

SUPPLEMENTARY MATERIAL

The following show MALDI-TOF mass spectra of all modified oligonucleotides prepared, ^{1}H NMR spectra of small molecules prepared, representative MALDI-TOF mass spectra from an exploratory nuclease selection assay involving a 20mer target duplex and a library of decamer strands, and a plot showing the correlation between melting points and protection factors obtained. MALDI-TOF spectra were acquired as described in the first paragraph of the Materials and Methods, except for those of compounds **13**, **16**, **17** and **18**, which were acquired using 6-aza-2-thiothymine as matrix.

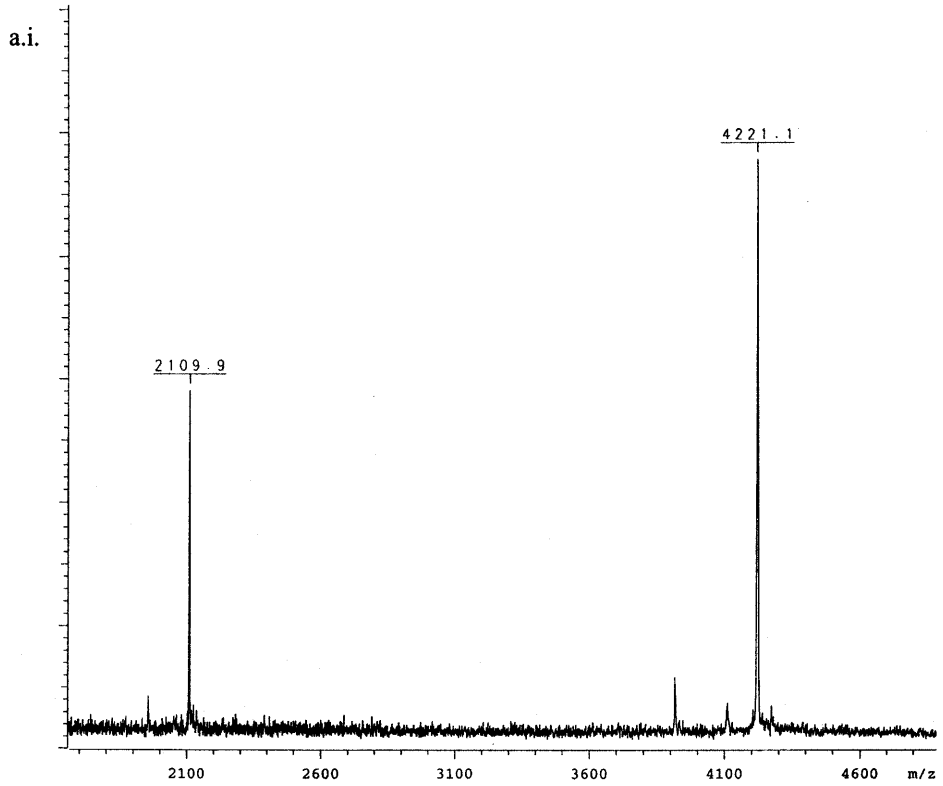

Figure S1. MALDI-TOF spectrum of **12l** (negative, linear mode).

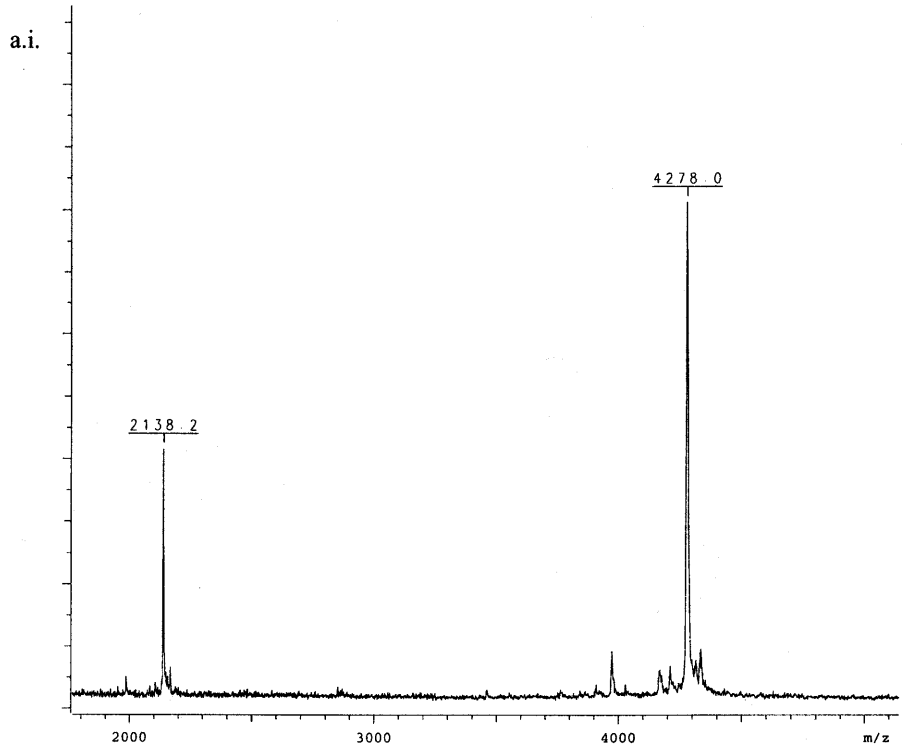

Figure S2. MALDI-TOF spectrum of **12m** (negative, linear mode).

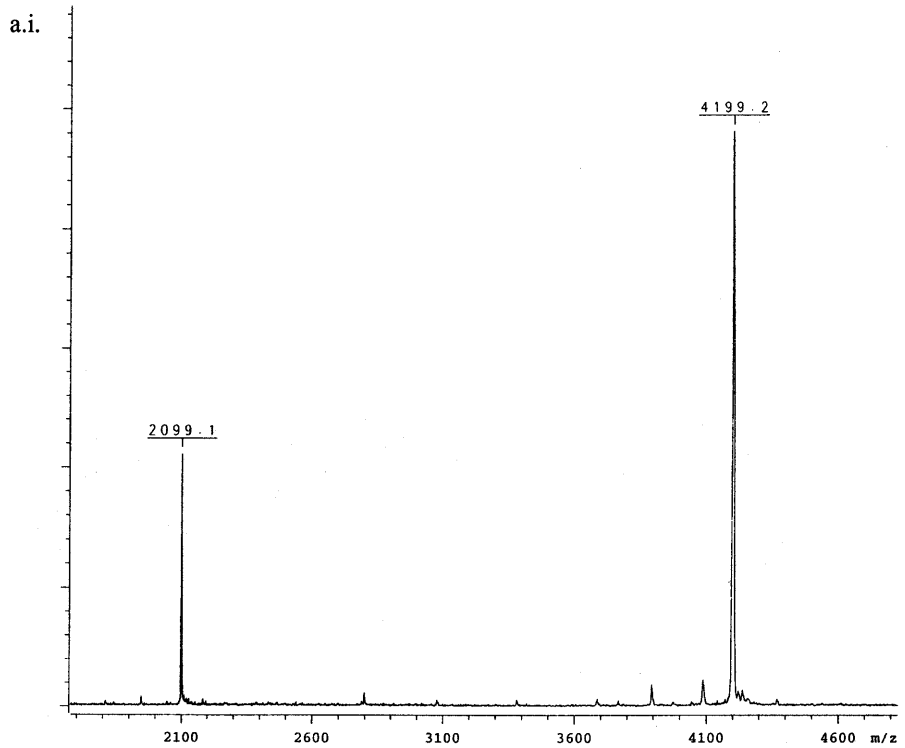

Figure S3. MALDI-TOF spectrum of **12h** (negative, linear mode).

