SUPPORTING INFORMATION FOR

Molecular dynamics simulations of viral neuraminidase inhibitors with the human neuraminidase enzymes: Insights into isoenzyme selectivity

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Synthesis of Oseltamivir-COOH (4)

Deprotection of oseltamivir phosphate to form **4** followed the previously described protocol.[1] Briefly, oseltamivir phosphate 30 mg was dissolved in 1 mL methanol and 500 µL 1 M NaOH was added. The mixture was stirred at room temperature for 30 min. After completion, Amberlite IR-120 (H^+) was added to adjust the pH to 2. Solvents were then removed under vacuum to provide **4** as a white solid. 27 mg (quant.) The spectral data corresponds with literature.[1]

^{1}H NMR (500 MHz, CD_3OD) δ 6.55 (s, 1H), 4.12 (d, J = 8.3 Hz, 1H), 3.90 (dd, J = 11.1, 8.3 Hz, 1H), 3.39 (dd, J = 11.1, 5.6 Hz, 1H), 2.84 (dd, J = 17.4, 5.4 Hz, 1H), 2.42 – 2.33 (m, 1H), 2.01 (s, 3H), 1.57 – 1.43 (m, 4H), 0.99 – 0.77 (m, 6H, 2 × CH_3). ^{13}C NMR (126 MHz, CD_3OD) δ 174.59, 173.95, 134.92, 133.19, 83.32, 76.59, 55.28, 51.33, 31.63, 27.27, 26.63, 23.20, 9.84, 9.61.

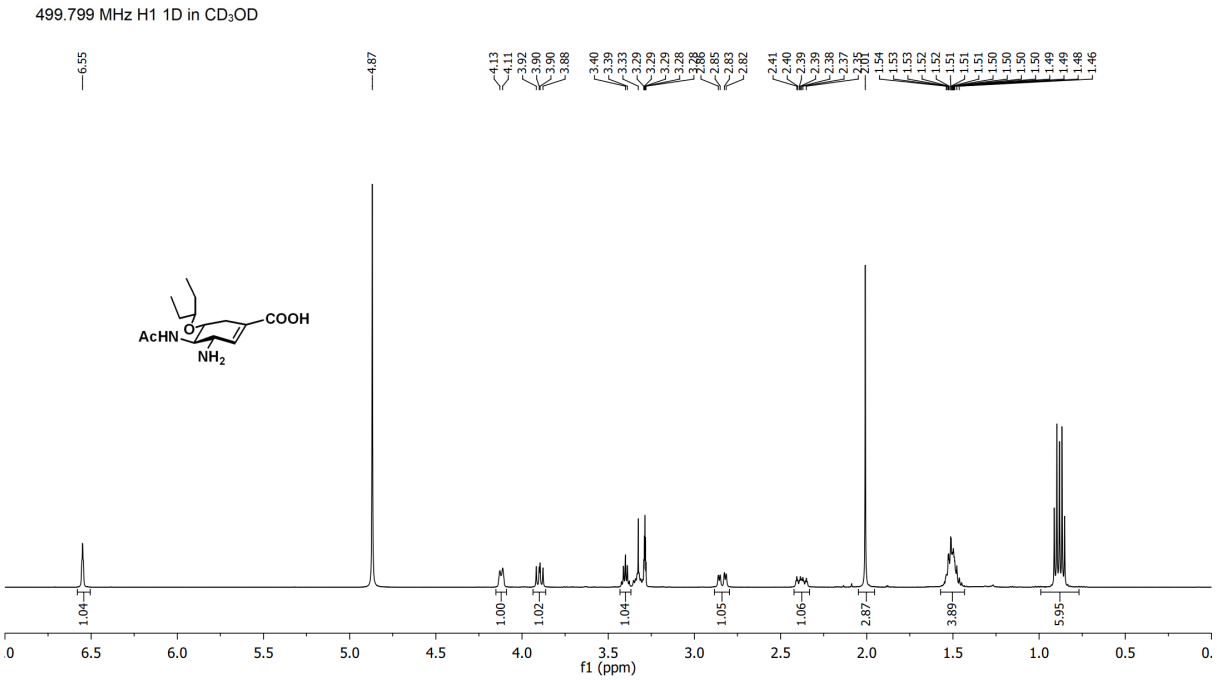

Figure S.1. ^{1}H NMR for Oseltamivir-COOH (**4**)

