

Electronic Supplementary Information

Versatile C₃-symmetric scaffolds and their use for covalent stabilization of the foldon trimer

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NMR spectra of 3 through 8

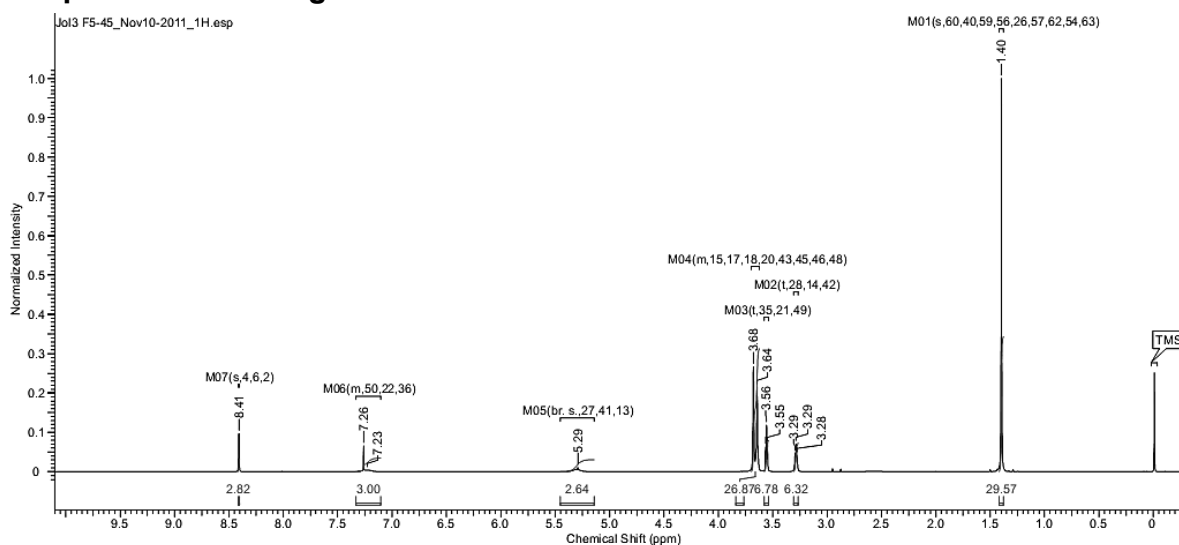


Figure S1. ¹H-NMR spectrum of **3**.

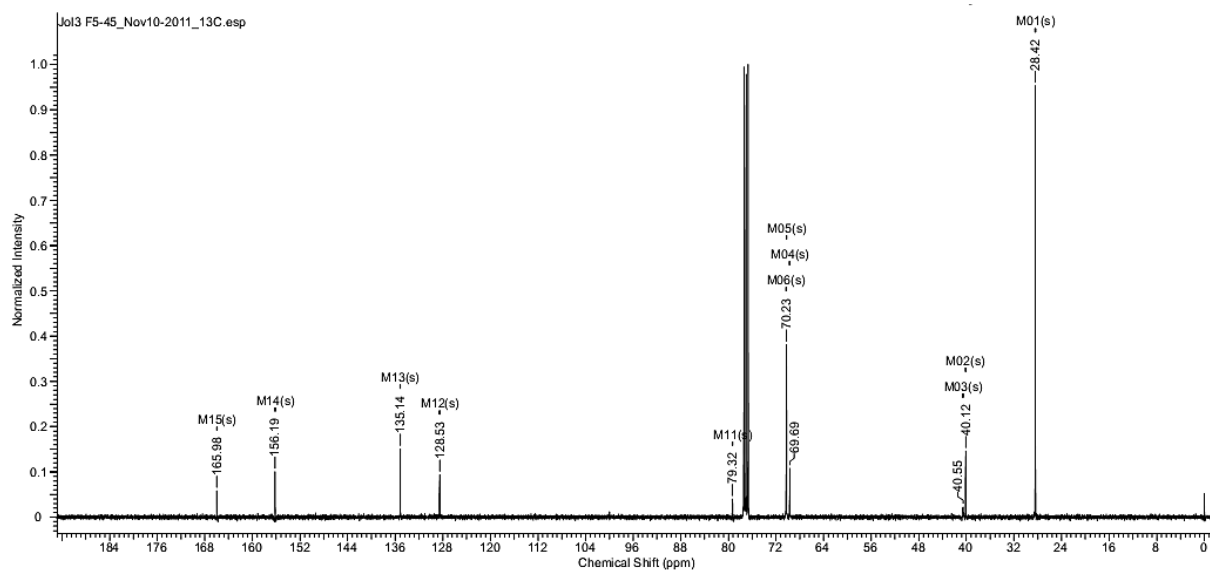


Figure S2. ^{13}C -NMR spectrum of 3.

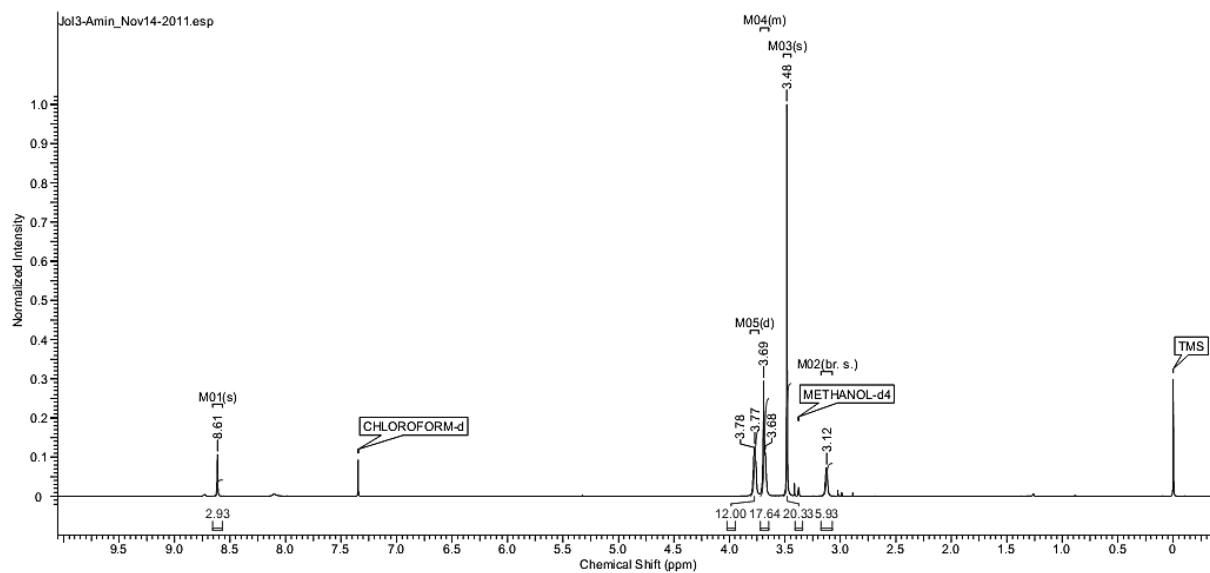


Figure S3. ^1H -NMR spectrum of 4.

