

Supporting Information

Designing Photoaffinity Tool Compounds for the Investigation of the DENV NS2B-NS3 Protease Allosteric Binding Pocket

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Content

Inhalt

Scans of SDS-PAGE gels.....	1
IC ₅₀ curves and Dixon Plot	2
NMR Spectra.....	4
HPLC Chromatograms.....	10

Scans of SDS-PAGE gels

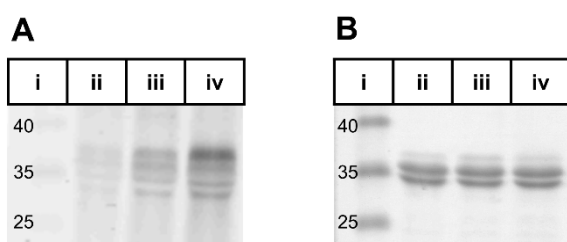


Figure S1. Scans of SDS-PAGE gels of PAL experiments with DENV NS2B-NS3 protease. **A** Typhoon scan of the gel. i) marker; ii) blind: DENV NS2B-NS3; iii) blind: DENV NS2B-NS3 + **4**; iv) irr: DENV NS2B-NS3 + **4**. **B** Scan of the Coomassie stained gel. i) marker; ii) blind: DENV NS2B-NS3; iii) blind: DENV NS2B-NS3 + **4**; iv) irr: DENV NS2B-NS3 + **4**.

IC₅₀ curves and Dixon Plot

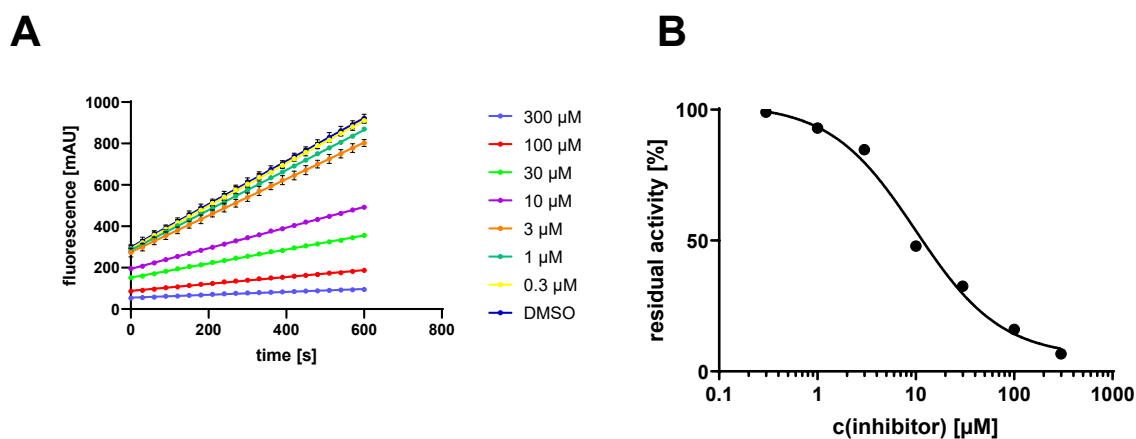


Figure S2. Fluorometric assay of compound **2**. **A** Fluorescence Increase over 600 s with different concentrations of compound **2**. **B** IC₅₀ curve of compound **2**. The IC₅₀ was determined to be $9.97 \pm 0.87 \mu\text{M}$.

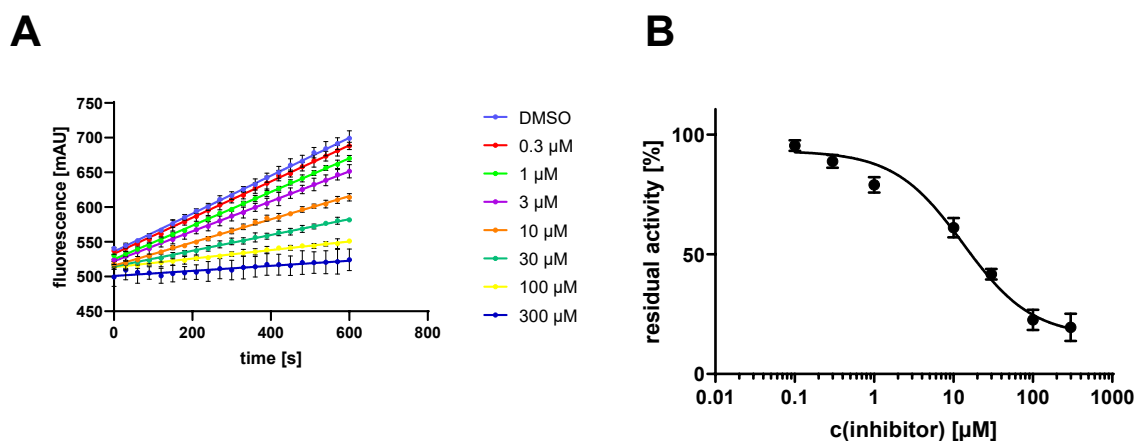


Figure S3. Fluorometric assay of compound **3a**. **A** Fluorescence Increase over 600 s with different concentrations of compound **3a**. **B** IC₅₀ curve of compound **3a**. The IC₅₀ was determined to be $13.4 \pm 2.1 \mu\text{M}$.

