30? water, twice with 0.5M HCl, twice with saturated $NaHCO_3$ and dried over $MgSO_4$. The organic phase is removed to dryness under vacuum, the residue is taken up in petrol ether, filtered and the organic phase removed to dryness again to yield tert-butyl (2-bromoacryloyl)alaninate.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.99 (d, *J* = 1.6 Hz, 1H), 6.04 (d, *J* = 1.6 Hz, 1H), 4.46 (dq, *J*= 7.1 Hz, 1H [rotamer]), 1.49 (s, 9H), 1.44 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 160.3, 127.6, 122.6, 82.5, 49.7, 27.9, 18.4; HRMS (m/z): $[M+Na]^+$ calcd. For $C_{10}H_{16}O_3NBr$ 300.0211; found: 300.0206.

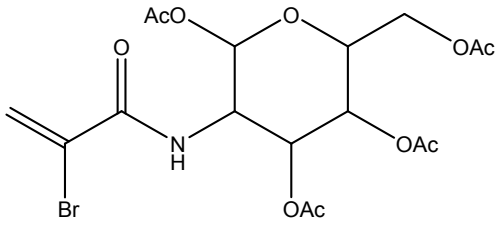
(2-bromoacryloyl)alanine



Removal of the *tert*-butyl protection group was accomplished by dissolving 1 mmol of tert-butyl (2-bromoacryloyl)alaninate in 10 ml DCM and 2 ml TFA. Stirring for 2 hours with subsequent removal of solvent and acid under vacuum yielded (2-bromoacryloyl)alanine which can be used without further purification.

^{1}H NMR (400 MHz, D_2O): δ 6.57 (d, *J* = 2.7 Hz, 1H), 6.09 (d, *J* = 2.7 Hz, 1H), 4.30 (q, *J* = 7.4Hz, 1H), 1.33 (d, *J* = 7.4Hz, 3H); ^{13}C NMR (100 MHz, D_2O): δ 175.9, 165.1, 127.6, 121.7, 49.6, 15.8; HRMS (m/z): $[M+Na]^+$ calcd. For $C_6H_8O_3NBr$ 243.9585; found: 243.9581.

6-(acetoxymethyl)-3-(2-bromoacrylamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate



1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride (1 equivalent, 1 mmol) was dissolved in 30 ml DMF with 2 equivalents DIPEA under argon atmosphere. The solution was cooled to 0°C and 1 equivalent of 2,3-dibromopropionyl chloride added dropwise. The reaction was stirred over night whilst slowly warming to room temperature. 50 ml ethyl acetate were added and the organic phase washed three times with 30 ml H_2O, twice with 15 ml 0.5 M HCl, twice with 15 ml saturated $NaHCO_3$ and once with 15 ml H_2O. Drying over $MgSO_4$, filtering and removal of the organic phase yields the pure product.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.96 (d, *J* = 1.7 Hz, 1H), 6.7 (d, *J* = 9.3 Hz, 1H), 6.06 (d, *J* = 1.7 Hz, 1H), 5.82 (d, *J* = 8.7 Hz, 1H), 5.31 (m, 1H), 5.16 (m, 1H), 4.30 (m, 2H), 4.15 (m, 1H), 3.86 (m, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.6, 169.3, 169.2, 161.4, 128.5, 121.9, 92.3, 73.0, 72.0, 67.7, 61.7, 54.0, 20.8, 20.7, 20.6, 20.5; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{17}H_{22}BrNO_{10}$ 502.0325; found: 502.0319.