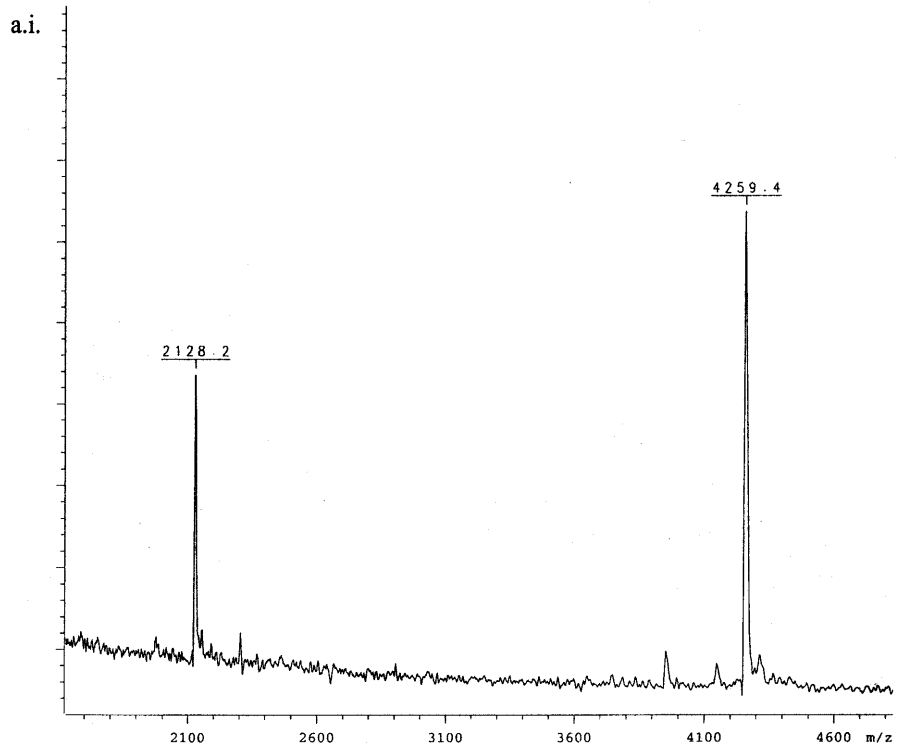

Figure S4. MALDI-TOF spectrum of **12i** (negative, linear mode).

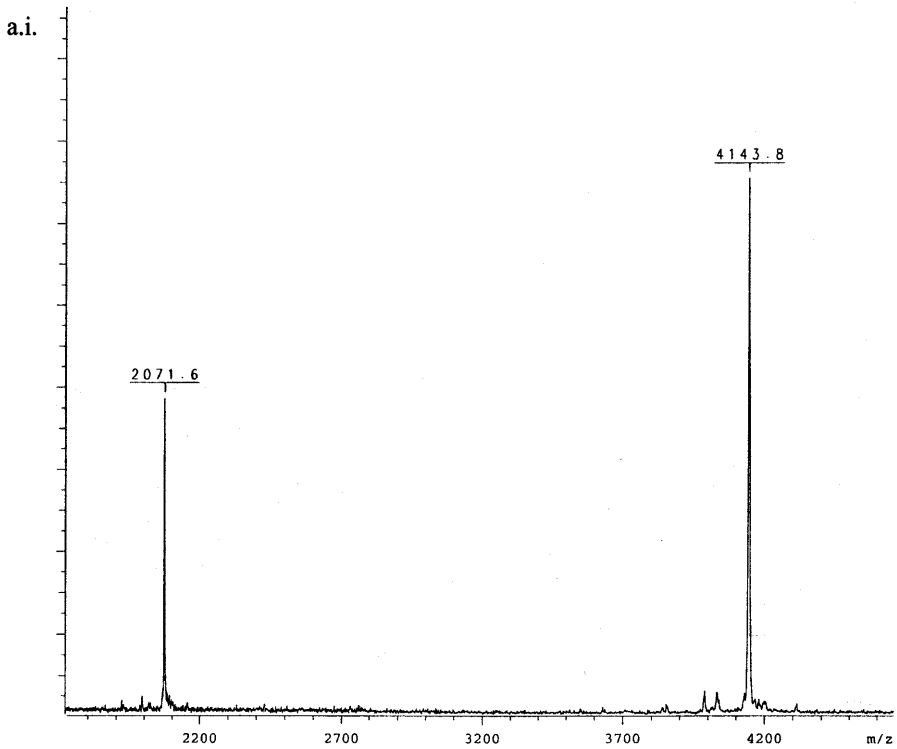

Figure S5. MALDI-TOF spectrum of **12d** (negative, linear mode).

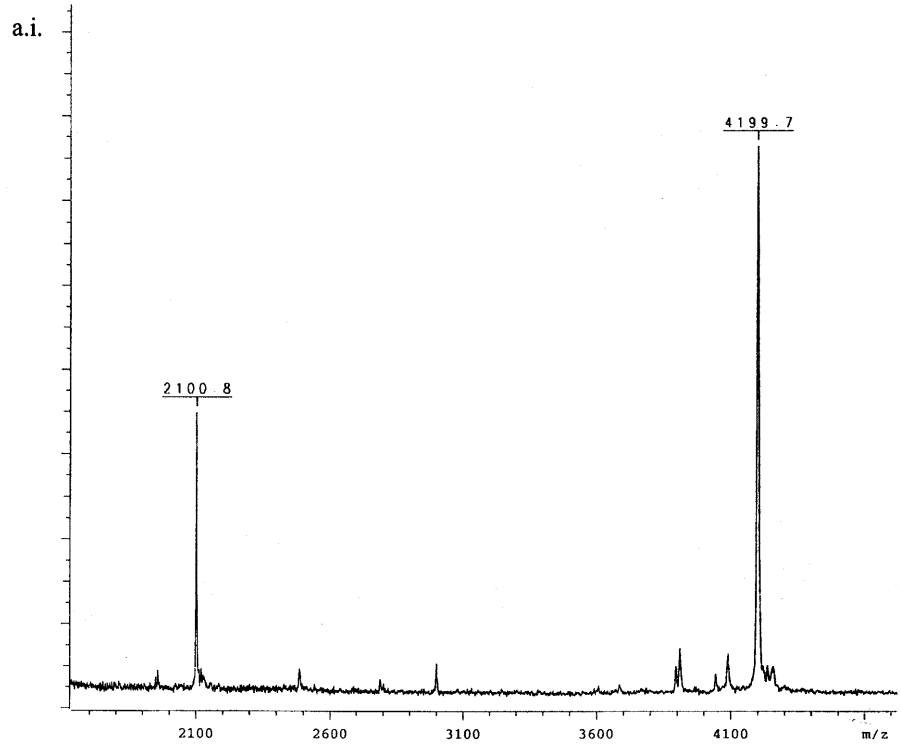

Figure S6. MALDI-TOF spectrum of **12e** (negative, linear mode).