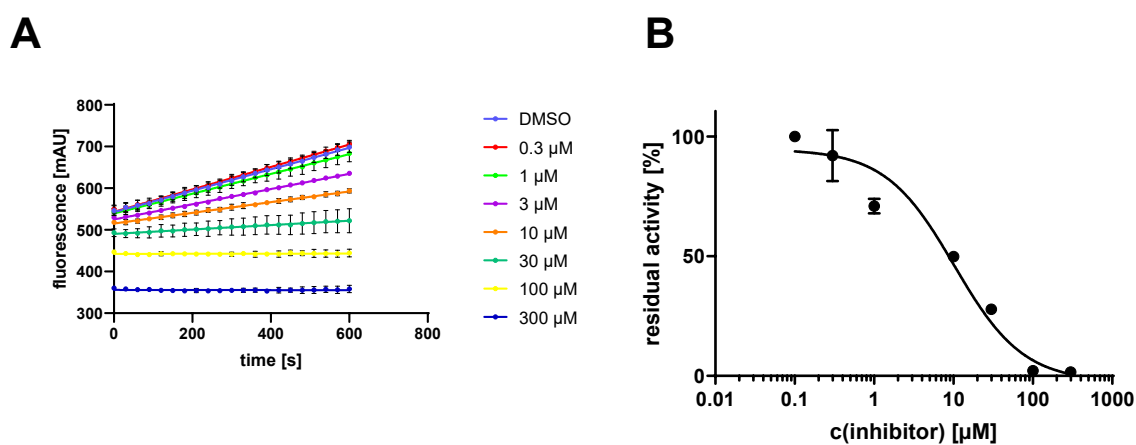


Figure S4. Fluorometric assay of compound **3b**. **A** Fluorescence Increase over 600 s with different concentrations of compound **3b**. **B** IC₅₀ curve of compound **3b**. The IC₅₀ was determined to be $10.2 \pm 1.5 \mu\text{M}$.

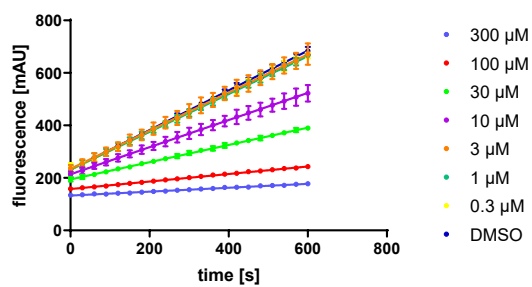
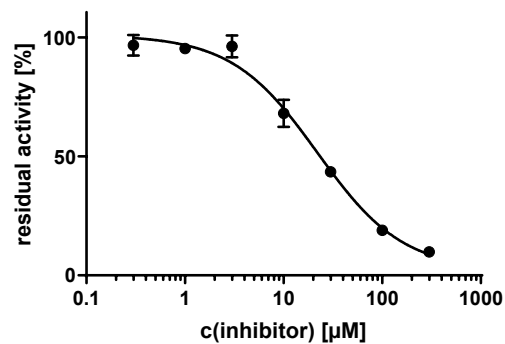
A**B**

Figure S5. Fluorometric assay of compound 3c. **A** Fluorescence Increase over 600 s with different concentrations of compound 3c. **B** IC₅₀ curve of compound 3c. The IC₅₀ was determined to be 20.0 \pm 2.0 μ M.

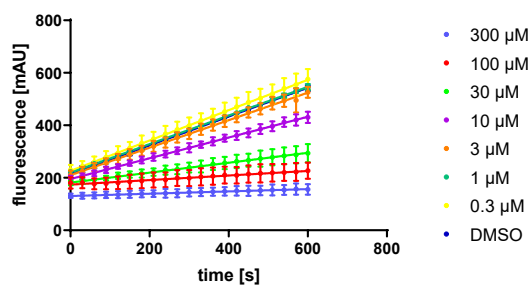
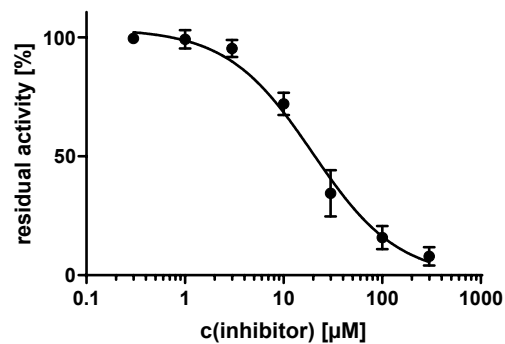
A**B**

Figure S6. Fluorometric assay of compound 4. **A** Fluorescence Increase over 600 s with different concentrations of compound 4. **B** IC₅₀ curve of compound 4. The IC₅₀ was determined to be 17.0 \pm 1.3 μ M.

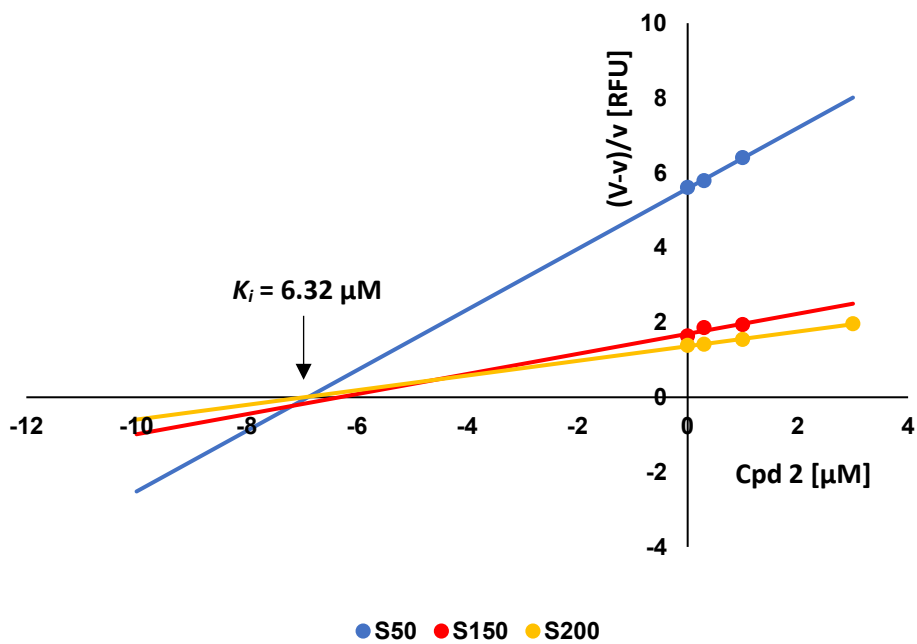


Figure S7. Dixon Plot of compound 2. The apparent K_i was determined from the intersection point of the straight lines to be $6.32 \pm 1.27 \mu\text{M}$.