4. NMR-Spectra of the synthesized compounds (acquired at 9.4 T and 25°C with 8 transients).

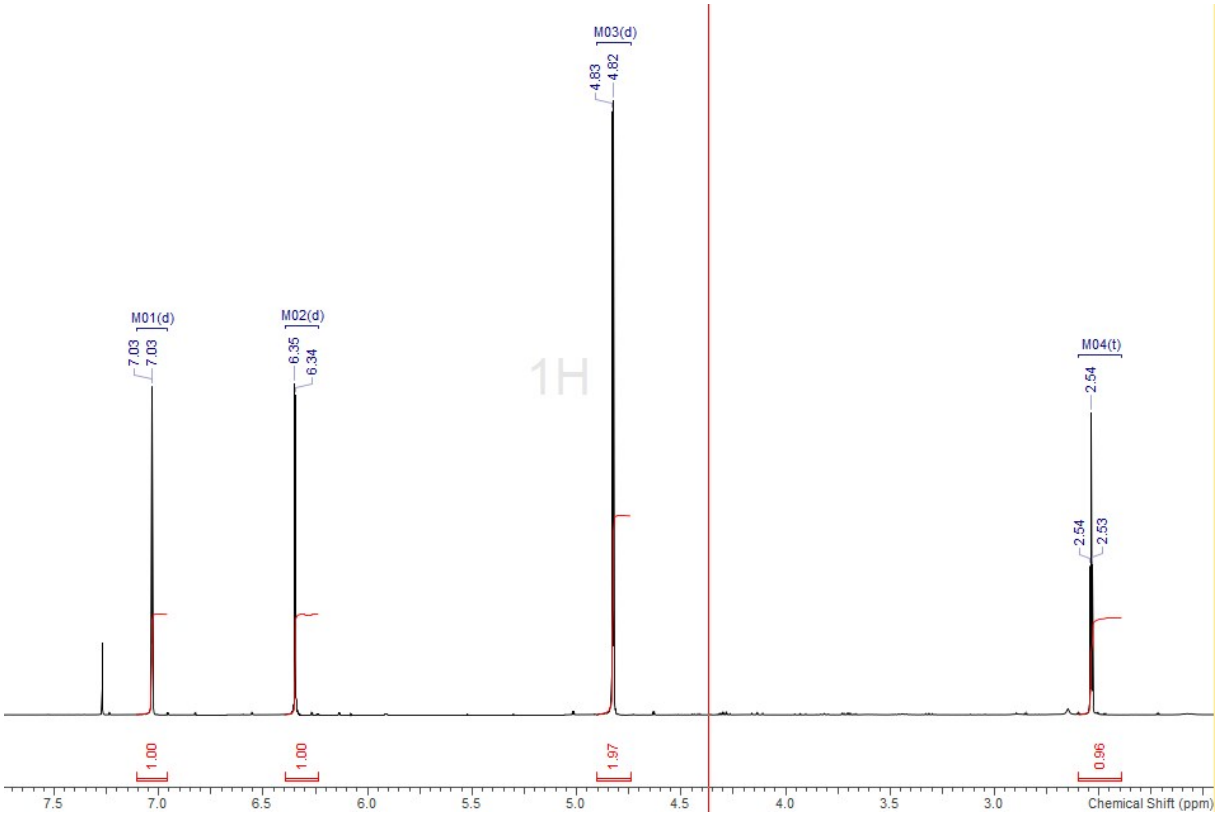


Figure S2. ^{1}H NMR spectrum of prop-2-yn-1-yl 2-bromoacrylate at 400 MHz in $CDCl_3$

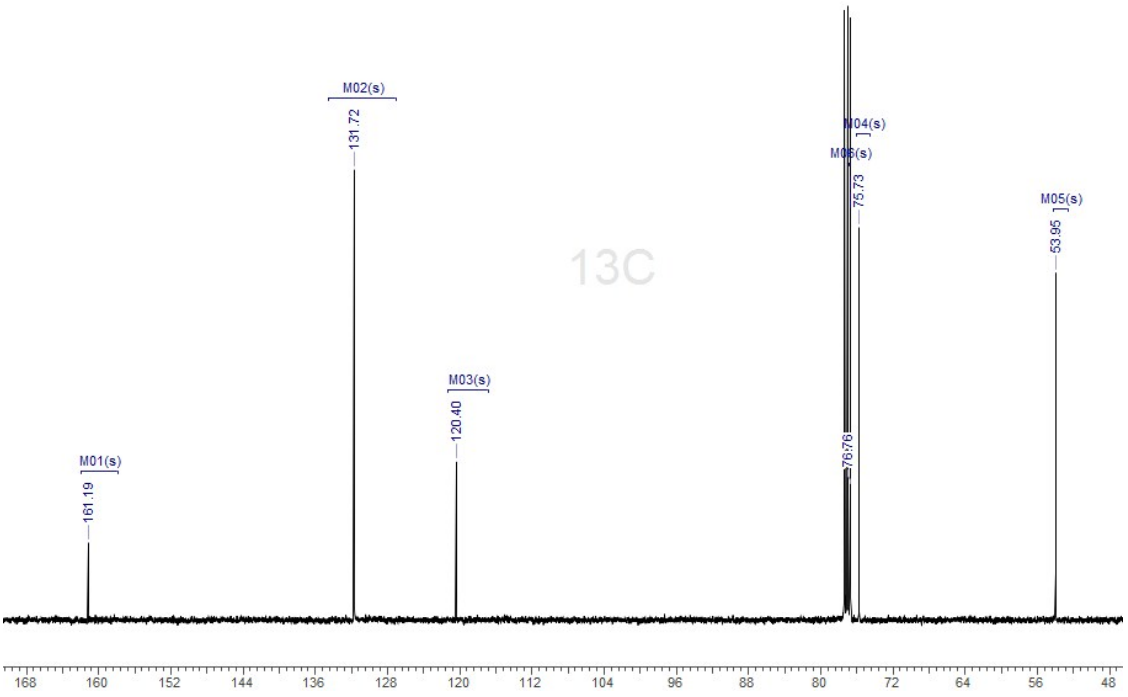


Figure S3. ^{13}C NMR spectrum of prop-2-yn-1-yl 2-bromoacrylate at 100 MHz in $CDCl_3$