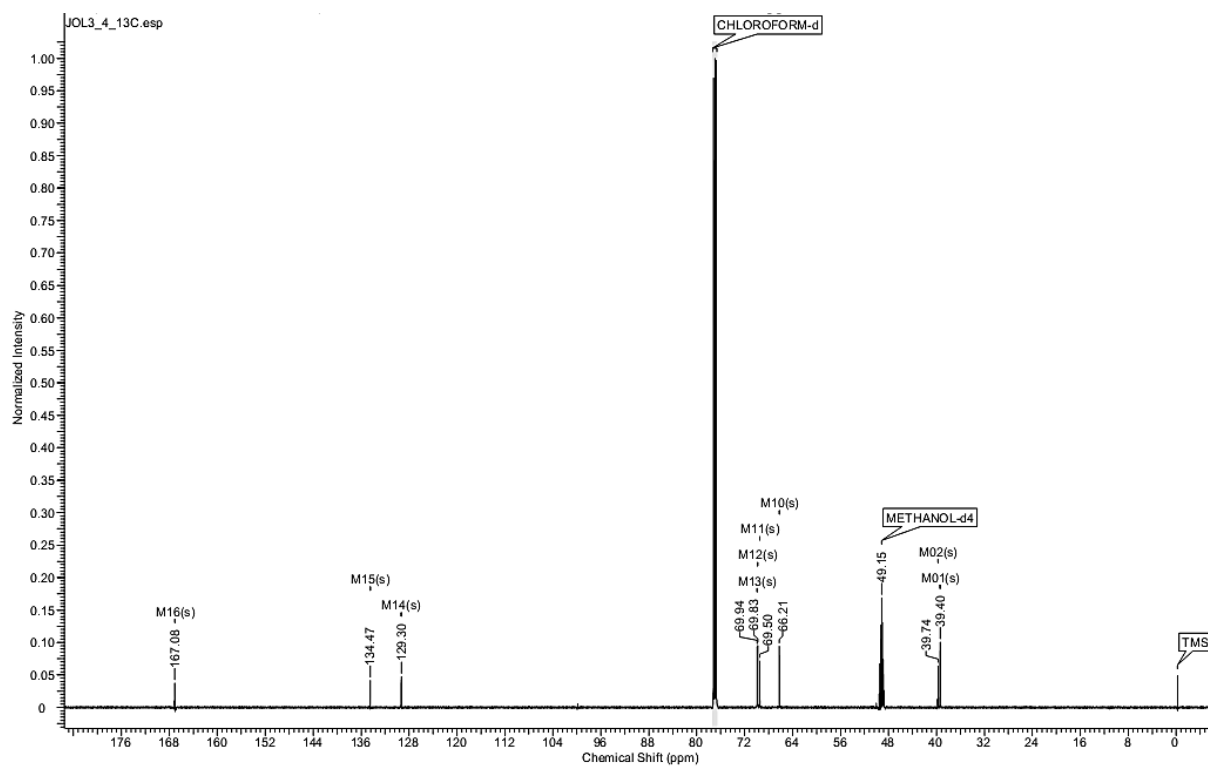


Figure S4. ^{13}C -NMR spectrum of **4**.

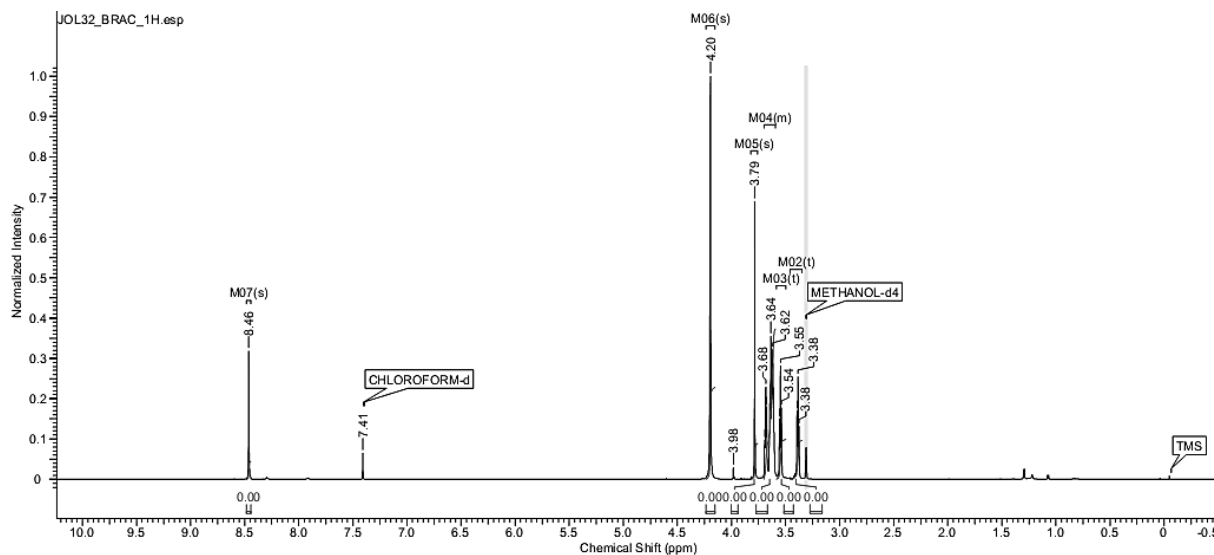


Figure S5. ^1H -NMR spectrum of **5**.

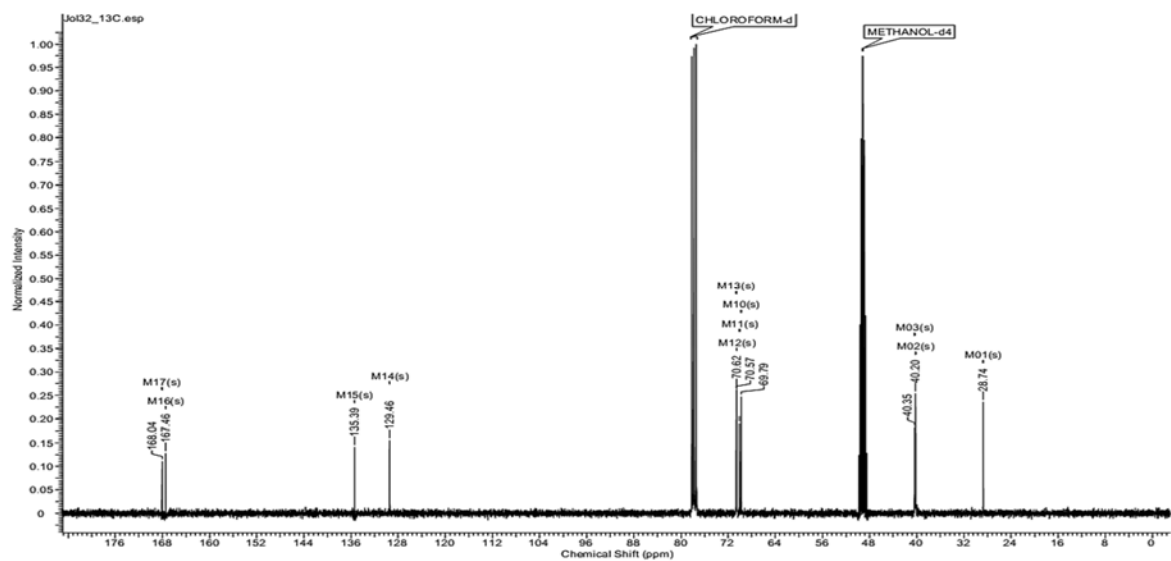


Figure S6. ¹³C-NMR spectrum of 5.