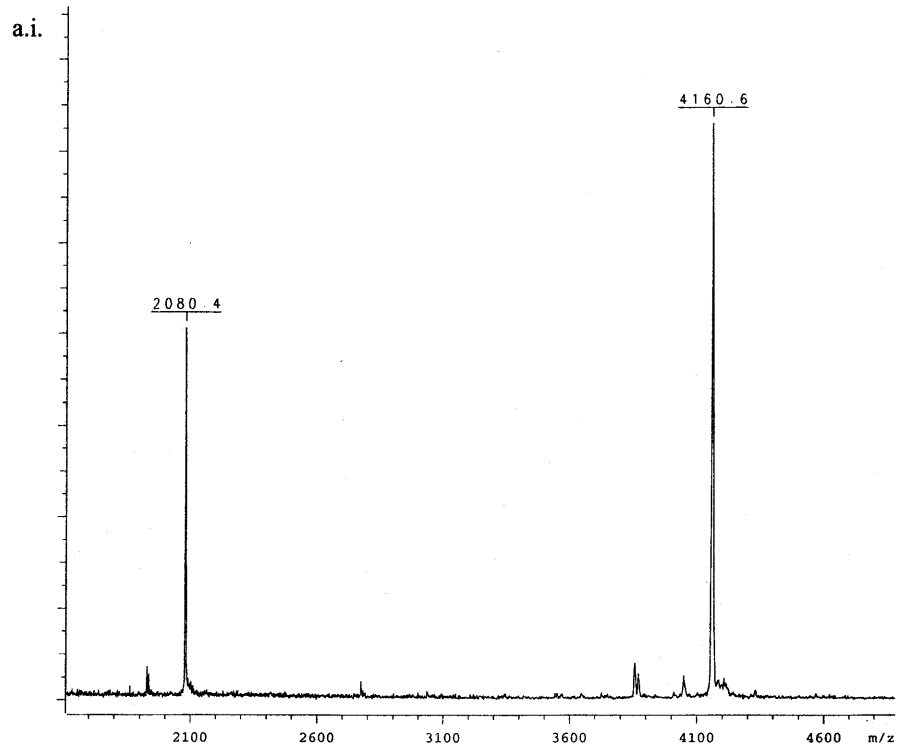

Figure S7. MALDI-TOF spectrum of **12j** (negative, linear mode).

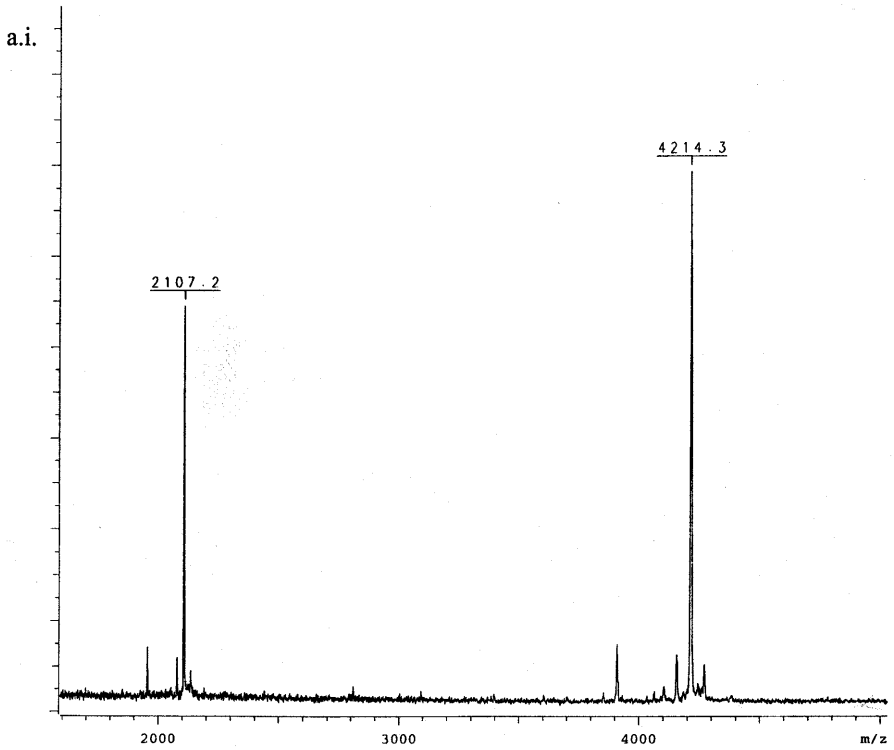

Figure S8. MALDI-TOF spectrum of **12k** (negative, linear mode).

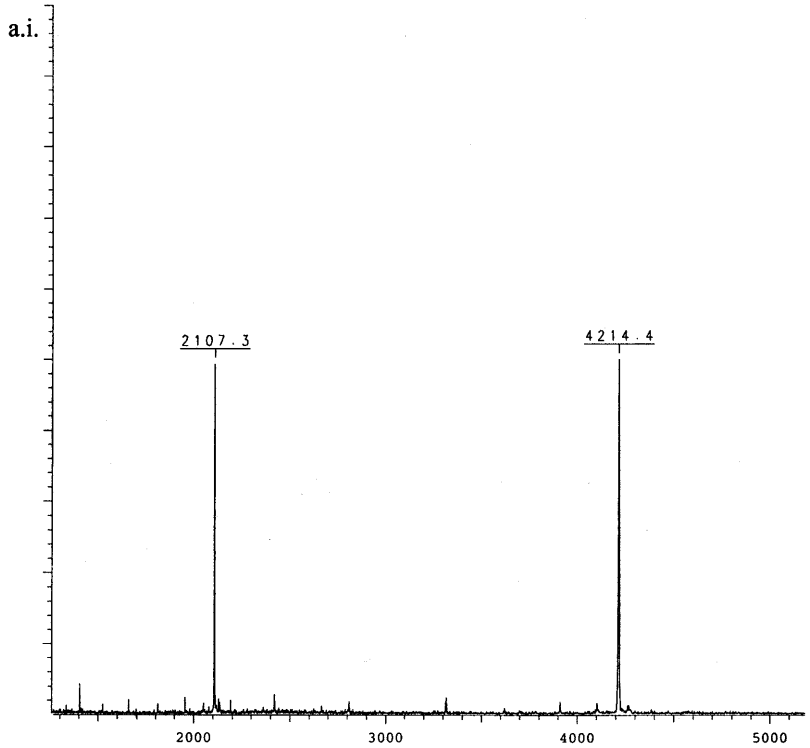

Figure S9. MALDI-TOF spectrum of **12n** (negative, linear mode).

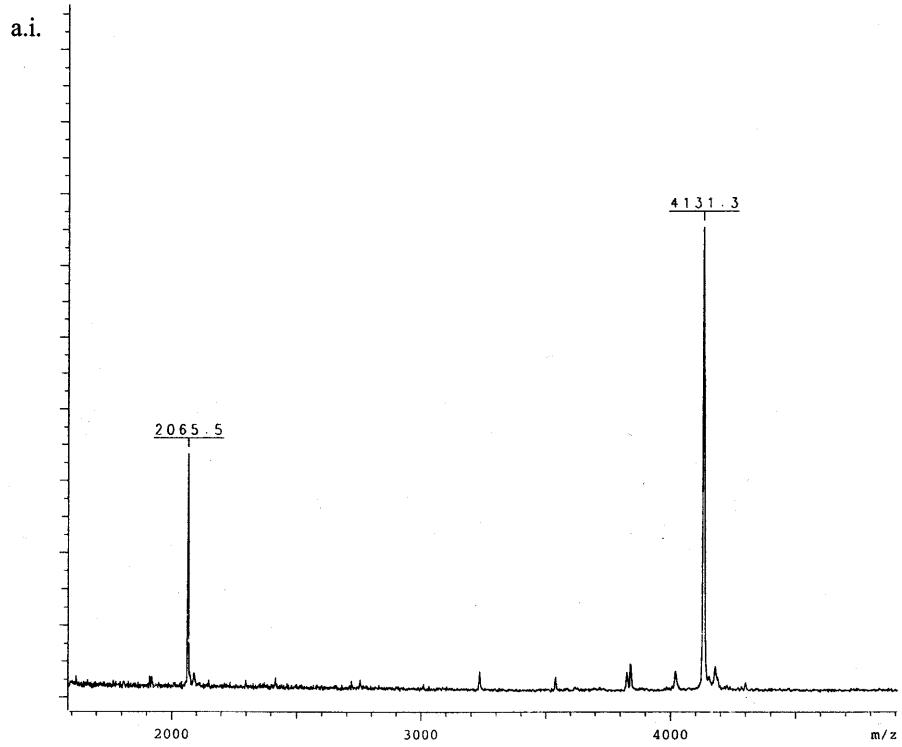

Figure S10. MALDI-TOF spectrum of **12f** (negative, linear mode).