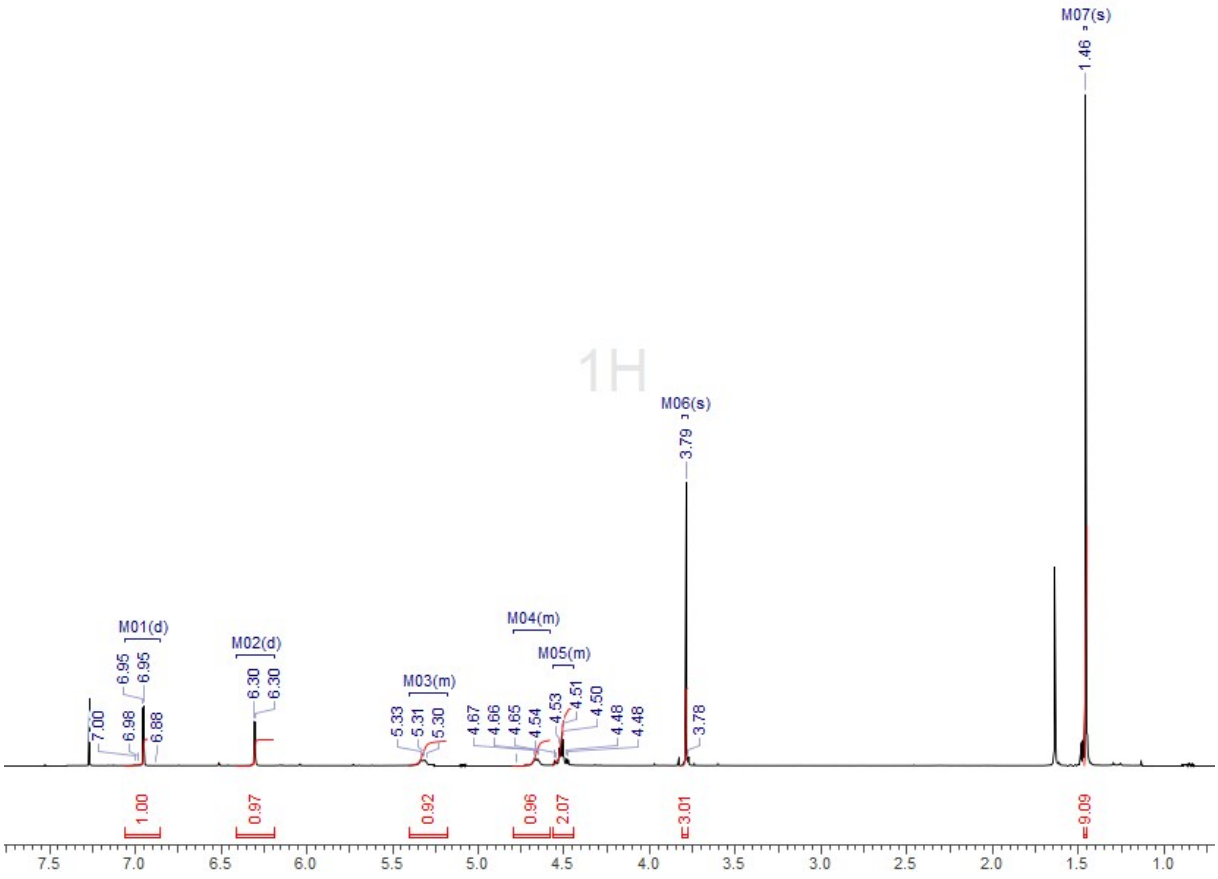


Figure S4. ^{1}H NMR spectrum of 2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl 2-bromoacrylate at 400 MHz in $CDCl_3$. The peak at 1.6 ppm represents an impurity (H_2O).