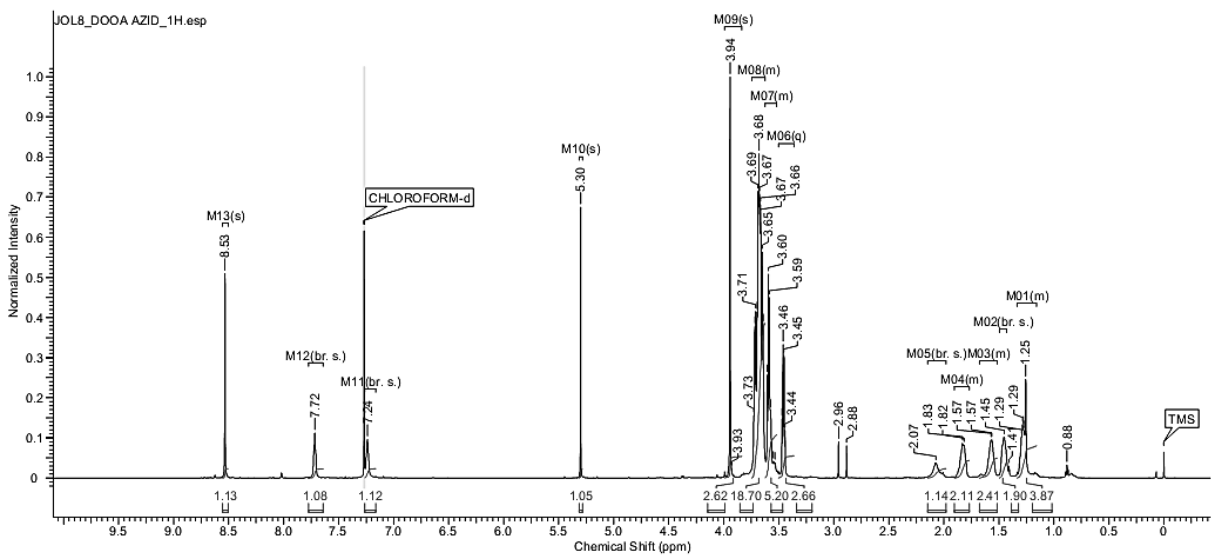


Figure S7. ¹H-NMR spectrum of 6.

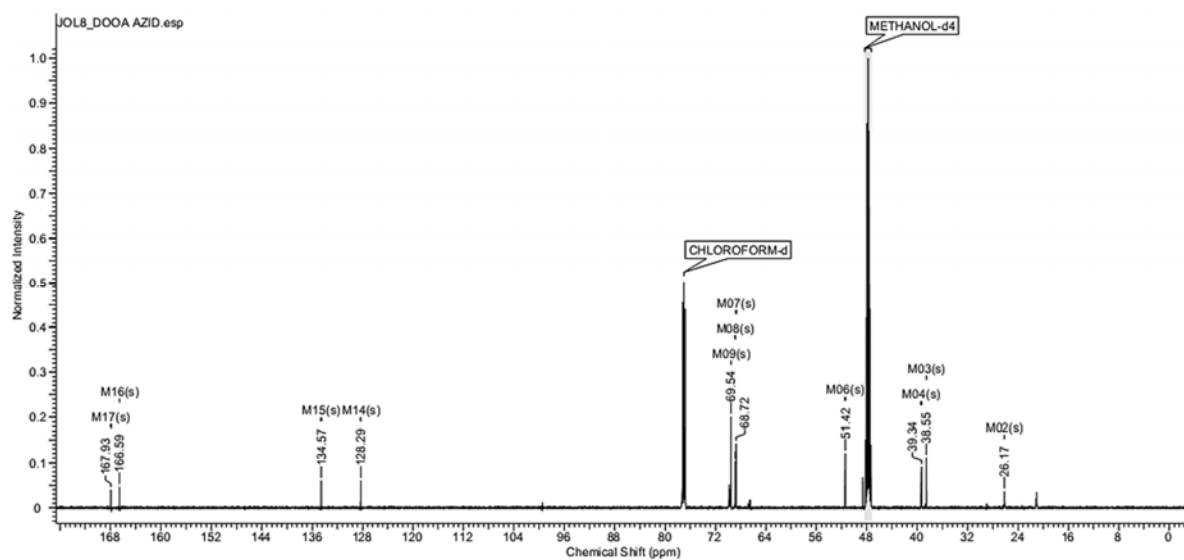


Figure S8. ^{13}C -NMR spectrum of **6**.

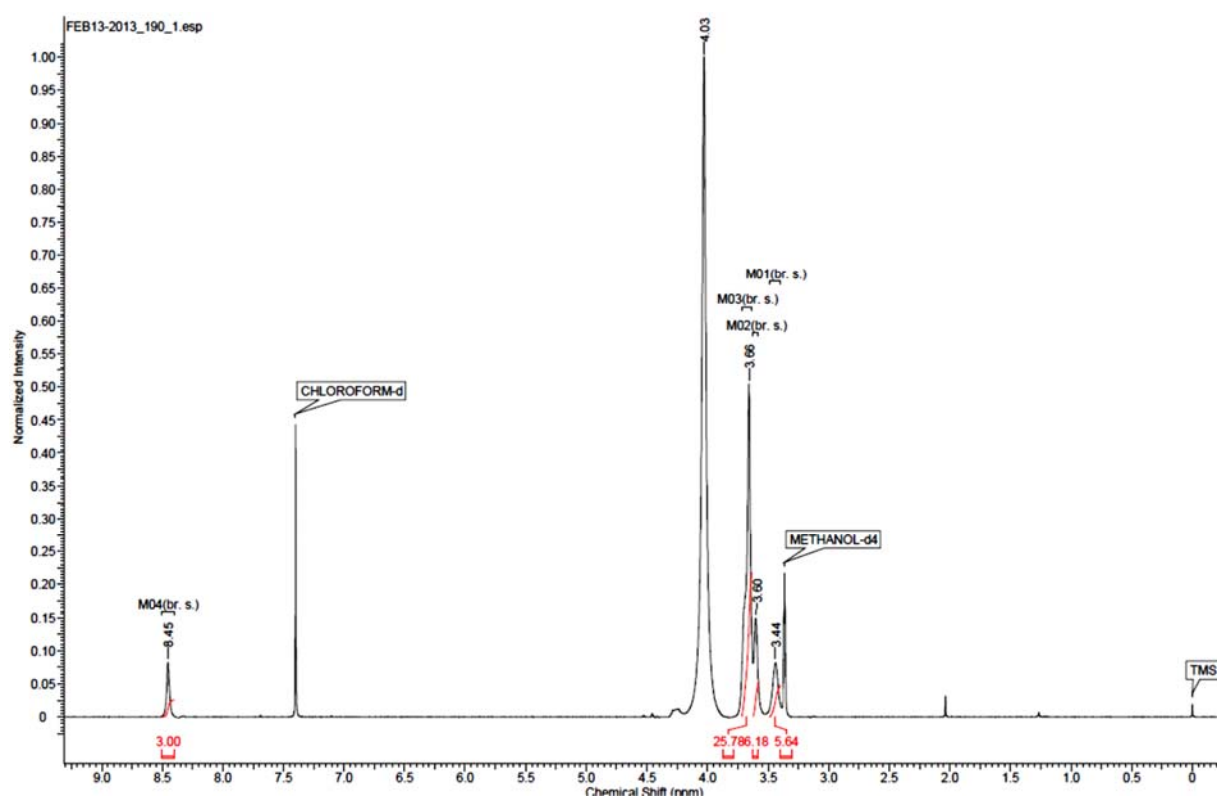


Figure S9. ^1H -NMR spectrum of **7**.