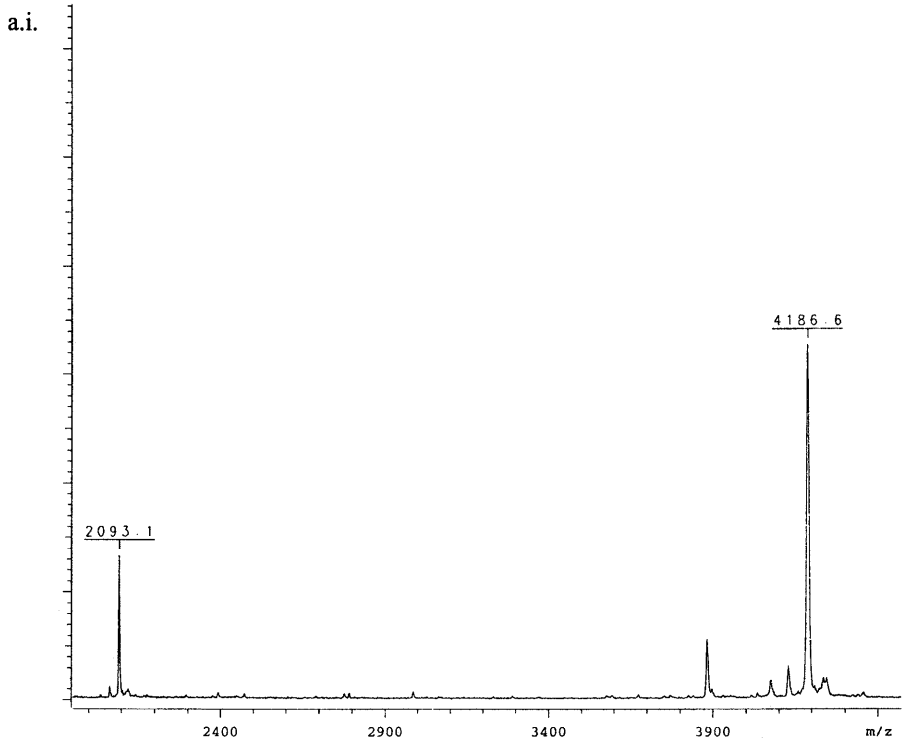

Figure S11. MALDI-TOF spectrum of **12g** (negative, linear mode).

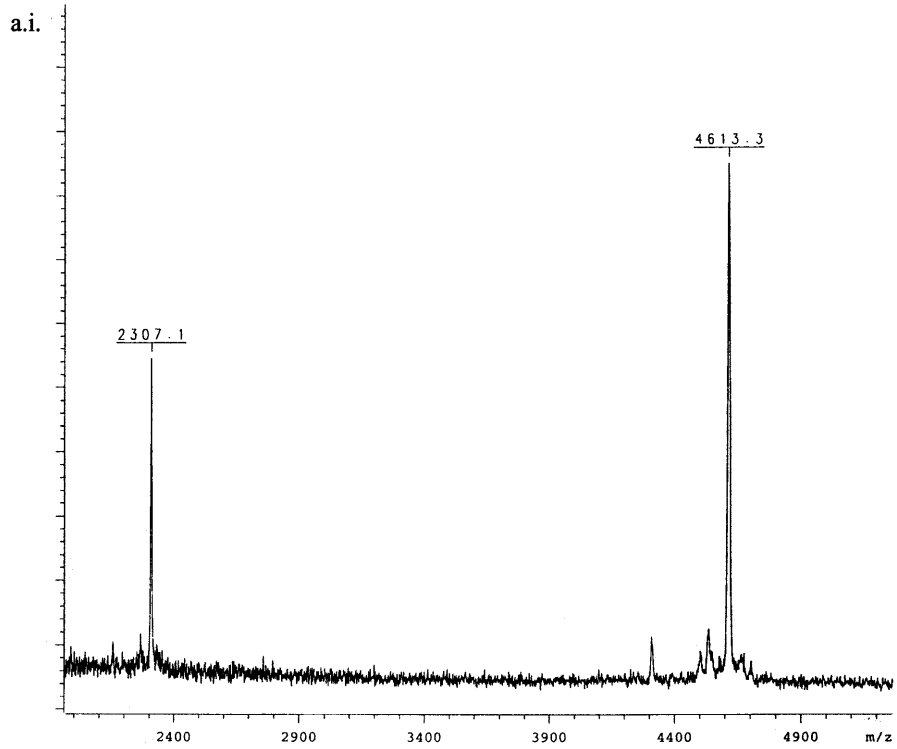

Figure S12. MALDI-TOF spectrum of **12o** (negative, linear mode).

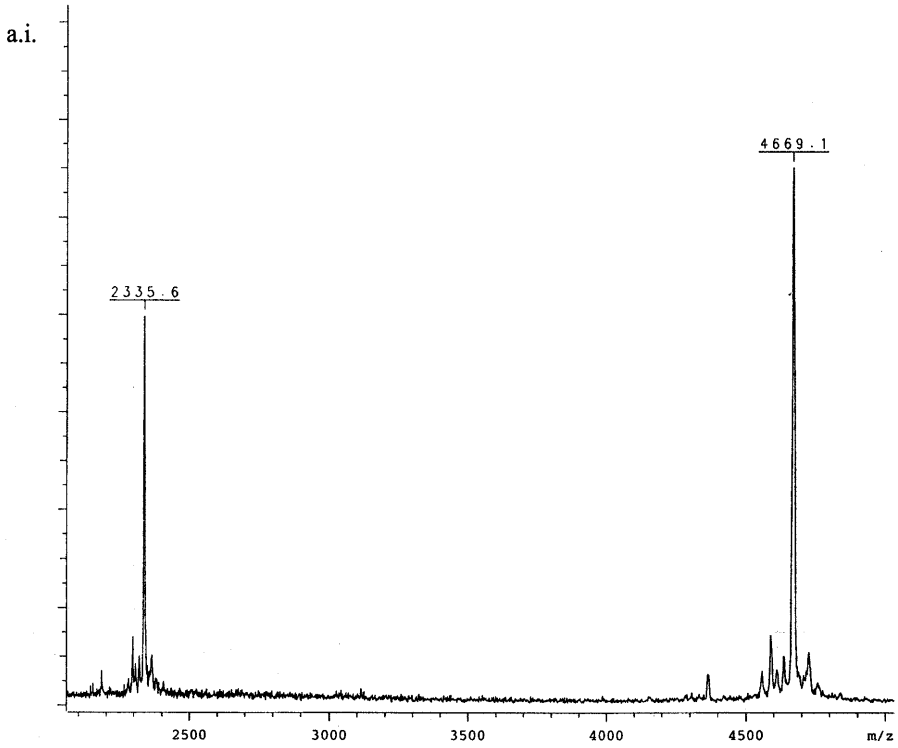

Figure S13. MALDI-TOF spectrum of **12p** (negative, linear mode).