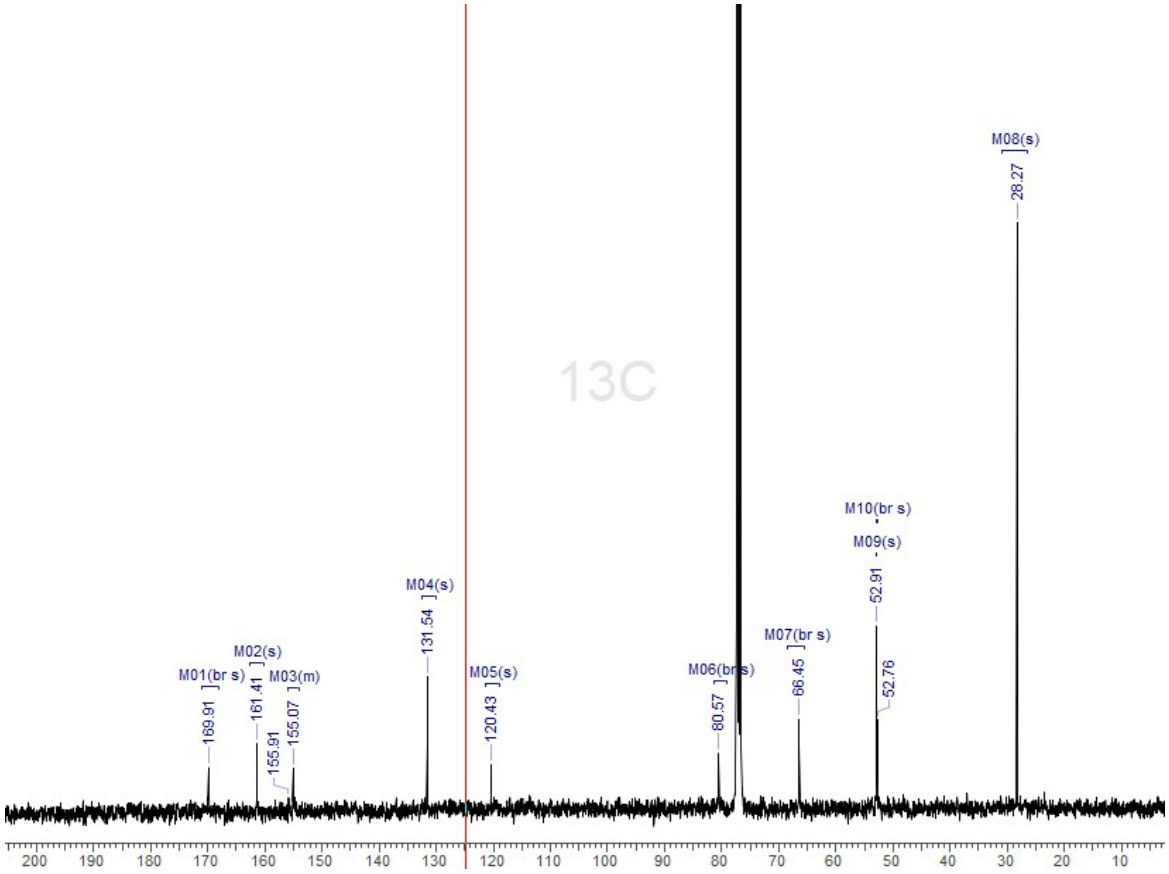


Figure S5. ^{13}C NMR spectrum of 2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl 2-bromoacrylate at 100 MHz in $CDCl_3$.