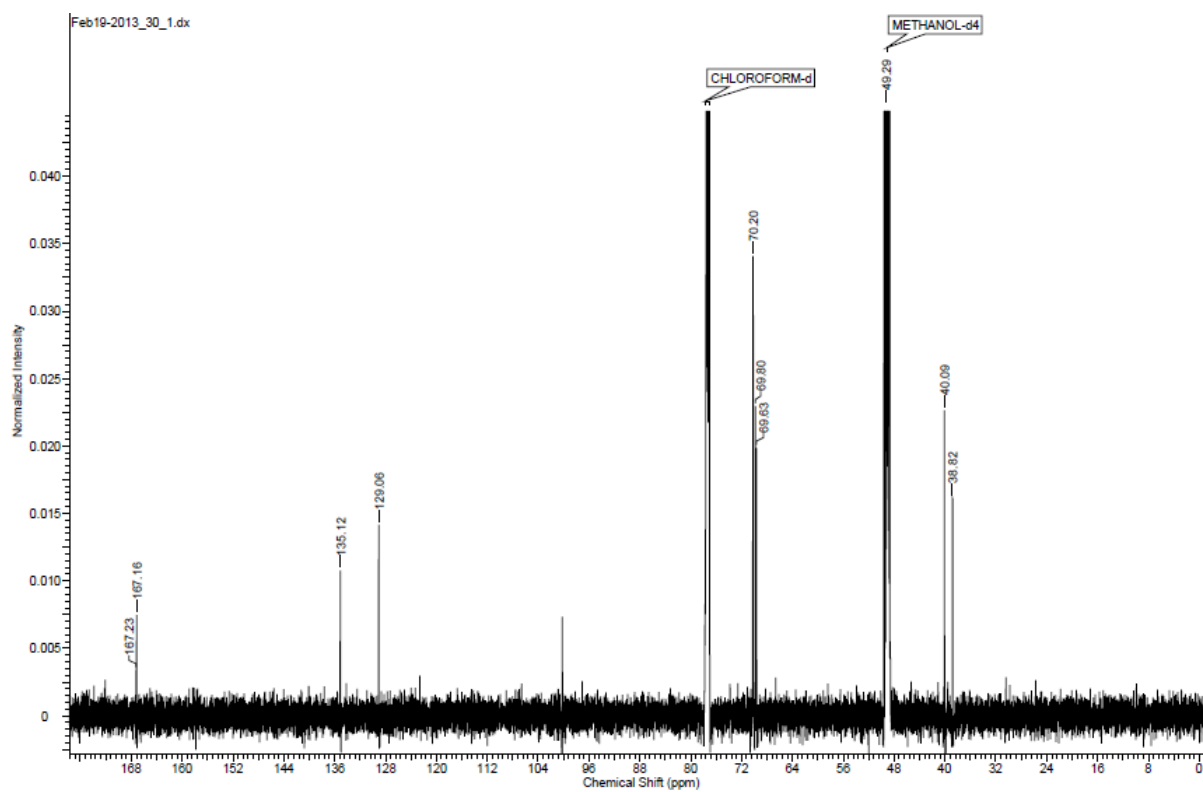


Figure S10. ^{13}C -NMR spectrum of 7.

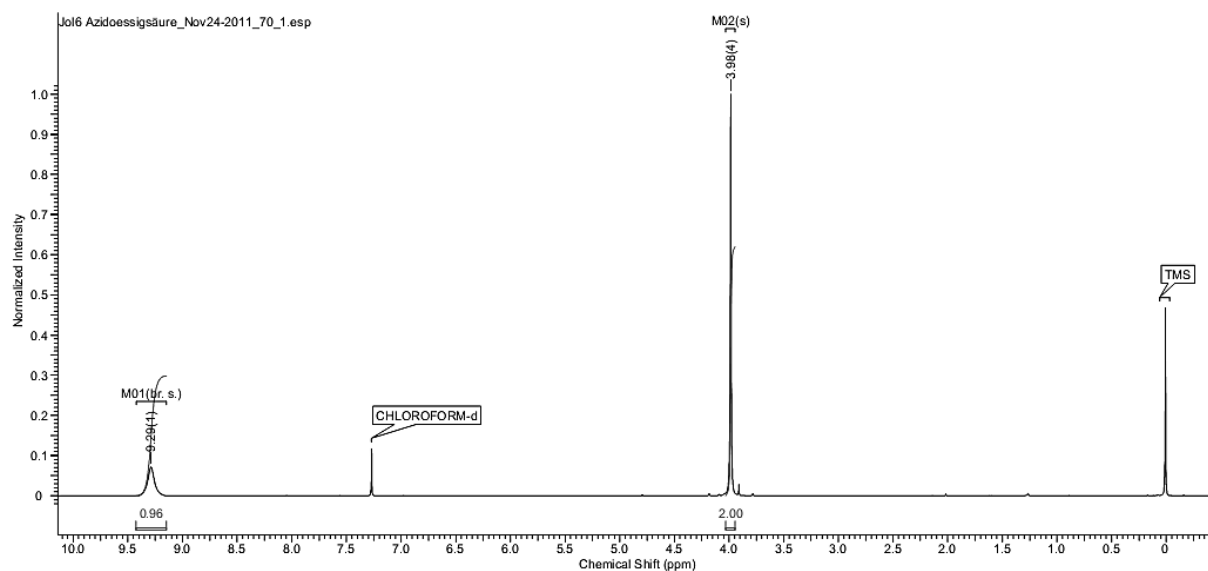


Figure S11. ^1H -NMR spectrum of 8.

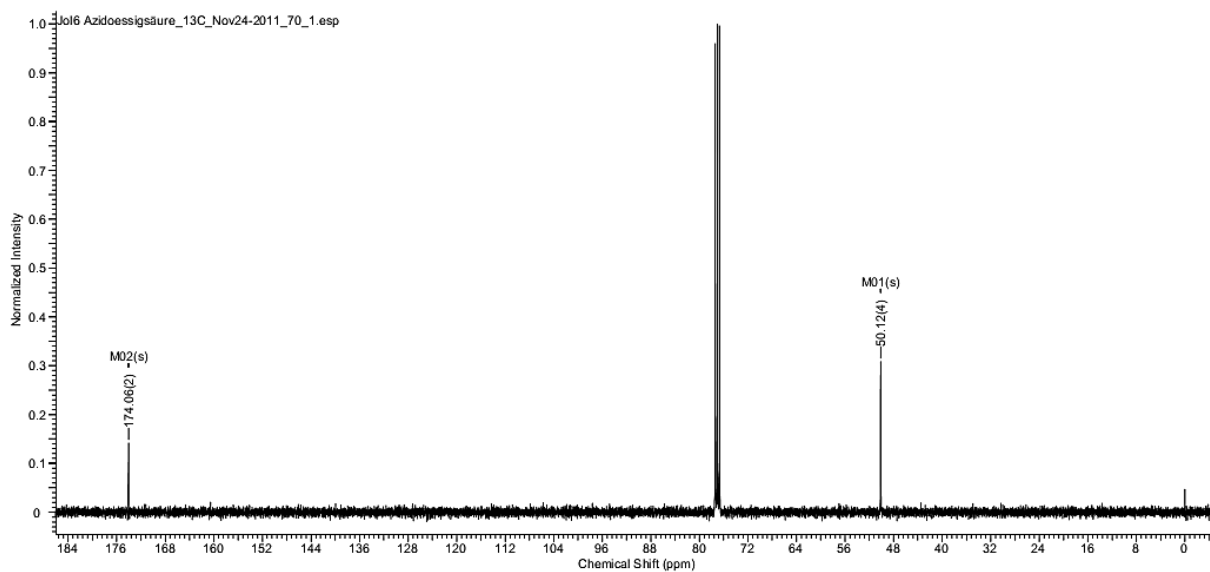


Figure S12. ^{13}C -NMR spectrum of 8.