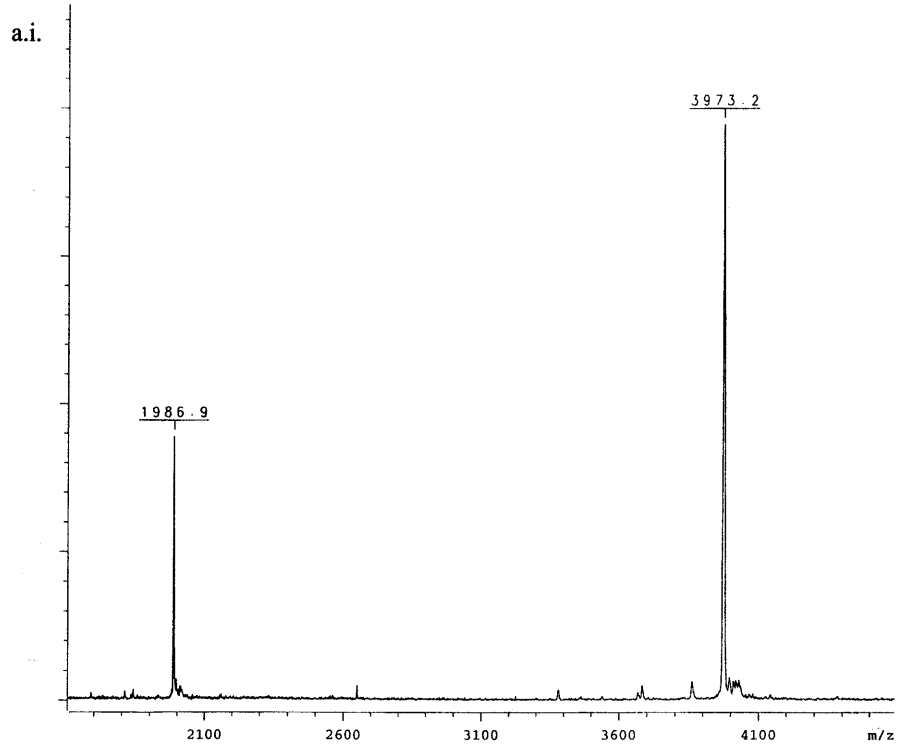

Figure S14. MALDI-TOF spectrum of **12a** (negative, linear mode).

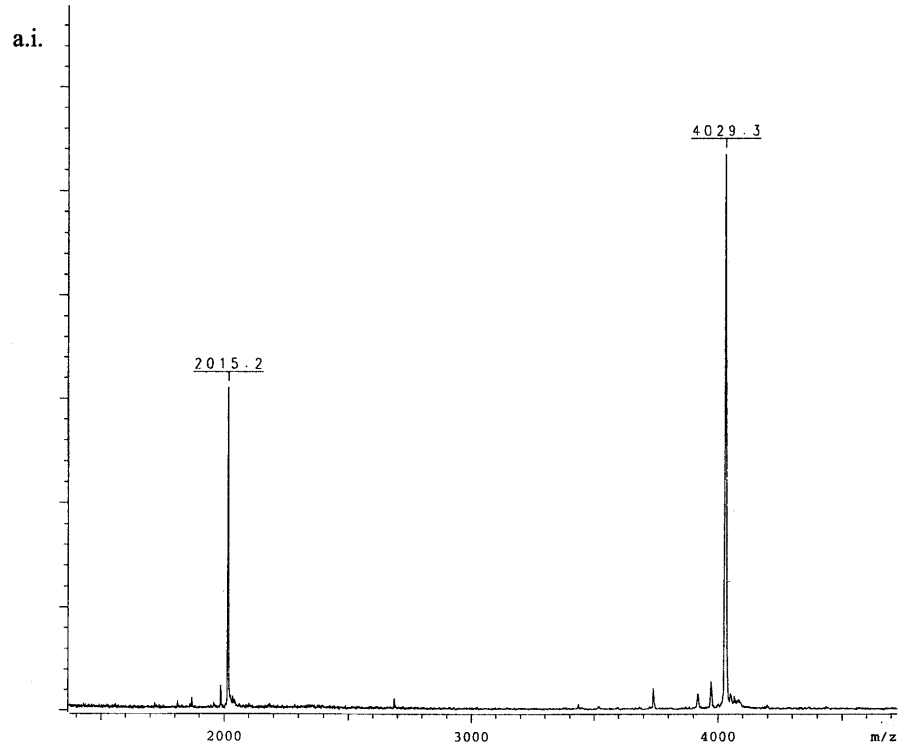

Figure S15. MALDI-TOF spectrum of **12b** (negative, linear mode).

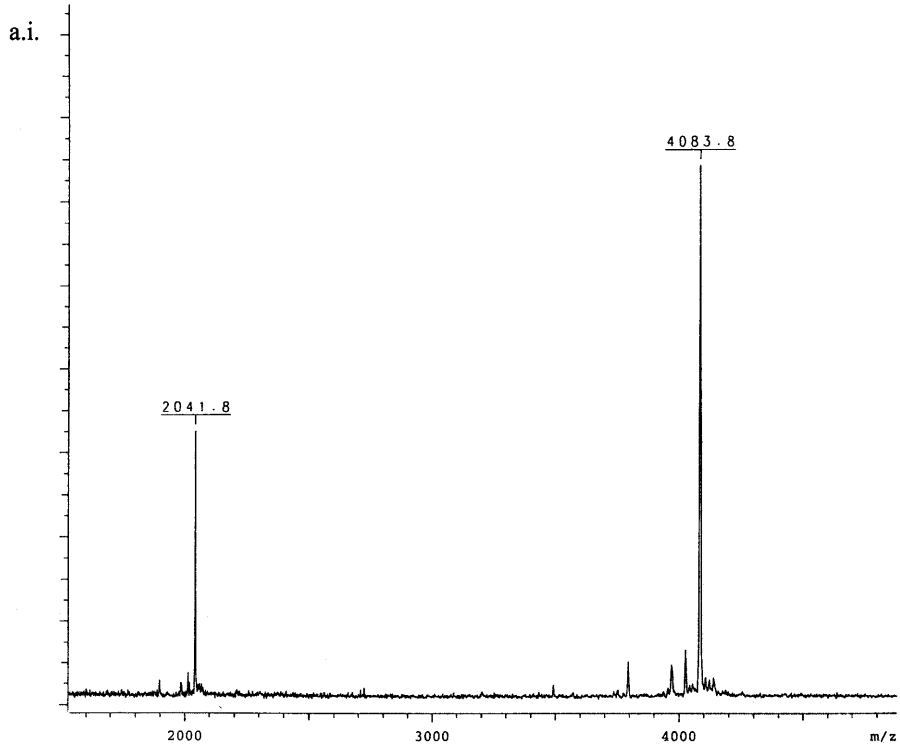

Figure S16. MALDI-TOF spectrum of **12c** (negative, linear mode).

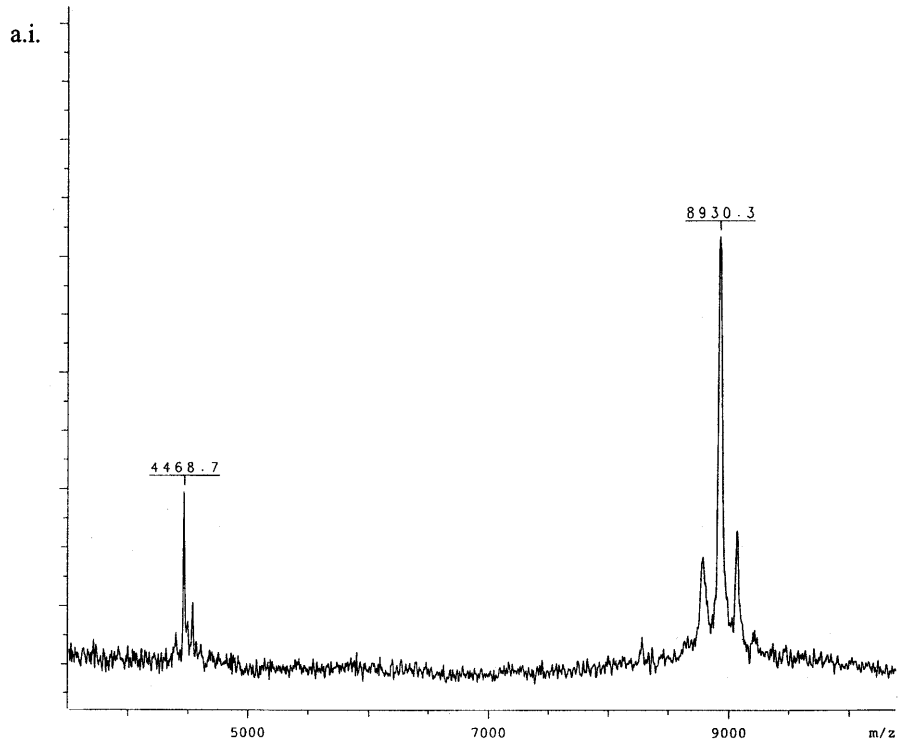

Figure S17. MALDI-TOF spectrum of compound **13** (negative, linear mode).