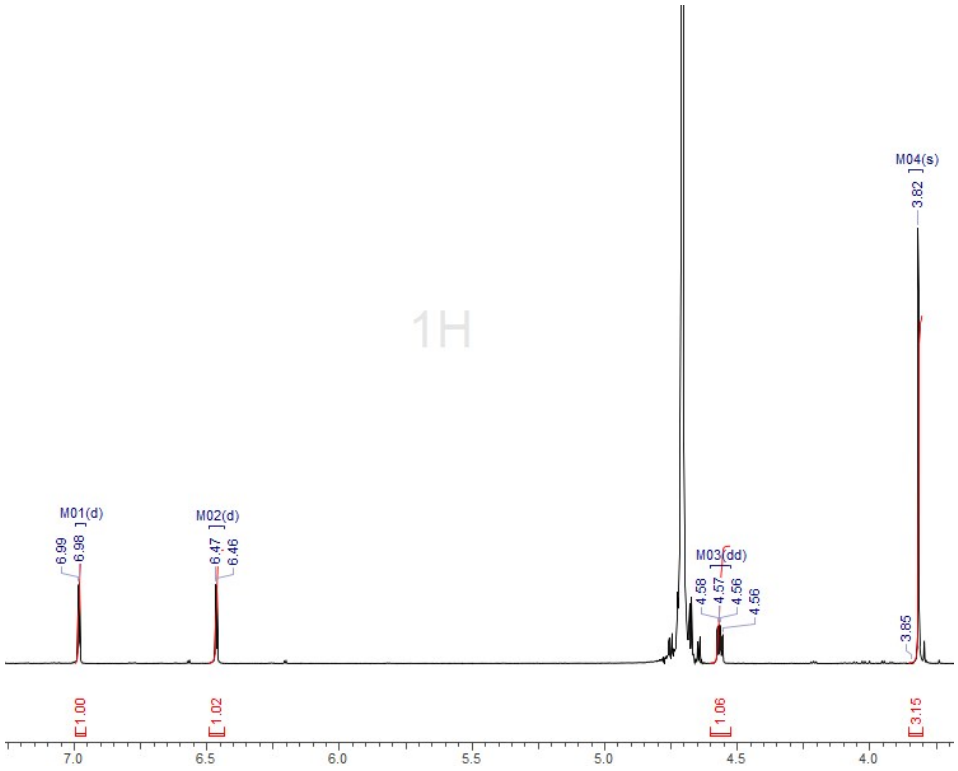


Figure S6. ^{1}H NMR spectrum of 2-amino-3-methoxy-3-oxopropyl 2-bromoacrylate at 400 MHz in D_2O. The CH_2-multiplet of the serine fragment is partly obscured by the D_2O peak.

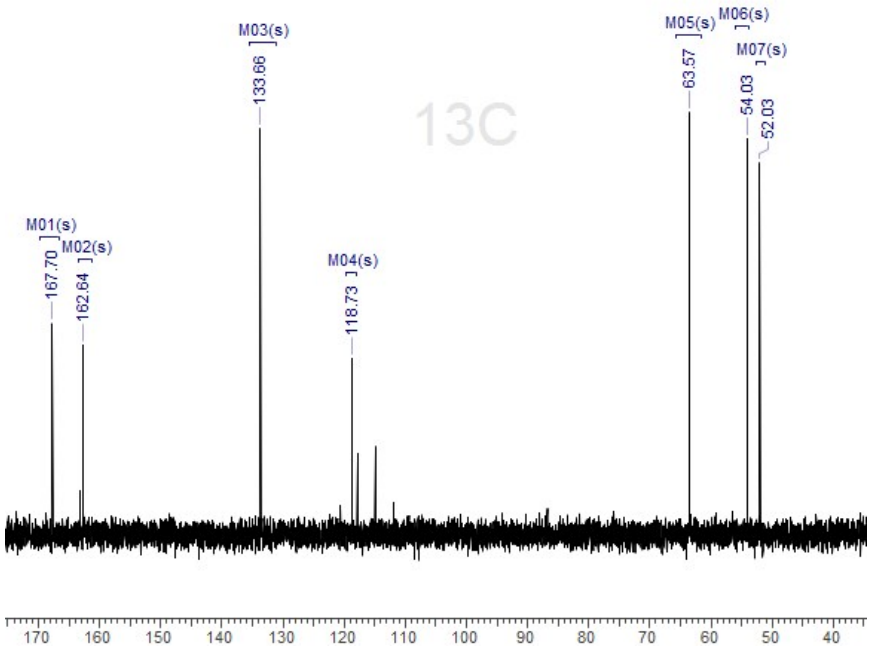


Figure S7. ^{13}C NMR spectrum of 2-amino-3-methoxy-3-oxopropyl 2-bromoacrylate at 100 MHz in D_2O. The quartet at 115 ppm and at 162 ppm correspond to residual trifluoroacetic acid.