LC-MS data of 9 through 17

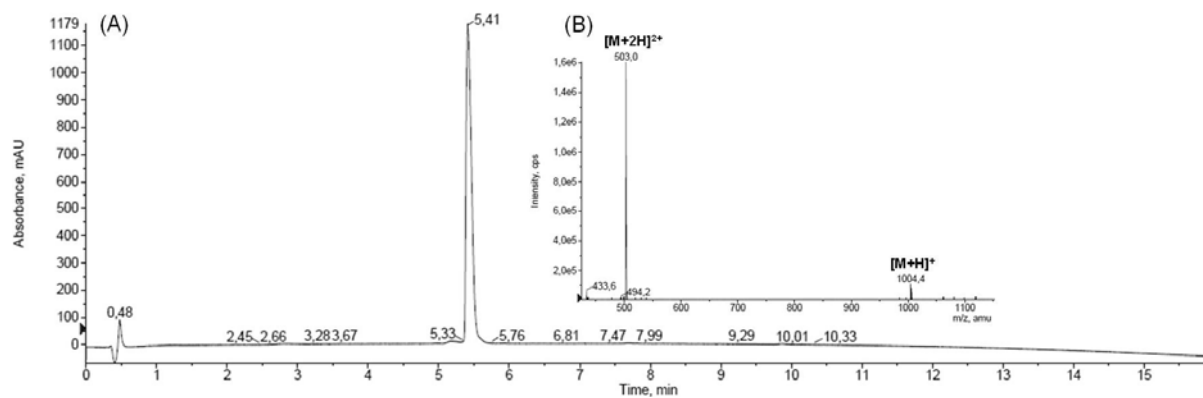


Figure S13. HPLC chromatogram (A) and ion masses from the ESI-mass spectrum (B) of 9 ($M = 1003.26$ g/mol).

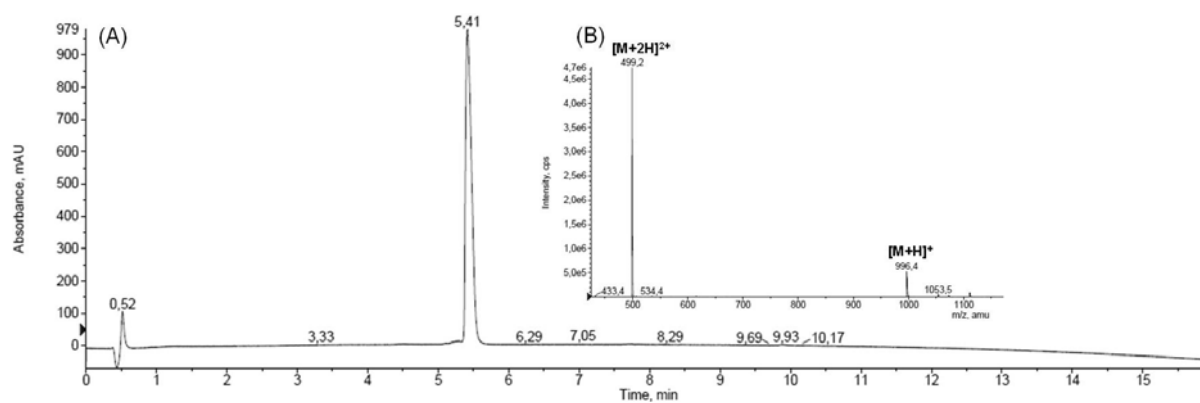


Figure S14. HPLC chromatogram (A) and ion masses from the ESI-mass spectrum (B) of **10** ($M = 996.23$ g/mol).

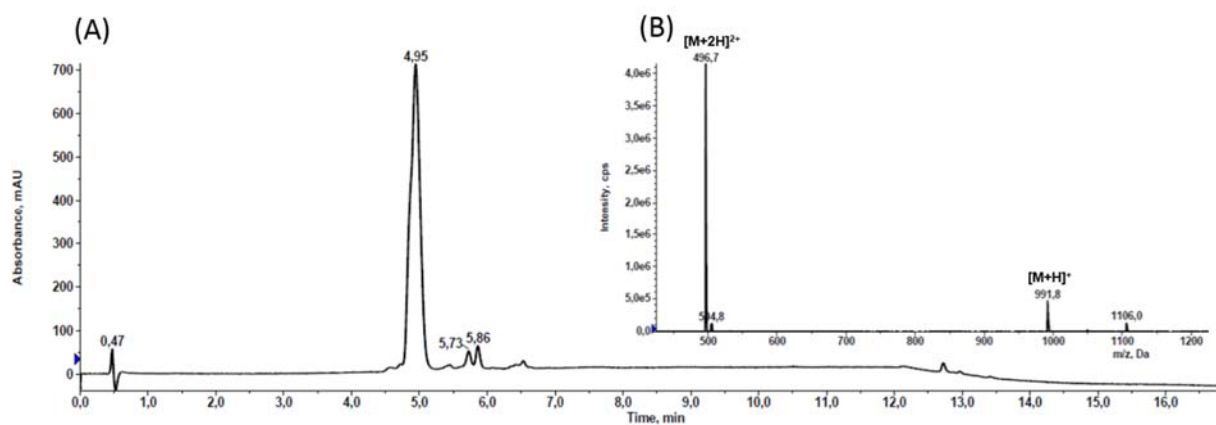


Figure S15. HPLC chromatogram (A) and ion masses from the ESI-mass spectrum (B) of **11** ($M = 991.21$ g/mol).

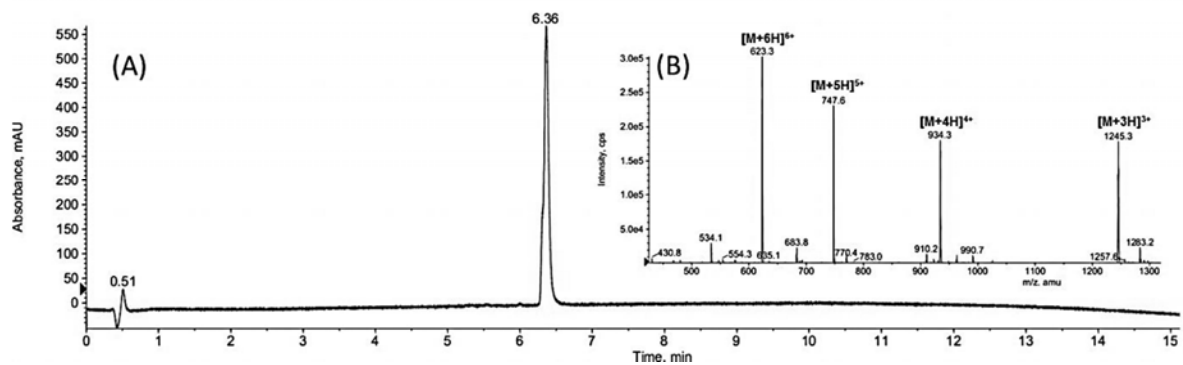


Figure S16. HPLC chromatogram (A) and ion masses from the ESI-mass spectrum (B) of **12** ($M_{\text{Calc}} = 3733.61$ g/mol).

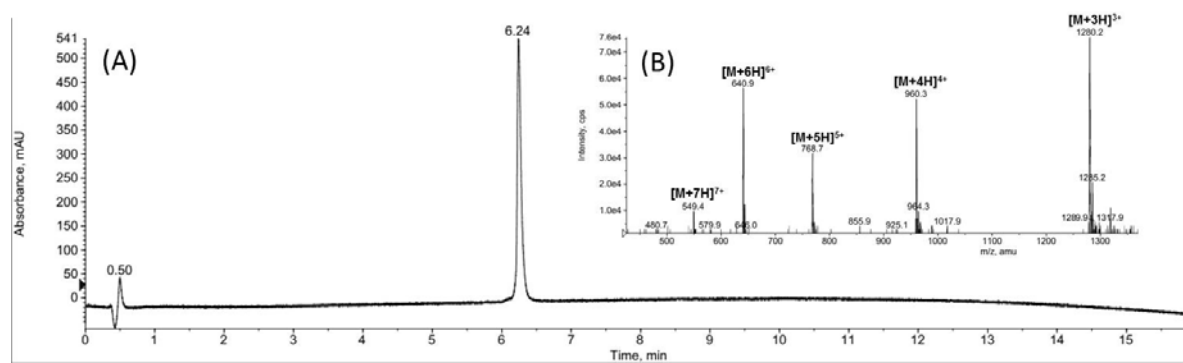


Figure S17. HPLC chromatogram (A) and ion masses from the ESI-mass spectrum (B) of **13** ($M_{\text{Calc}} = 3838.14$ g/mol).