NMR Spectra

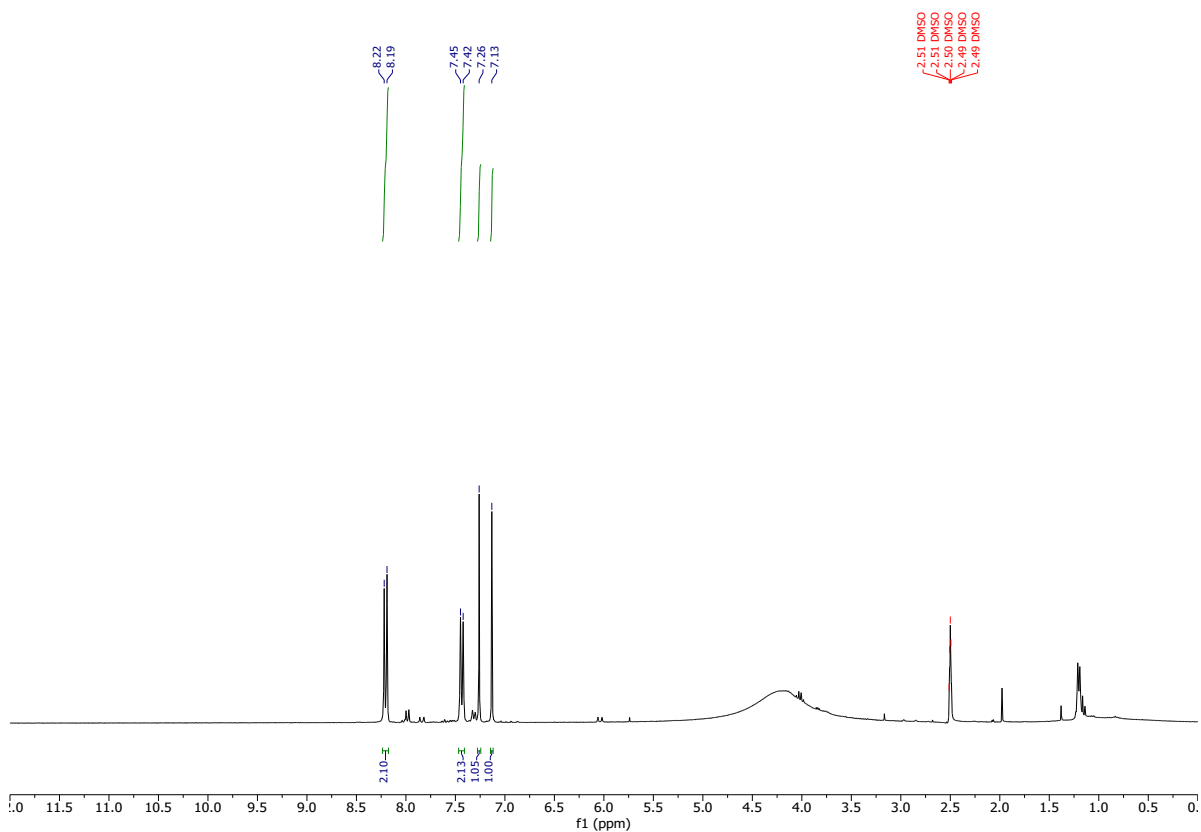


Figure S8. ^1H NMR spectrum of compound 2.

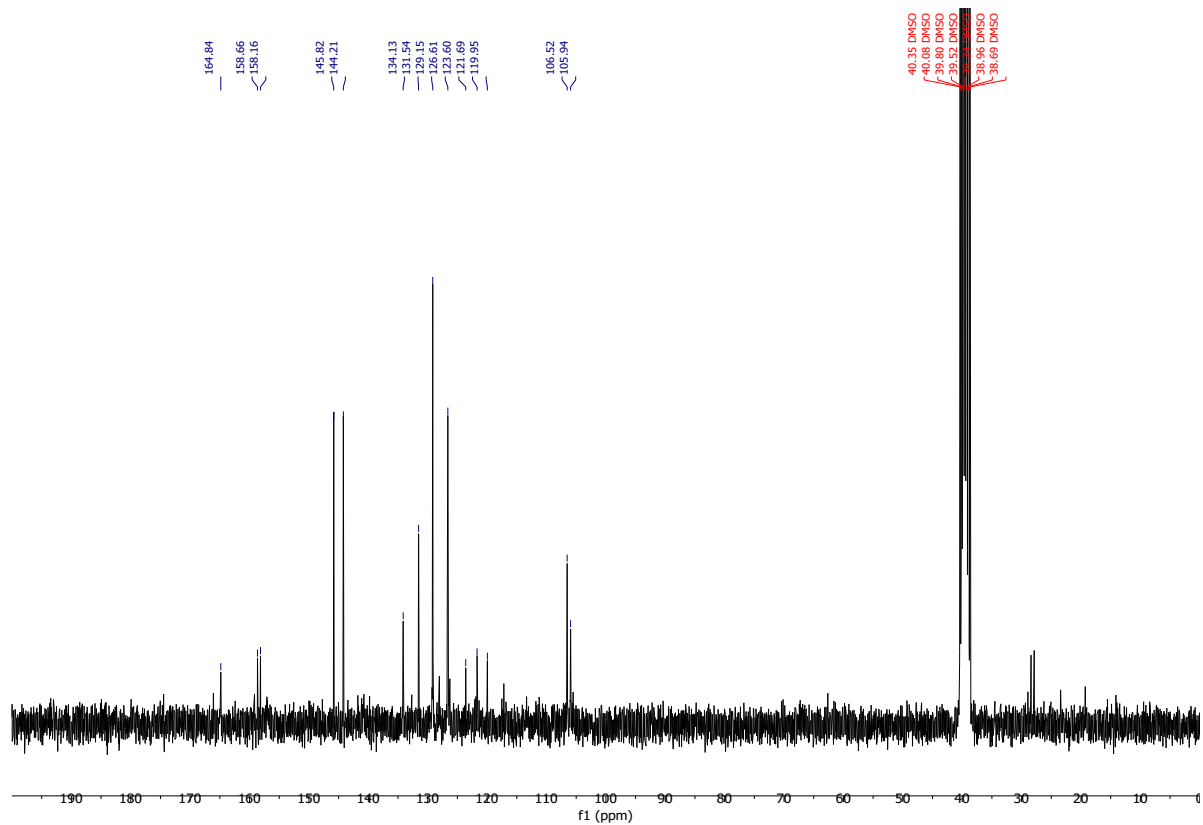


Figure S9. ¹³C NMR spectrum of compound 2.