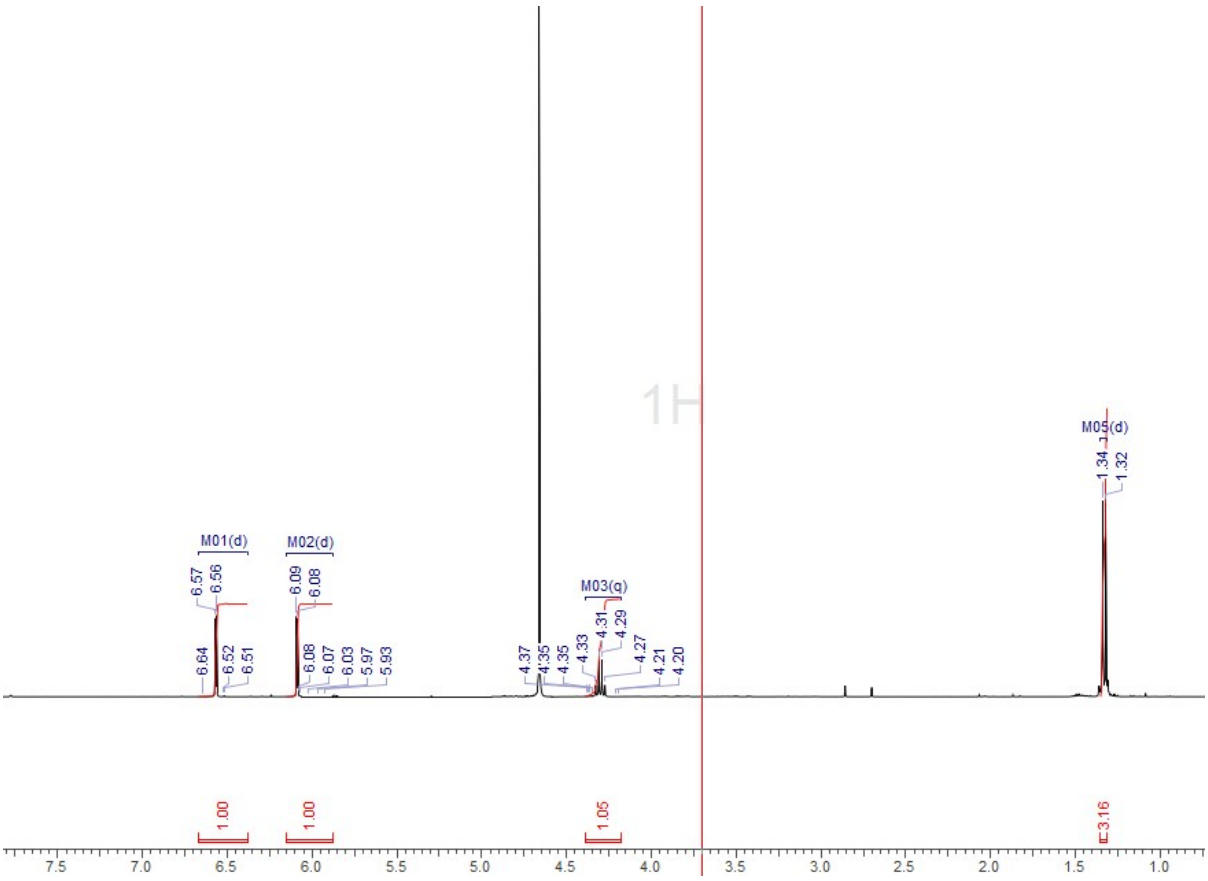


Figure S8. ^{1}H NMR spectrum of tert-butyl (2-bromoacryloyl)alaninate at 400 MHz in $CDCl_3$.