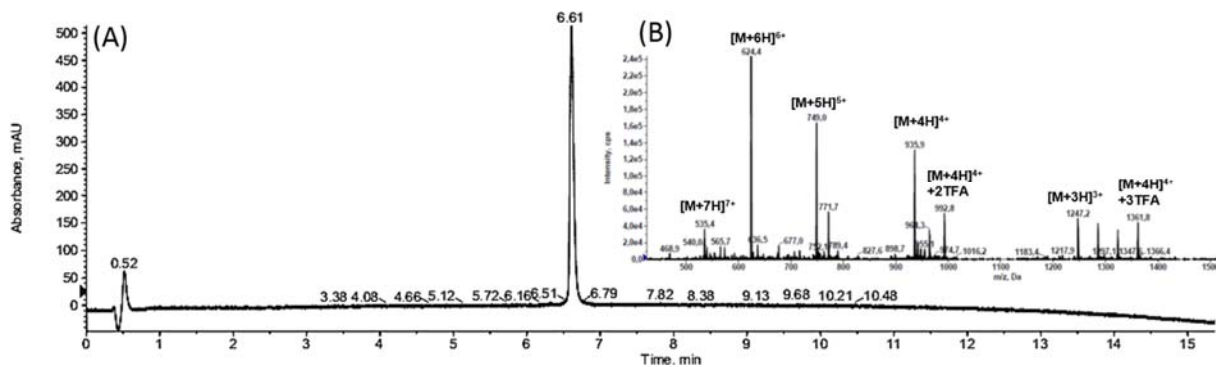


Figure S18. HPLC chromatogram (A) and ion masses from the ESI-mass spectrum (B) of **14** ($M = 3740.16$ g/mol).

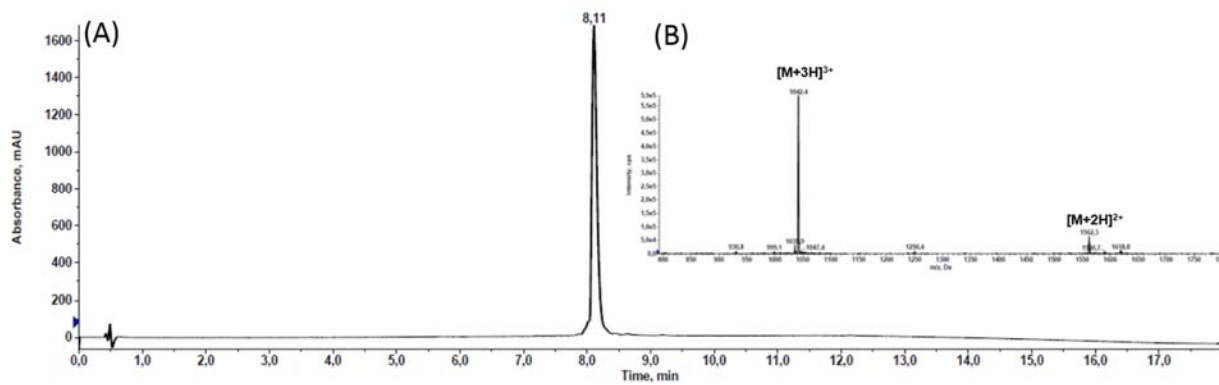


Figure S19. HPLC chromatogram (A) and ion masses from the ESI-mass spectrum (B) of **15** ($M = 3122.55$ g/mol).

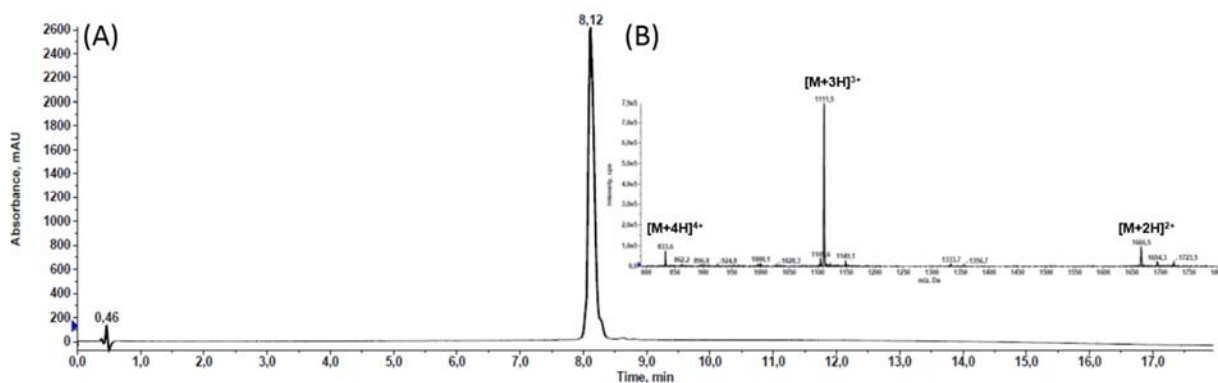


Figure S20. HPLC chromatogram (A) and ion masses from the ESI-mass spectrum (B) of **16** ($M = 3330.81$ g/mol).

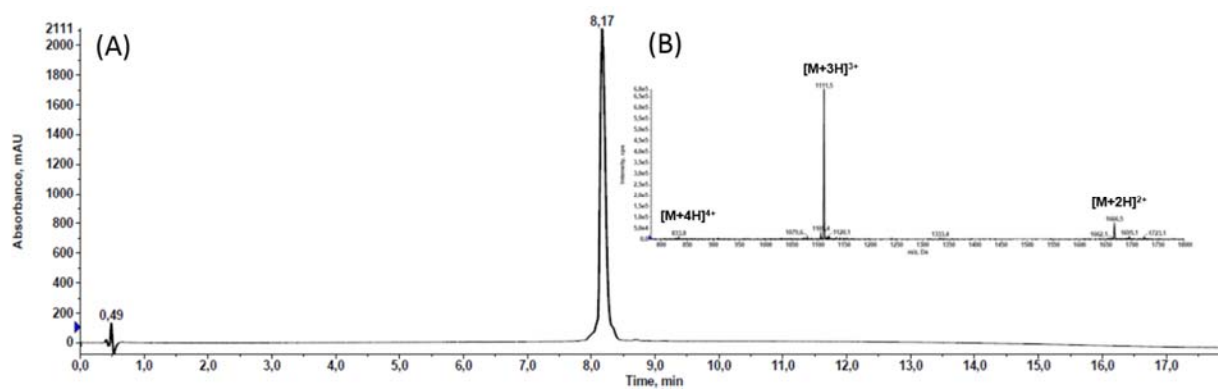


Figure S21. HPLC chromatogram (A) and ion masses from the ESI-mass spectrum (B) of **17** ($M = 3330.81$ g/mol).