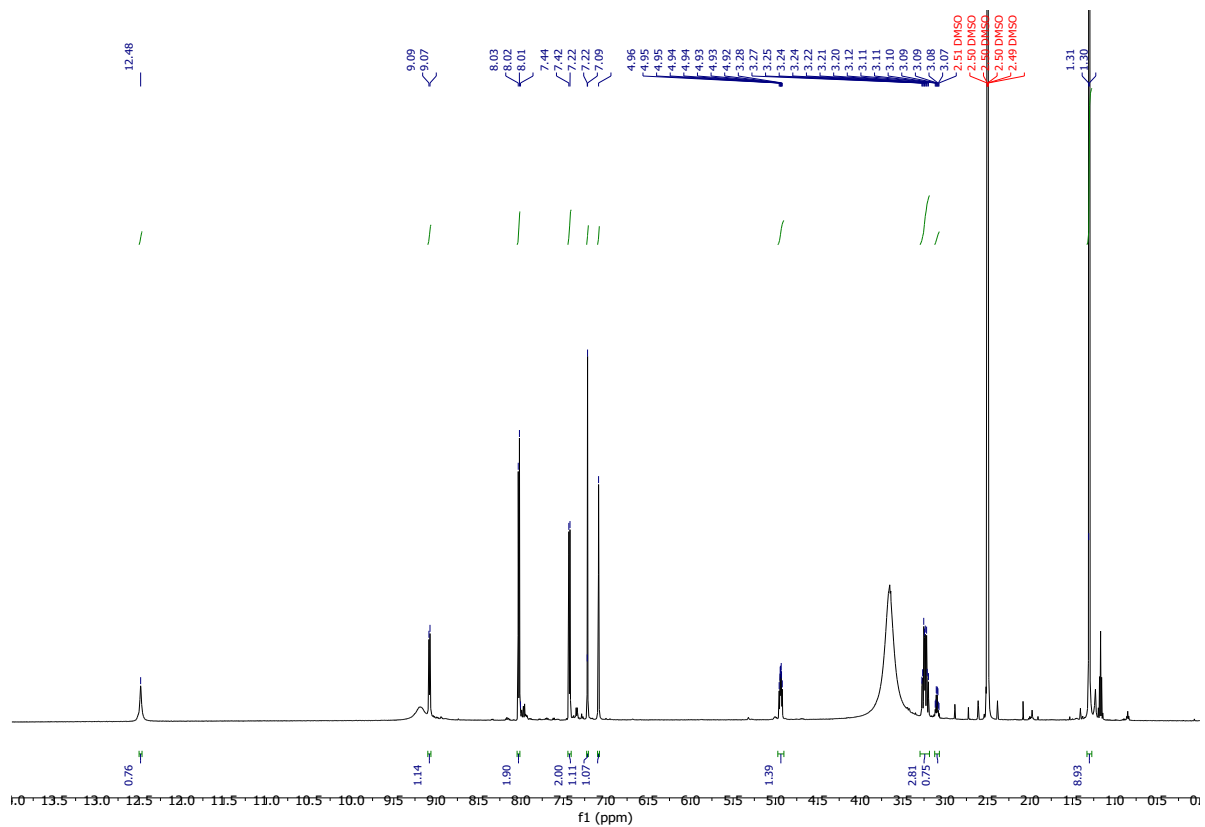


Figure S12. ¹H NMR spectrum of compound **3b**.