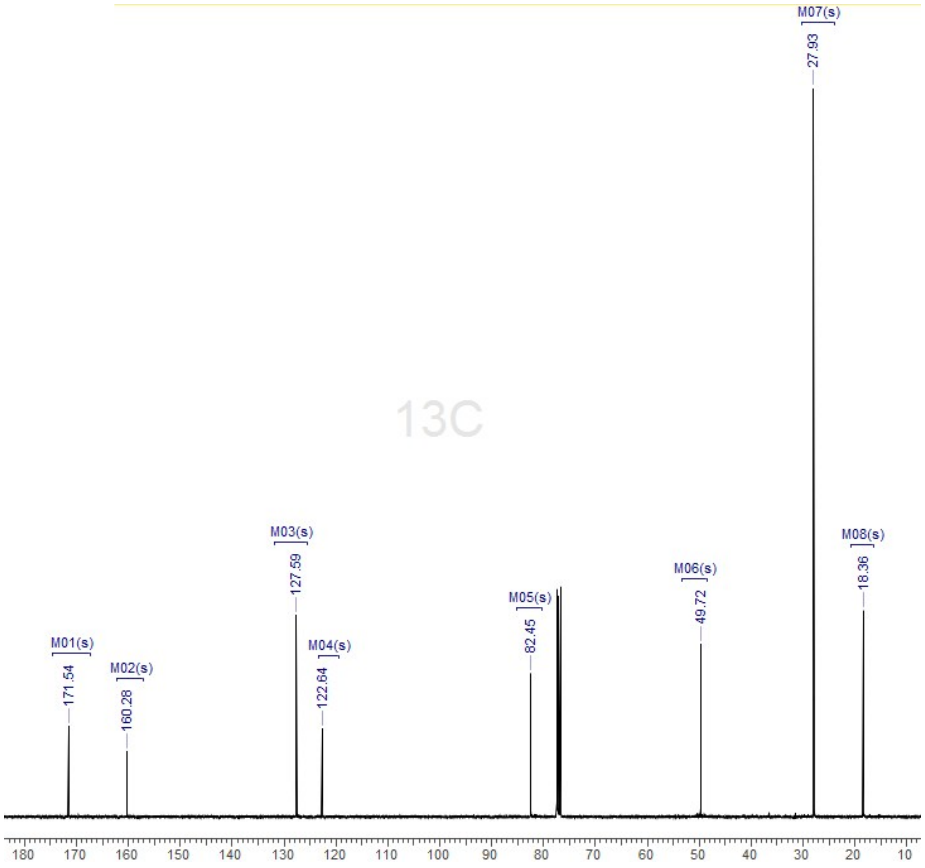


Figure S9. ^{13}C NMR spectrum of tert-butyl (2-bromoacryloyl)alaninate at 100 MHz in $CDCl_3$.

Figure S10. ^{1}H NMR spectrum of (2-bromoacryloyl)alanine at 400 MHz in D_2O.

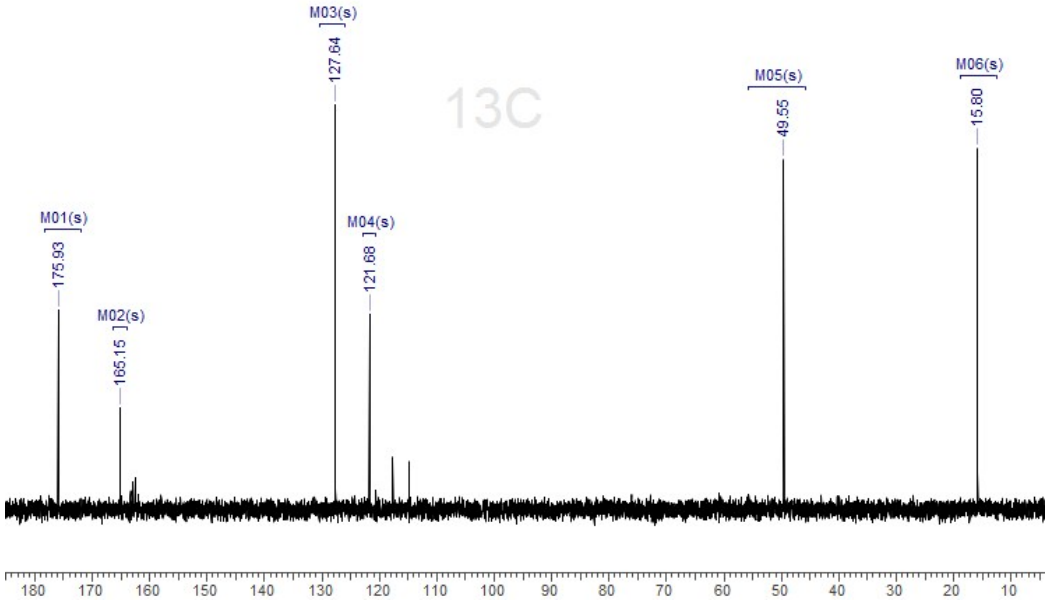


Figure S11. ^{13}C NMR spectrum of (2-bromoacryloyl)alanine at 100 MHz in D_2O. The quartet at 115 ppm and at 162 ppm correspond to residual trifluoroacetic acid.