HPLC chromatograms and high resolution mass spectra of 18 and 19

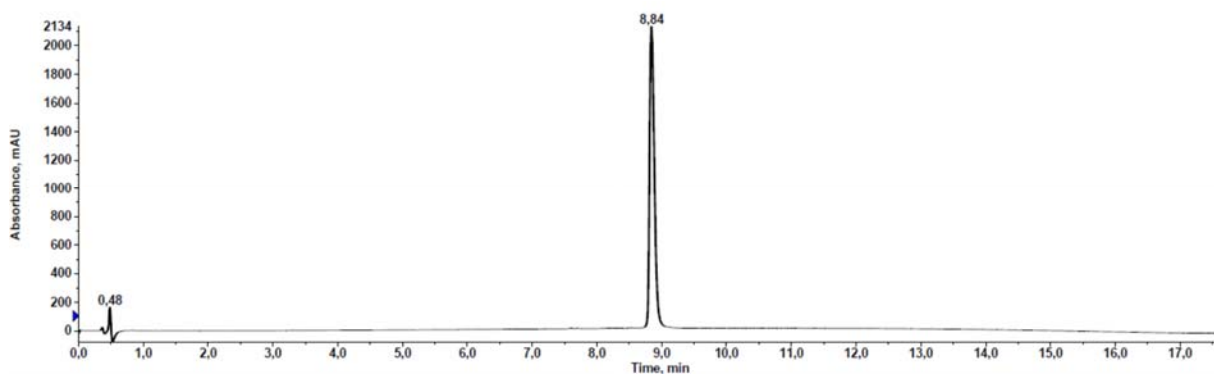


Figure S22. HPLC chromatogram of **18**.

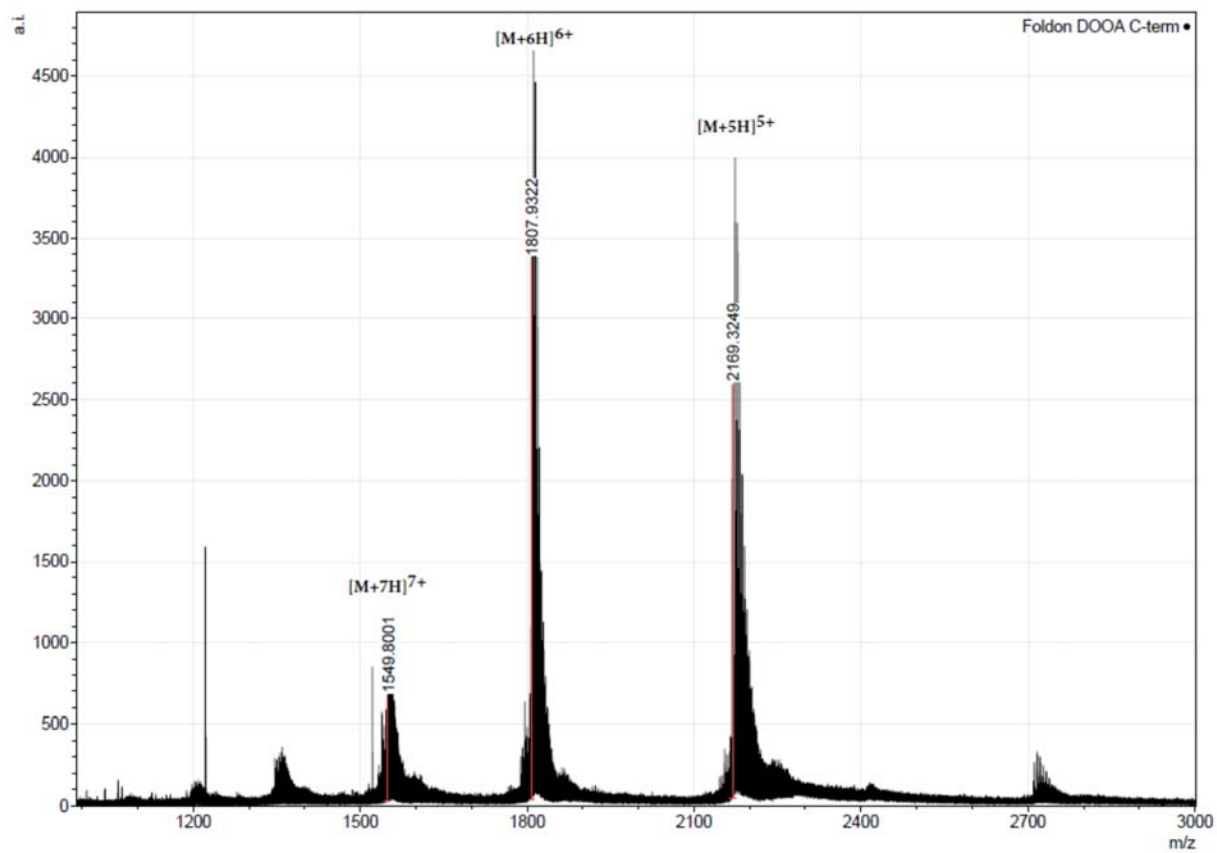


Figure S23. High resolution ESI-mass spectrum of **18** ($M = 10841.30$ g/mol).

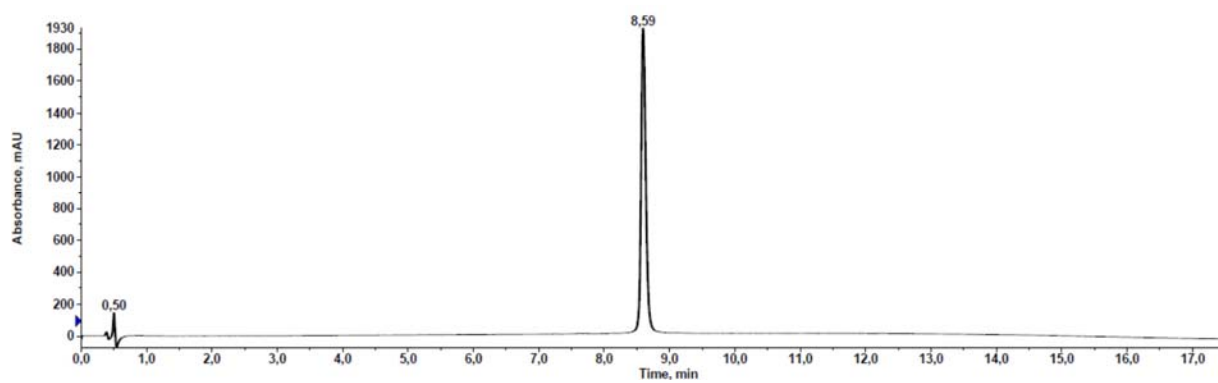


Figure S24. HPLC chromatogram of **19**.