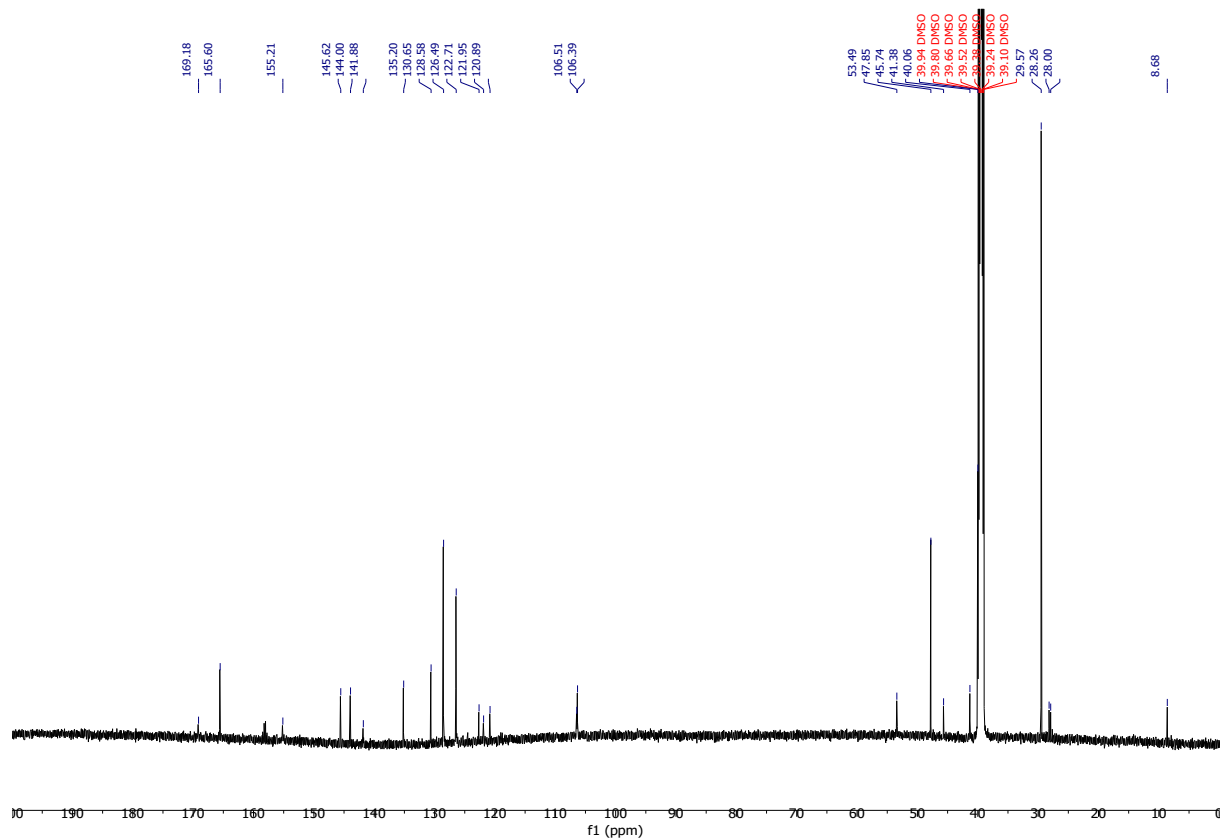


Figure S13. ¹³C NMR spectrum of compound **3b**.

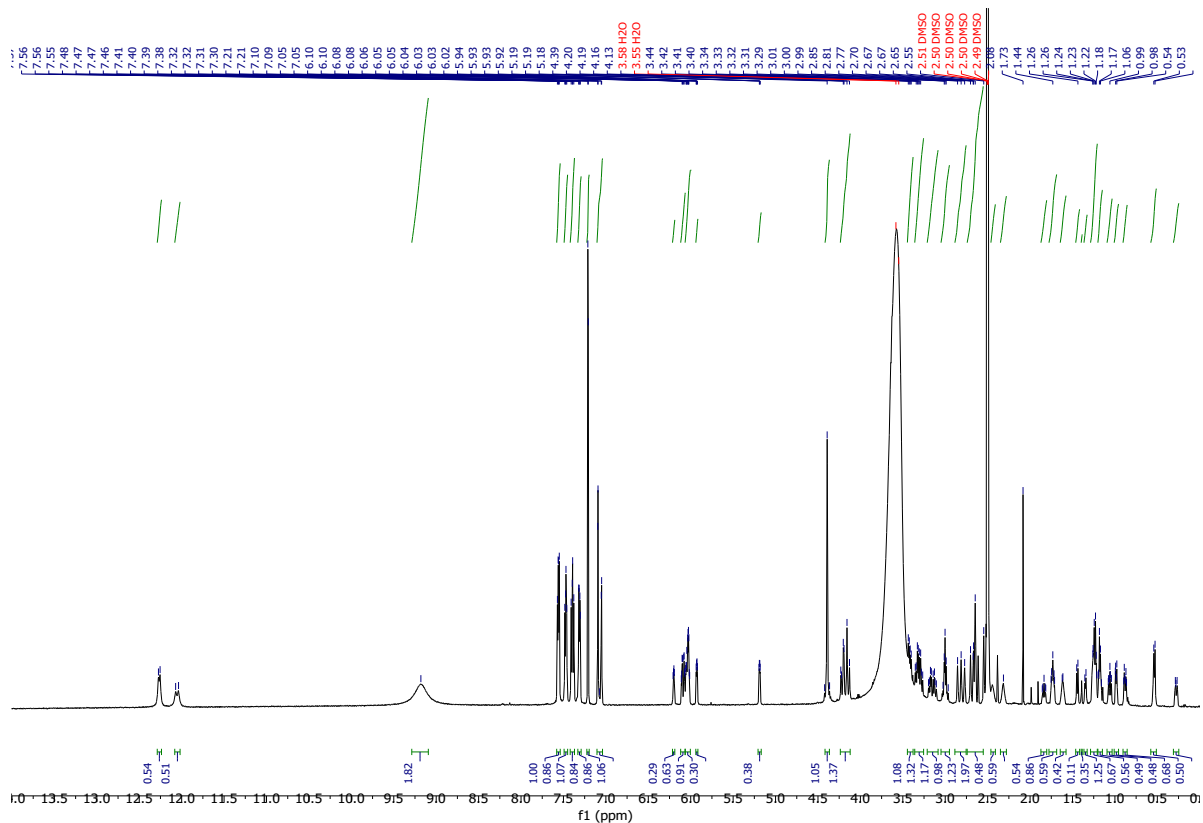


Figure S16. ¹H NMR spectrum of compound 4.