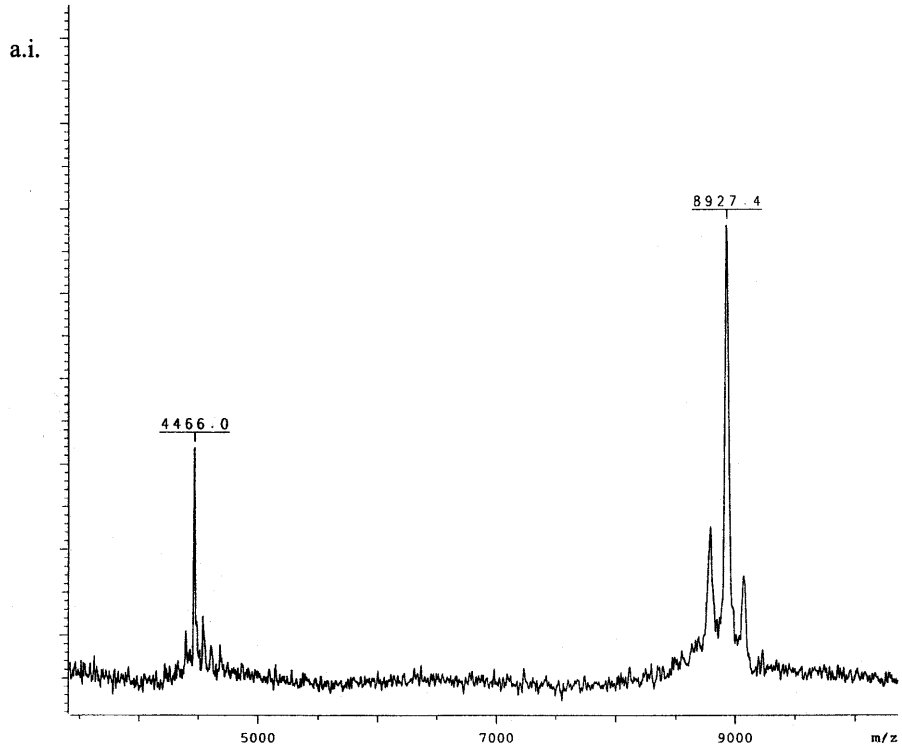

Figure S18. MALDI-TOF spectrum of compound **16** (negative, linear mode).

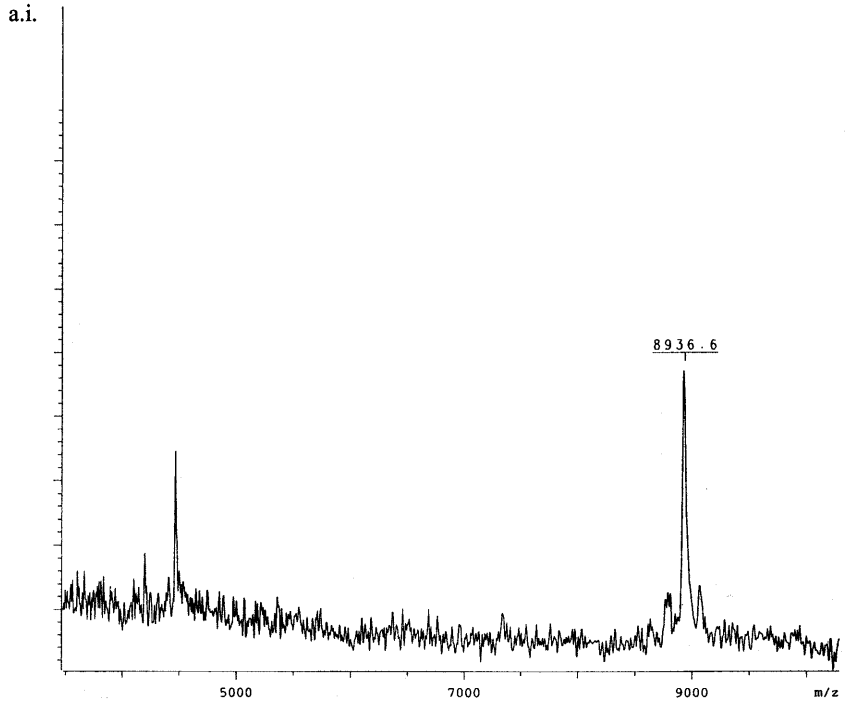

Figure S19. MALDI-TOF spectrum of compound **18** (negative, linear mode).

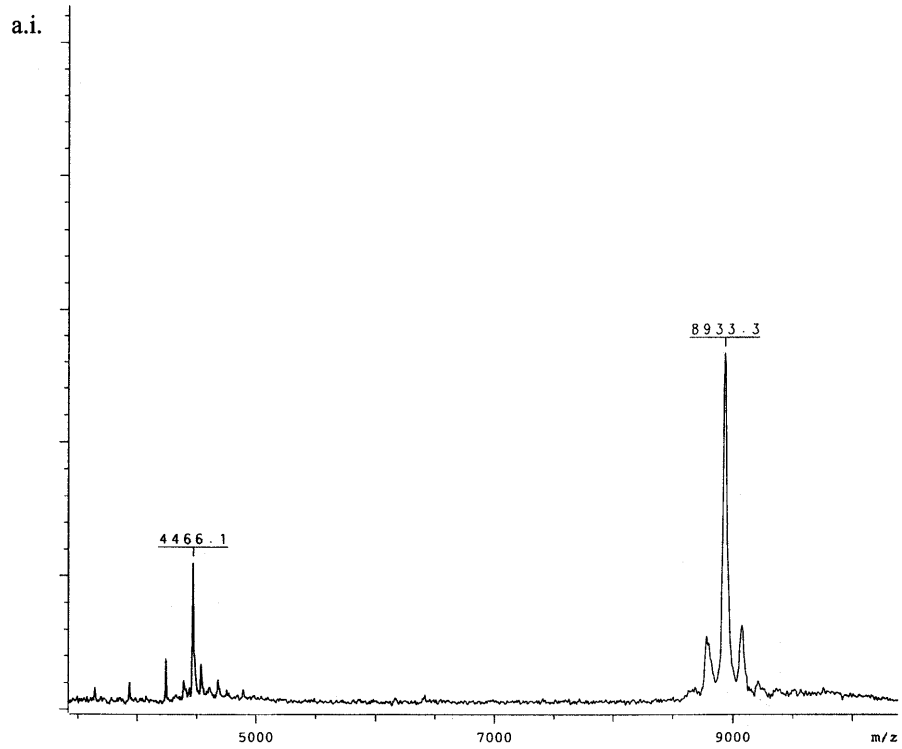

Figure S20. MALDI-TOF spectrum of compound **17** (negative, linear mode).