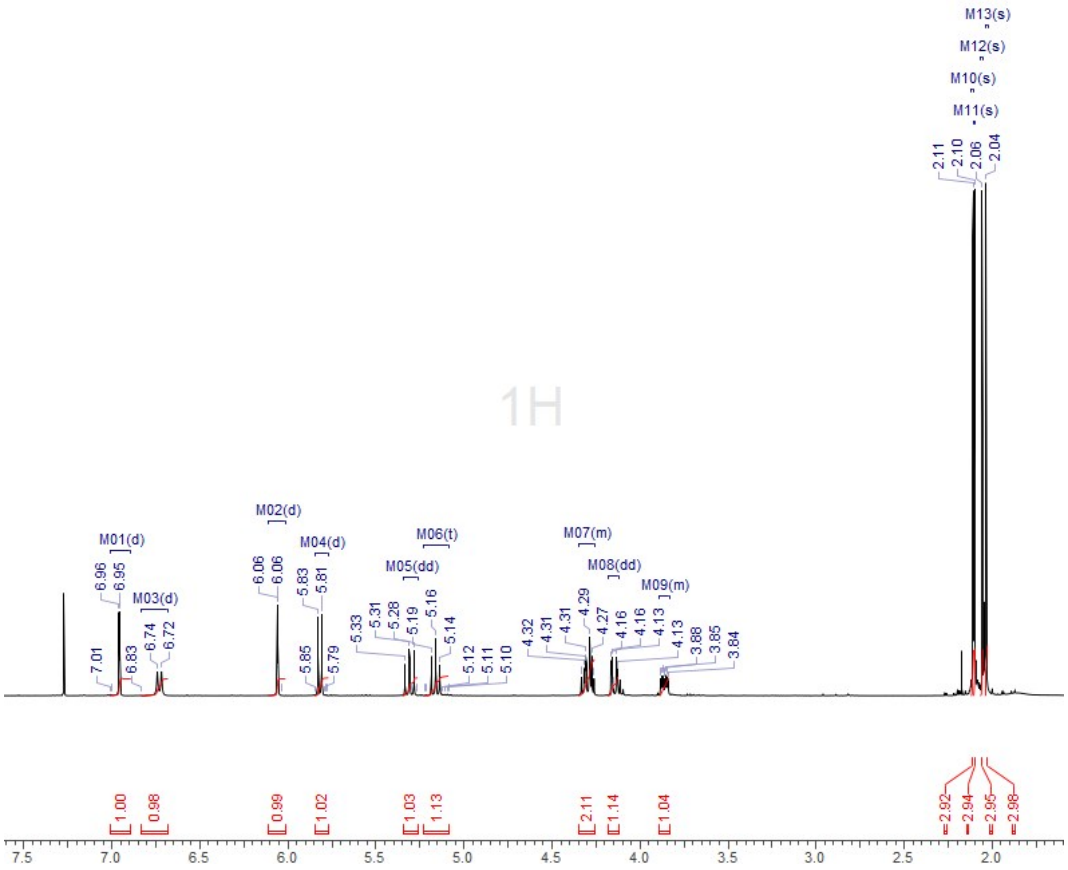


Figure S12. ^{1}H NMR spectrum of 6-(acetoxymethyl)-3-(2-bromoacrylamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate at 400 MHz in $CDCl_3$.

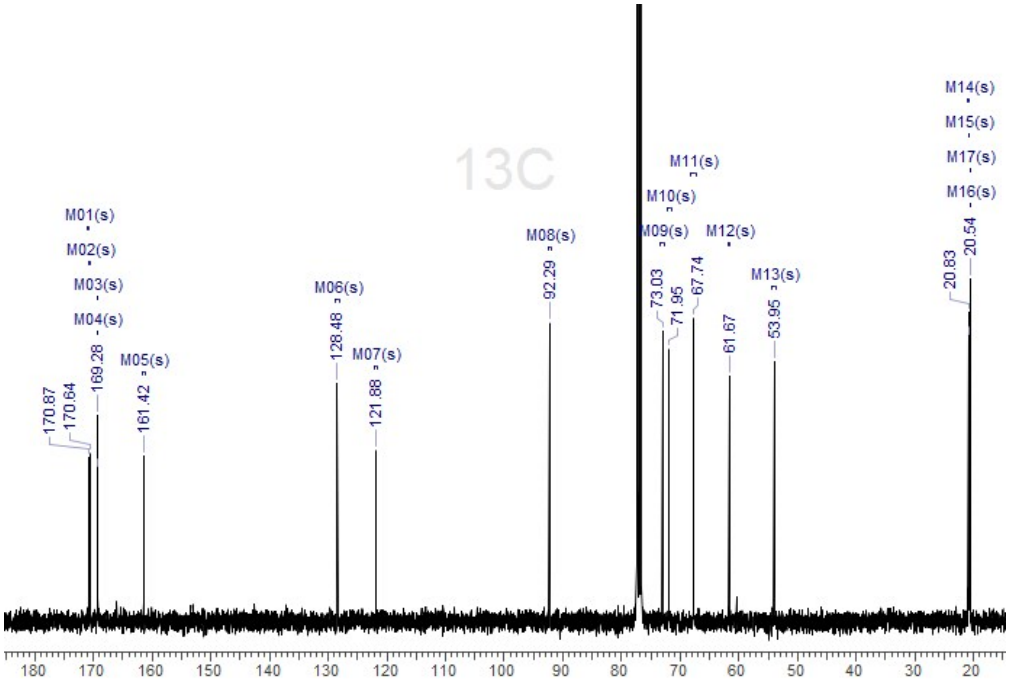


Figure S13. ^{13}C NMR spectrum of 6-(acetoxymethyl)-3-(2-bromoacrylamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate at 100 MHz in $CDCl_3$.

5. References

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