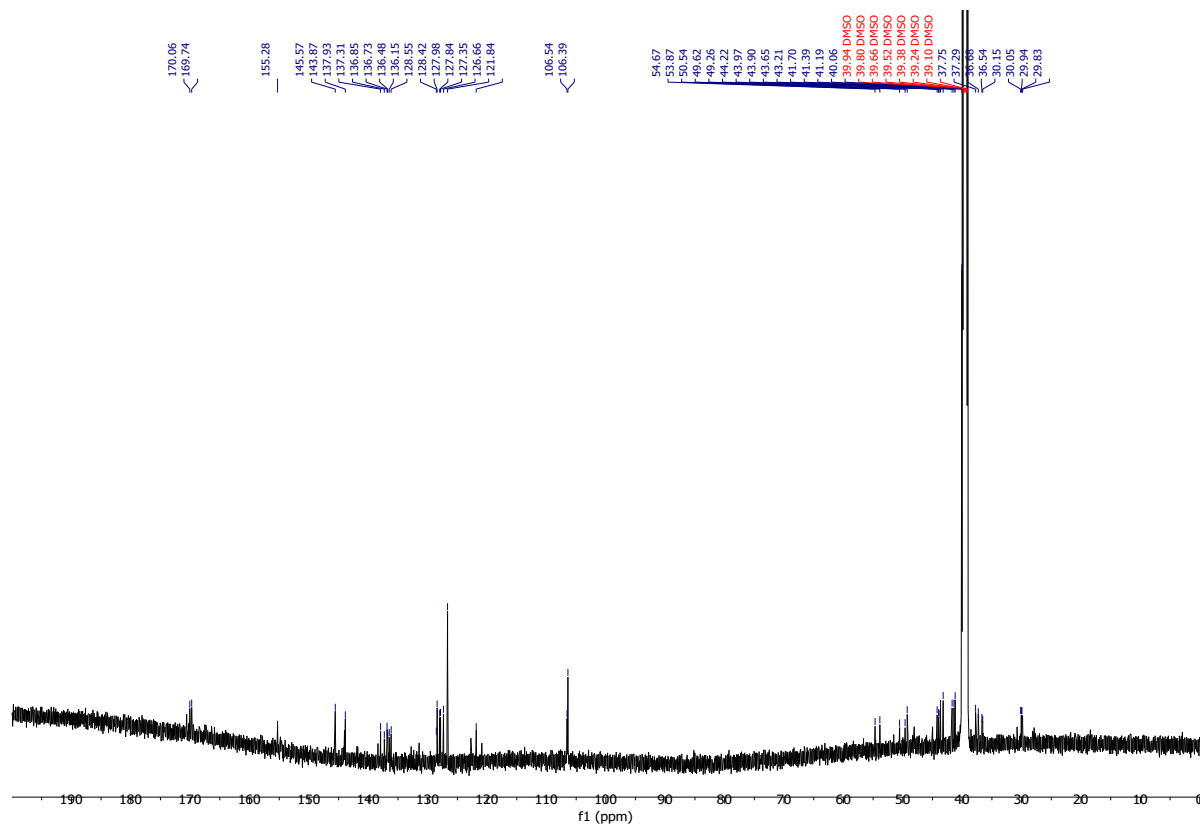


Figure S17. ¹³C NMR spectrum of compound 4.

HPLC Chromatograms

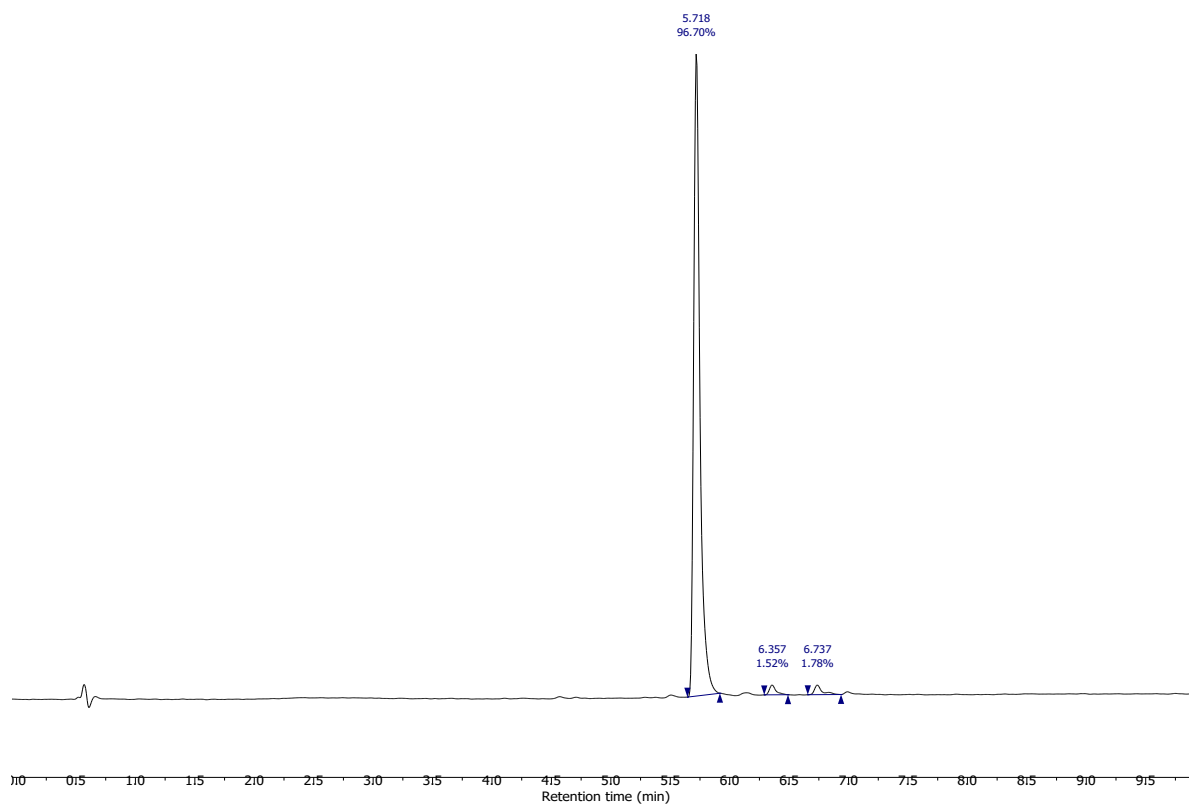


Figure S18. HPLC chromatogram of compound **2** at a wavelength of 254 nm.