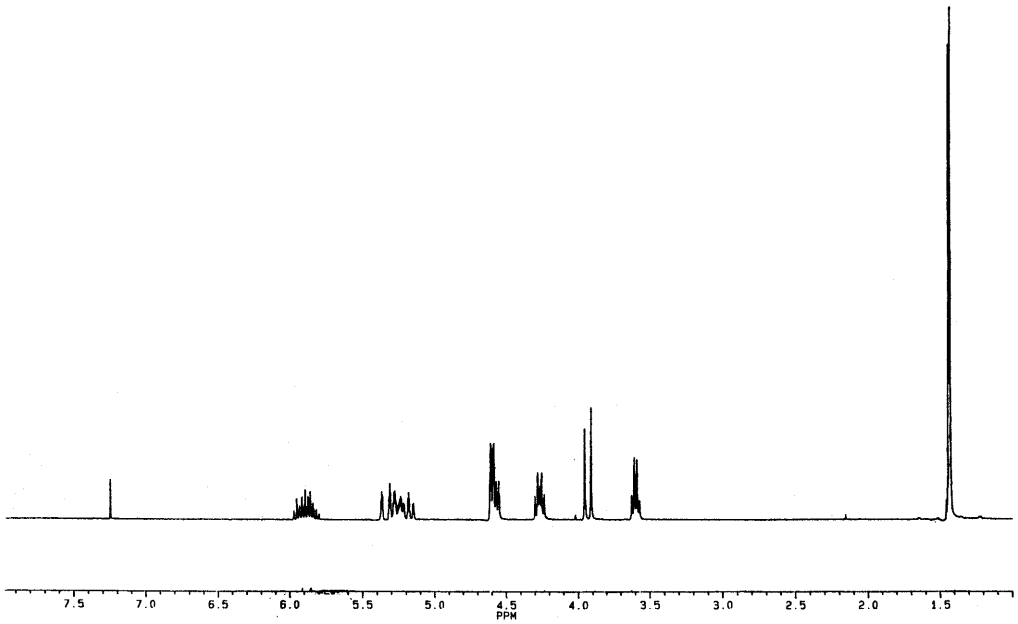

Figure S21. ^{1}H NMR spectrum (250 MHz, $CDCl_3$) of *N*-allyloxycarbonyl-*N*-(2-allyloxycarbonyloxyethyl)glycine *tert*-butyl ester (**2**).

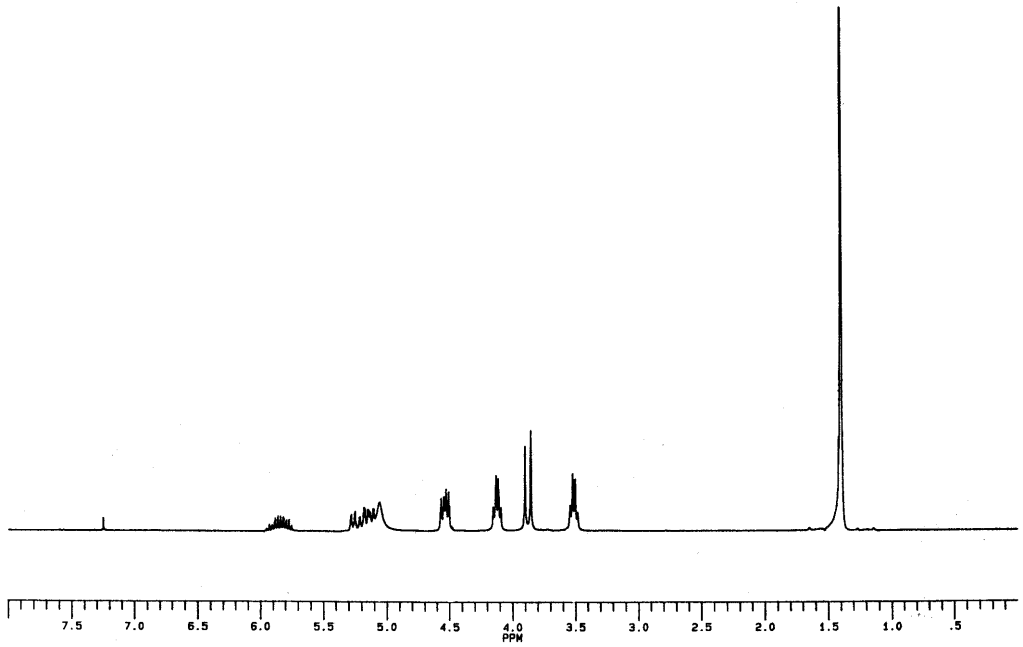

Figure S22. ^{1}H NMR spectrum (250 MHz, $CDCl_3$) of *N*-allyloxycarbonyl-*N*-(2-hydroxyethyl)glycine *tert*-butyl ester (**3**).

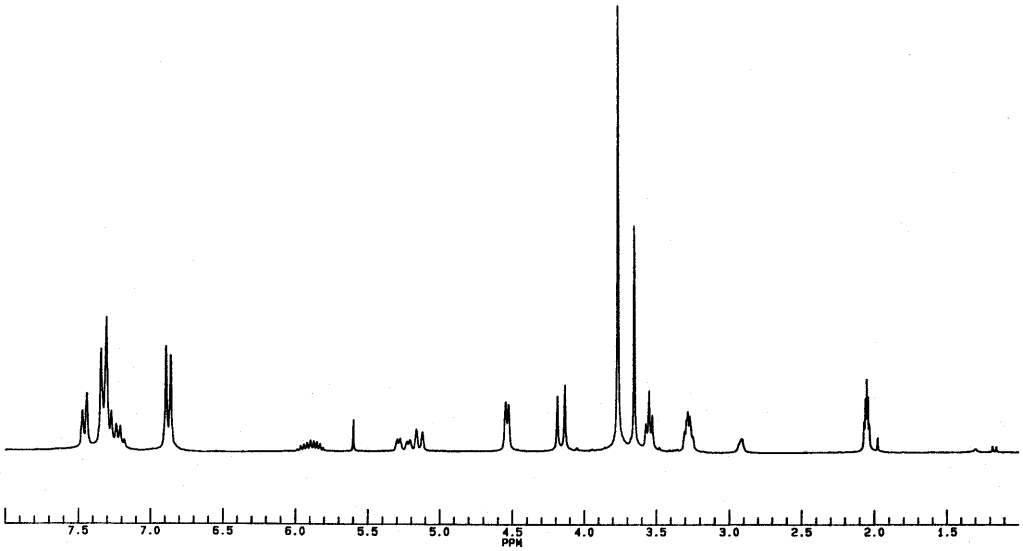

Figure S23. ^{1}H NMR spectrum (250 MHz, acetone-d_6) of *N*-allyloxycarbonyl-*N*-(2-(4,4′-dimethoxytriphenylmethoxy)ethyl)glycine methyl ester (**4**).