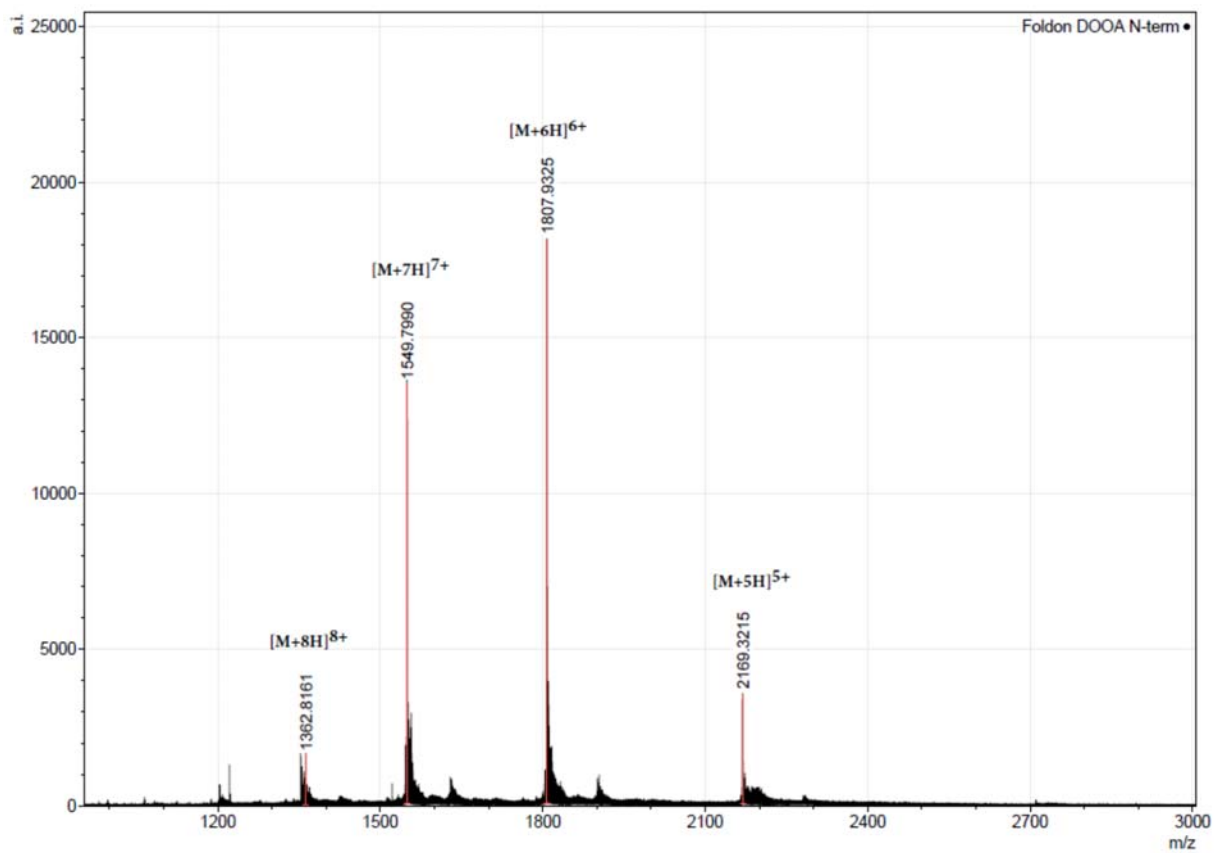


Figure S25. High resolution ESI-mass spectrum of **19** ($M = 10841.30$ g/mol).

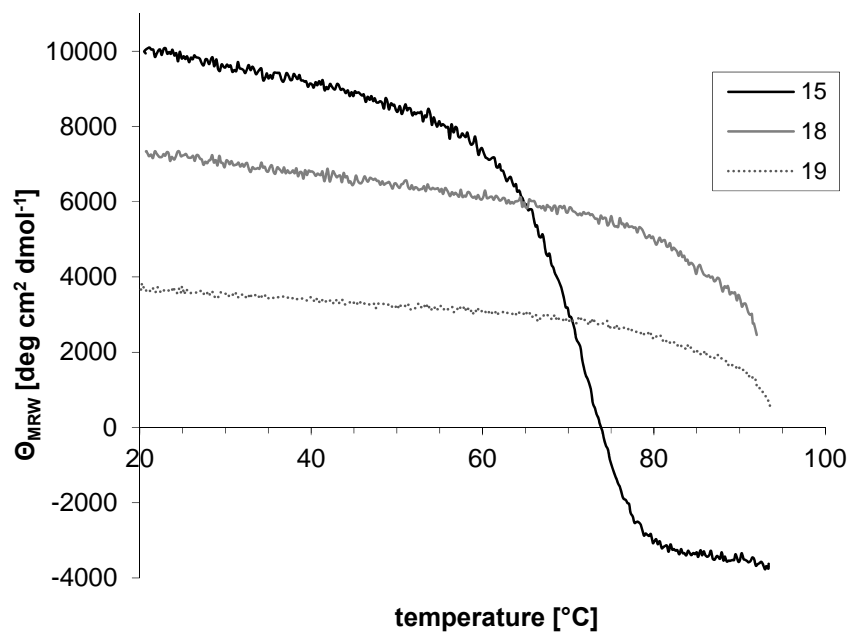


Figure S26. Thermal unfolding of the non-covalent foldon (**15**), as well as the covalently stabilized trimers **18** and **19**, in phosphate buffer without detergent.

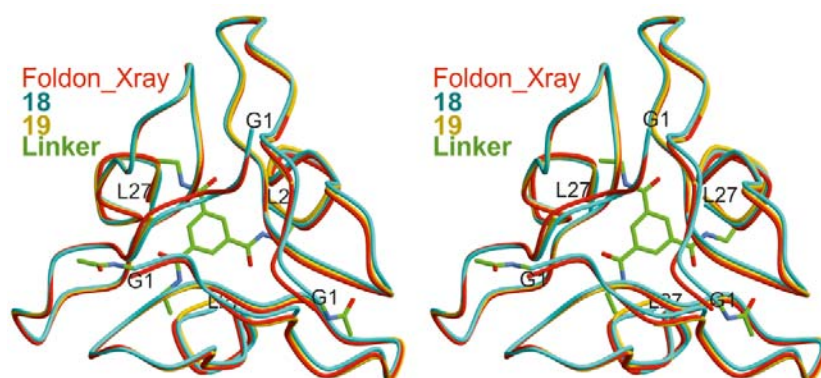


Figure S27. Backbone superposition of *wt* foldon (red, PDB ID 4NCU) with the covalent conjugates **18** (cyan) and **19** (gold).

Table S1. X-ray data collection and refinement statistics of foldon-scaffold conjugates **18** and **19**.

	18	19
<i>Crystal parameters</i>		
Space group	P2 ₁	C2
Cell constants	a=28.0 Å; b=47.9 Å, c=27.9 Å; β=92.8 °	a=48.7 Å; b=28.1 Å, c=56.0 Å; β=106.8 °
CPs / AU ^a	3	3
<i>Data collection</i>		
Beam line	X06SA, SLS	X06DA, SLS
Wavelength (Å)	1.0	1.0
Resolution range (Å) ^b	30-1.2 (1.3-1.2)	30-1.3 (1.4-1.3)
No. observations	62797	49197
No. unique reflections ^c	22182	17100
Completeness (%) ^b	95.8 (91.9)	94.4 (92.5)
R _{merge} (%) ^{b, d}	3.2 (49.5)	7.2 (38.9)
I/σ (I) ^b	15.7 (2.3)	9.3 (3.6)
<i>Refinement (REFMAC5)</i>		
Resolution range (Å)	15-1.2	15-1.3
No. refl. working set	21062	16244
No. refl. test set	1109	855
No. non hydrogen	663	593
Solvent (H ₂ O, Na ⁺ , Cl)	126	143
Ligand (non hydrogen)	3	21
R _{work} /R _{free} (%) ^e	13.3 / 16.3	16.8 / 19.3
r.m.s.d. bond (Å) / (°) ^f	0.007 / 1.233	0.014 / 1.771
Average B-factor (Å ²)	16.4	16.6
Ramachandran Plot (%) ^g	97.3 / 2.7 / 0	95.5 / 4.5 / 0.3
PDB accession code	4NCV	4NCW

^a Asymmetric unit.

^b The values in parentheses of resolution range, completeness, R_{merge} and I/σ (I) correspond to the last resolution shell.

^c Friedel pairs were treated as identical reflections.

^d $R_{\text{merge}}(I) = \frac{\sum_{\text{hkl}} \sum_j |I(\text{hkl})_j - \langle I(\text{hkl}) \rangle|}{\sum_{\text{hkl}} I(\text{hkl})}$, where $I(\text{hkl})_j$ is the j^{th} measurement of the intensity of reflection hkl and $\langle I(\text{hkl}) \rangle$ is the average intensity.

^e $R = \frac{\sum_{\text{hkl}} (|F_{\text{obs}}| - |F_{\text{calc}}|)}{\sum_{\text{hkl}} |F_{\text{obs}}|}$, where R_{free} is calculated for a randomly chosen 5% of reflections, which were not used for structure refinement, and R_{work} is calculated for the remaining reflections.

^f Deviations from ideal bond lengths/angles.

^g Number of residues in favored region/allowed region/outlier region.