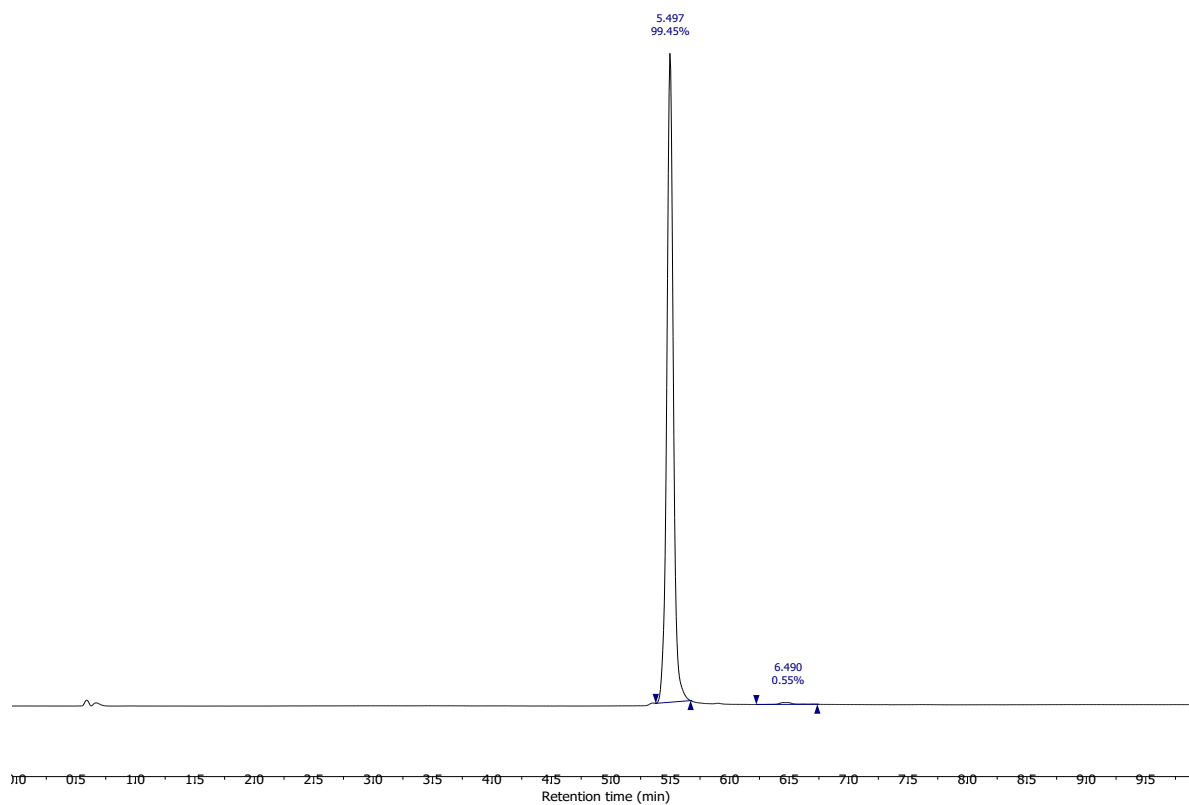


Figure S19. HPLC chromatogram of compound **3a** at a wavelength of 254 nm.

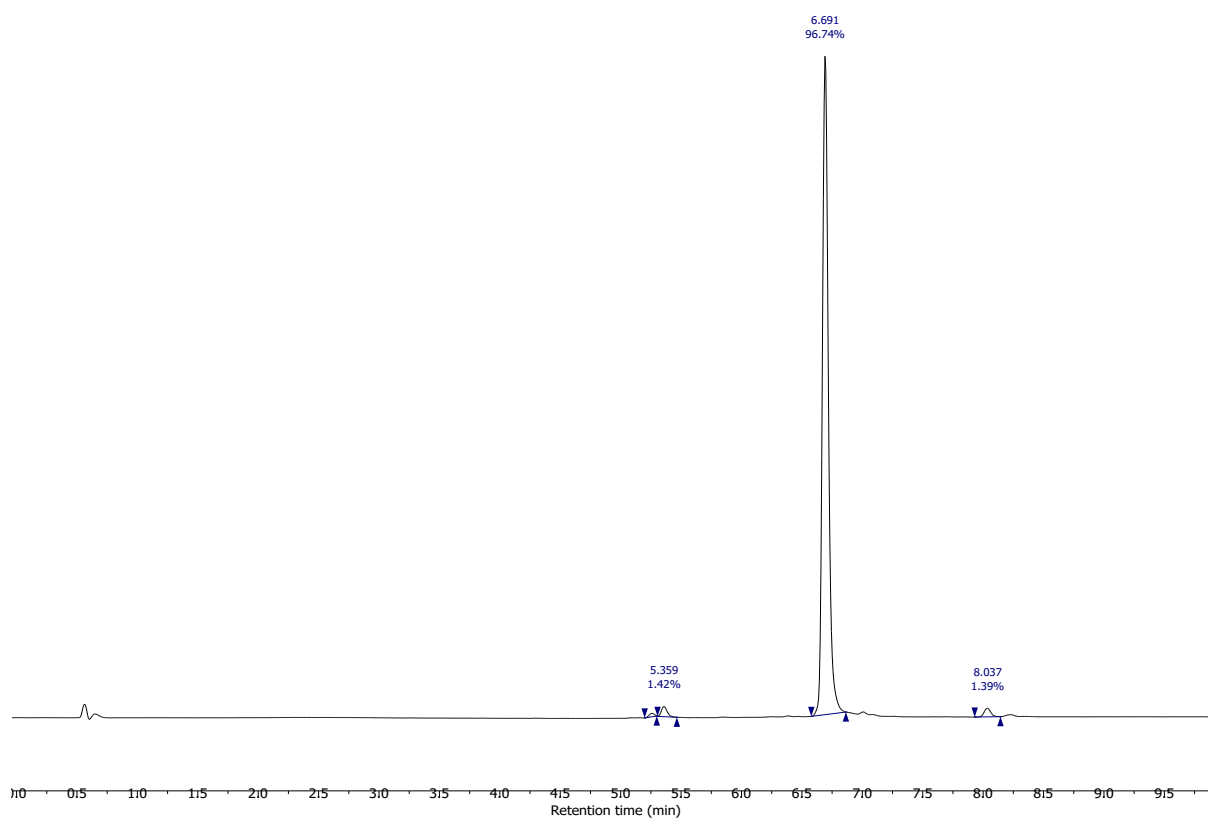


Figure S20. HPLC chromatogram of compound **3b** at a wavelength of 254 nm.