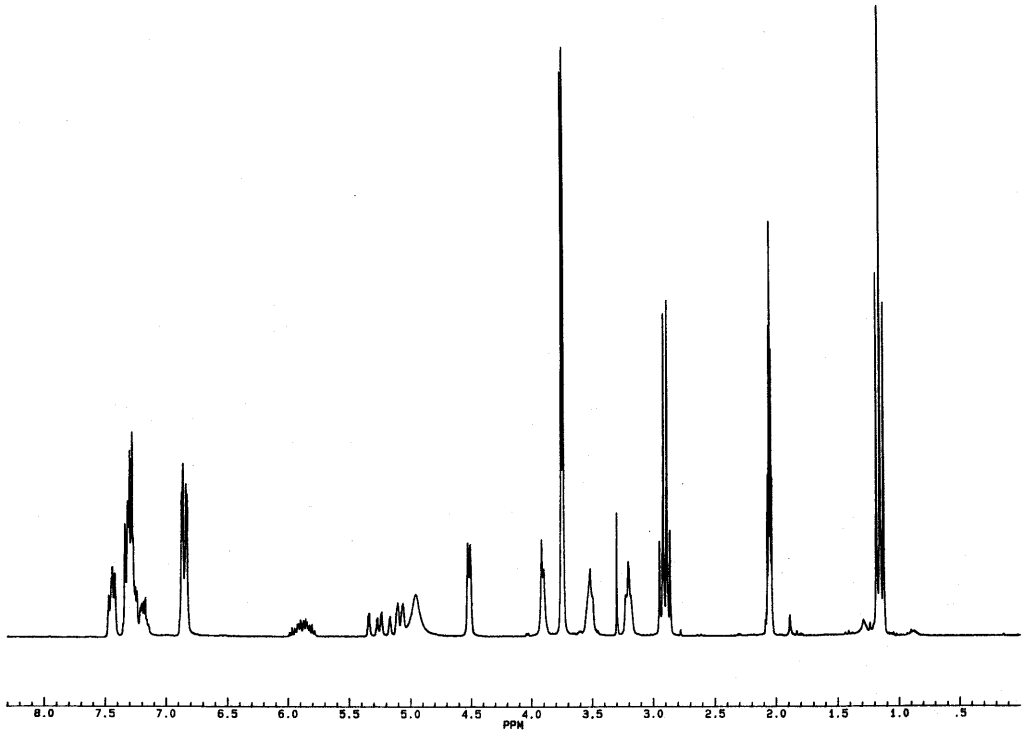

Figure S24. ^{1}H NMR spectrum (250 MHz, acetone-d_6) of *N*-allyloxycarbonyl-*N*-(2-(4,4′-dimethoxytriphenylmethoxy)ethyl)glycine triethylammonium salt (**5**).

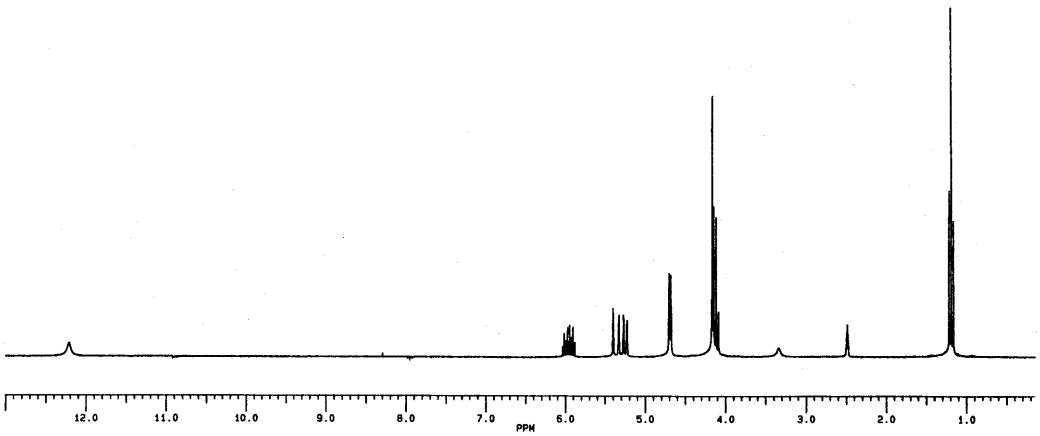

Figure S25. ^{1}H NMR spectrum (250 MHz, $CDCl_3$) of ethyl 5-*N*-allyloxycarbonylamino-1,3,4-thiadiazol-2-yl-acetate (building block for the preparation of **12n**).

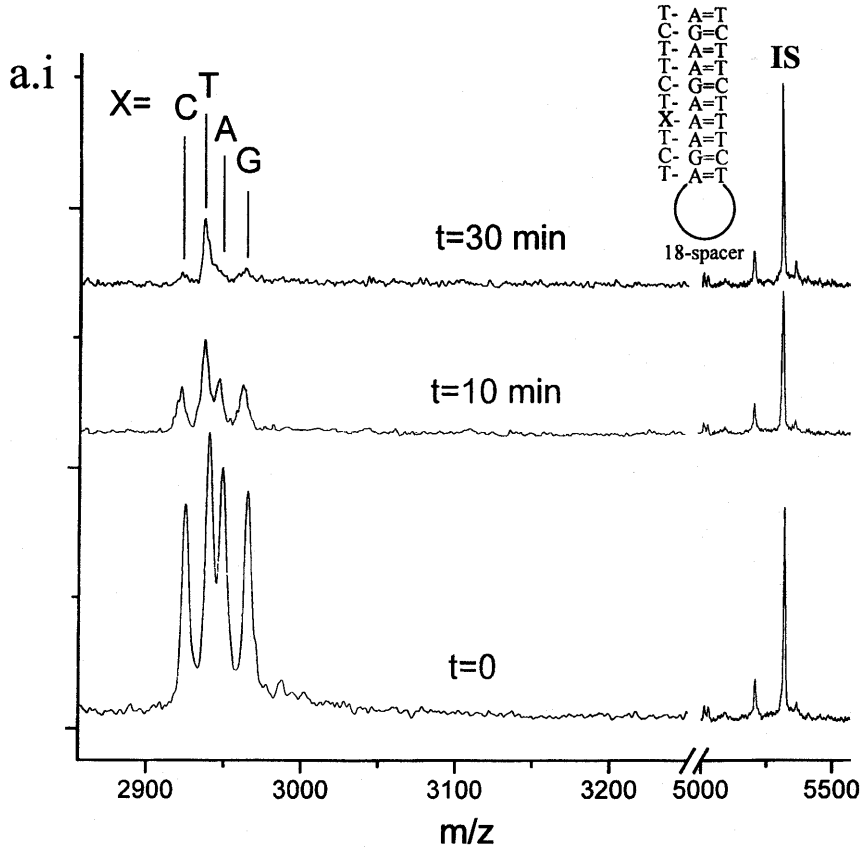

Figure S26. Representative MALDI-TOF mass spectra from a monitored nuclease selection involving an intramolecular decamer duplex of the sequence shown in the upper right corner and four strands competing for this duplex target. The selection was performed with nuclease S1 as the selecting enzyme, 10°C as the assay temperature, 250 mM ammonium sulfate buffer, adjusted to pH 6, and 50 pmol of each strand. IS denotes the peak of the internal standard. Note that the strand fully complementary in the Hoogsteen sense is surviving selectively.

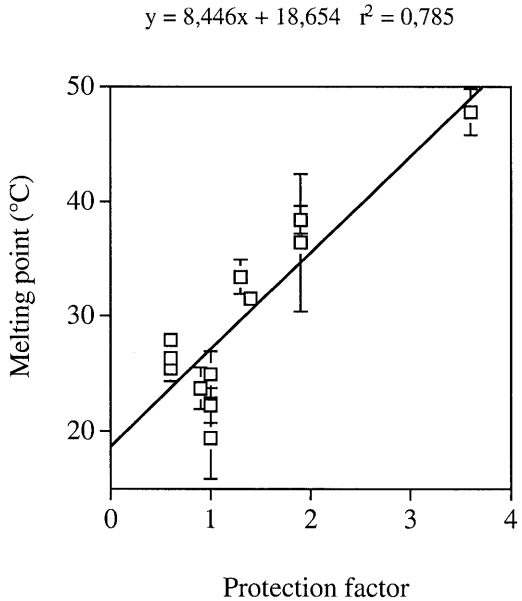

Figure S27. Correlation of protection factors obtained from exploratory nuclease selection assays and UV melting experiments for those triplexes of **13** and derivatives of **12** for which both data are available. Data points can also be found in Tables 1 and 3. The equation at the top shows the results of the linear fit.