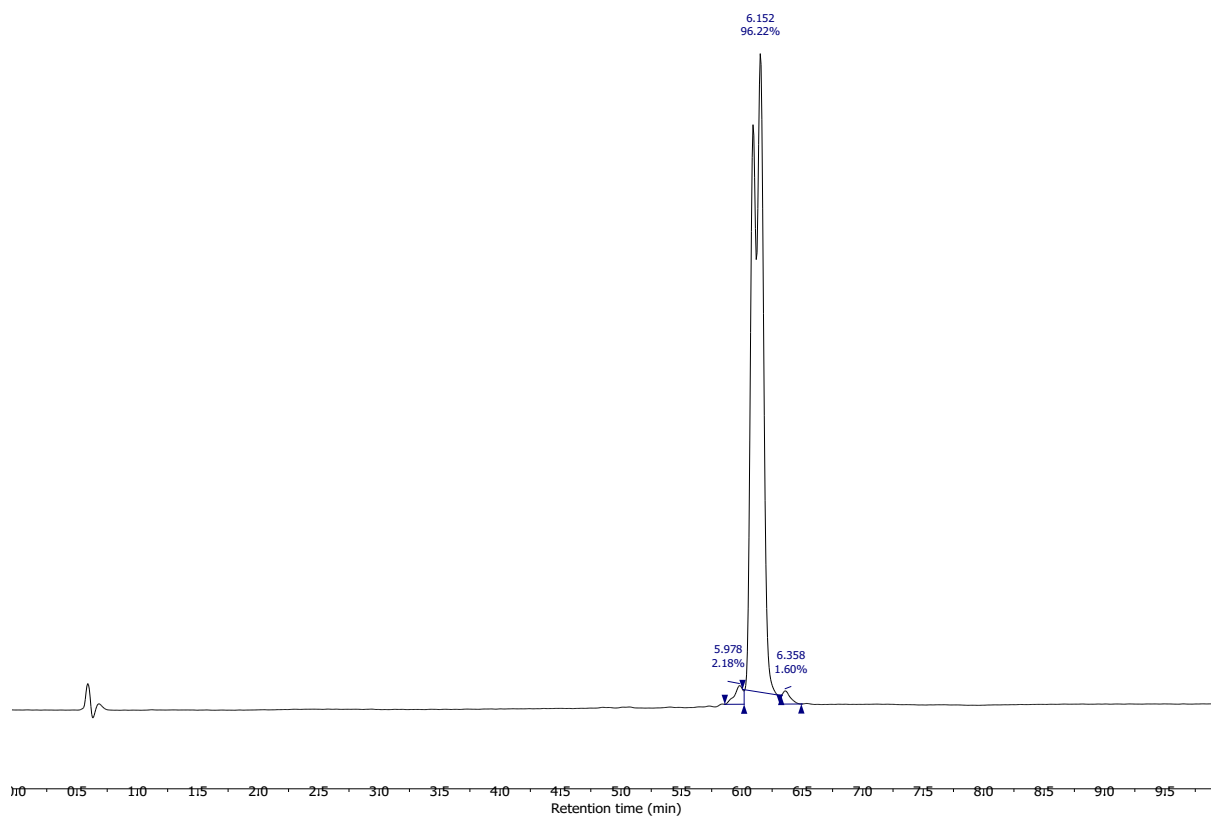


Figure S21. HPLC chromatogram of compound **3c** at a wavelength of 254 nm. Double peak due to the formation of diastereomers since SSMot-protecting group was used as a racemate.

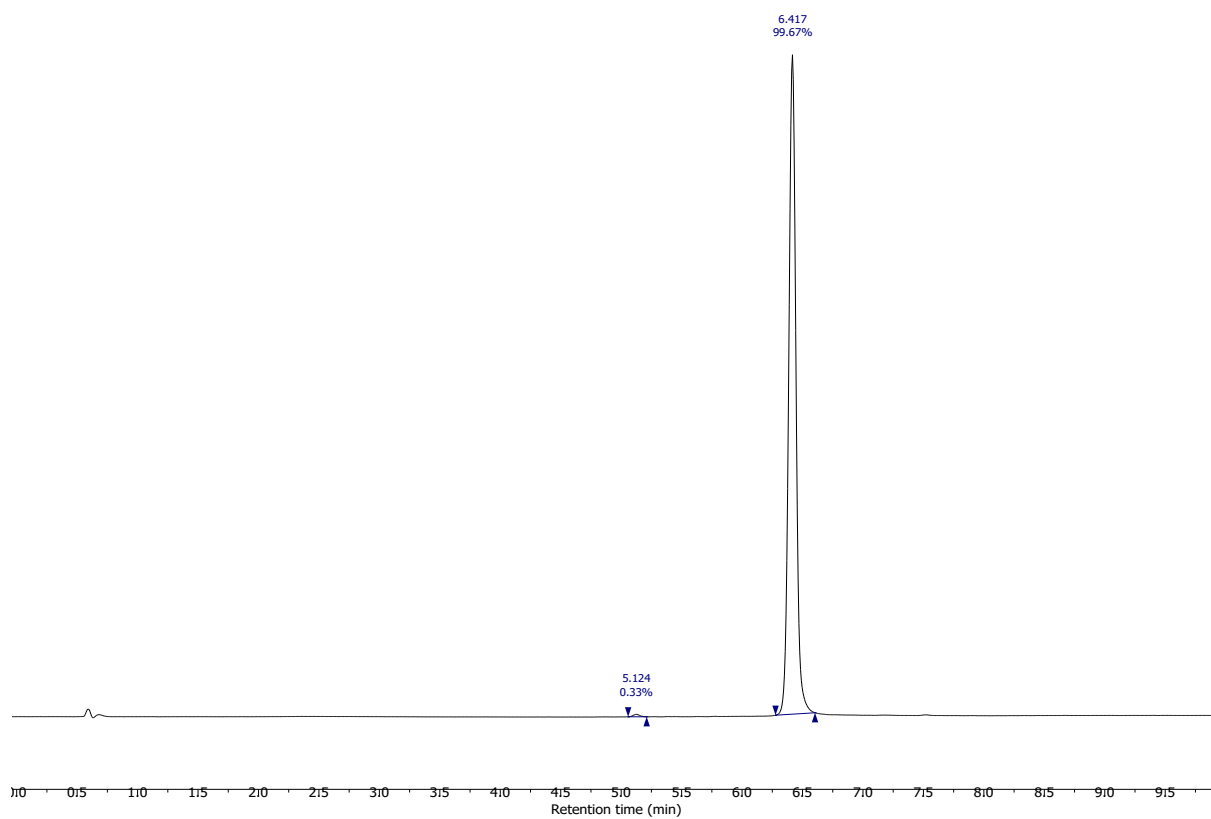


Figure S22. HPLC chromatogram of compound **4** at a wavelength of 254 nm.