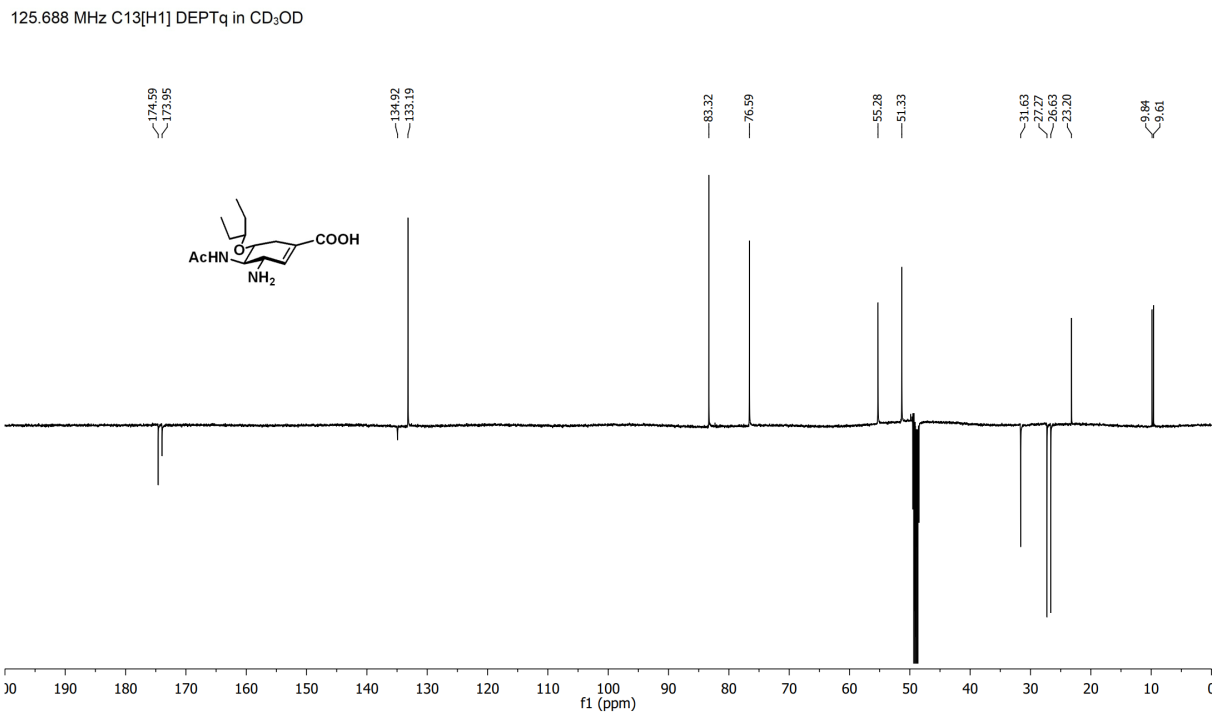

Figure S.2. ^{13}C NMR for Oseltamivir-COOH (**4**)

IC_{50} curves

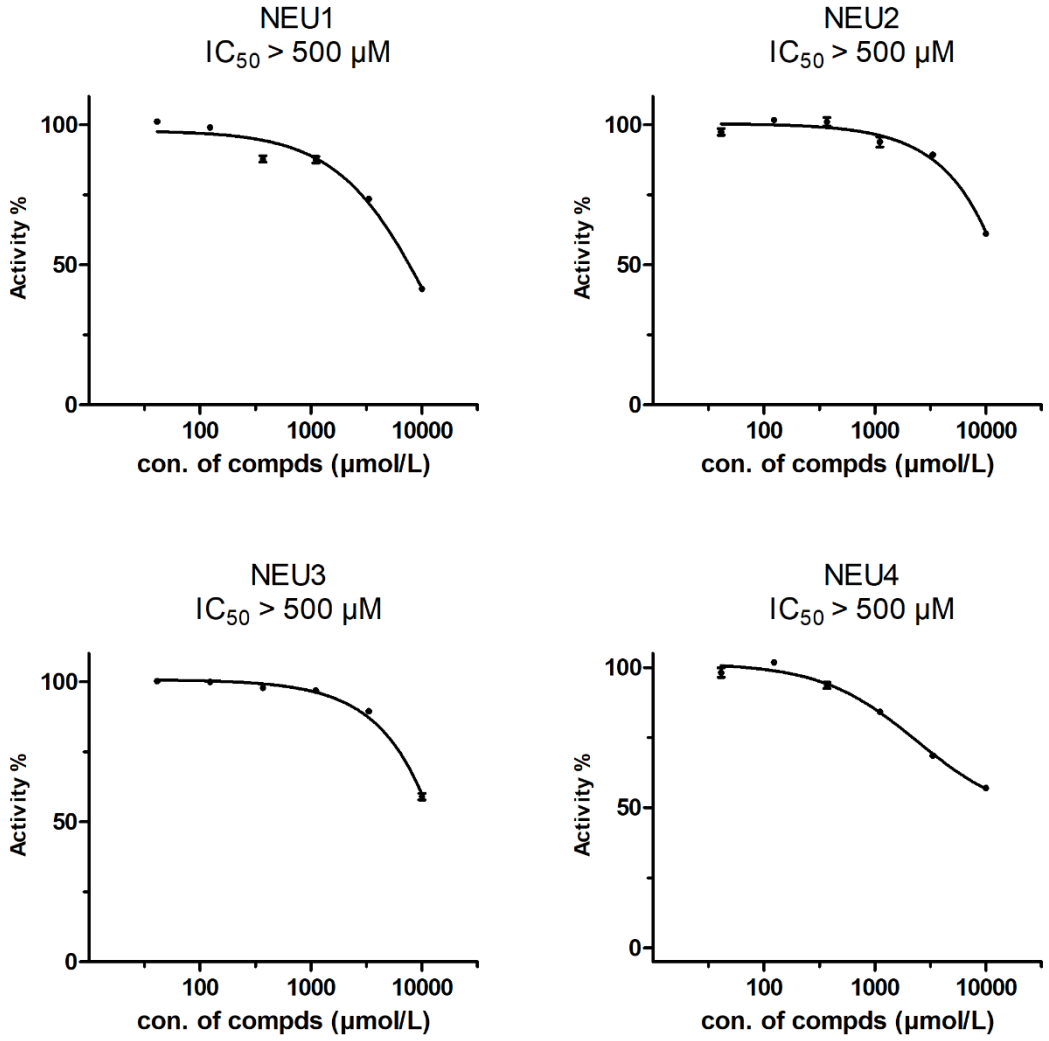

Figure S.3. IC_{50} curves for Neu5Ac (**1**)

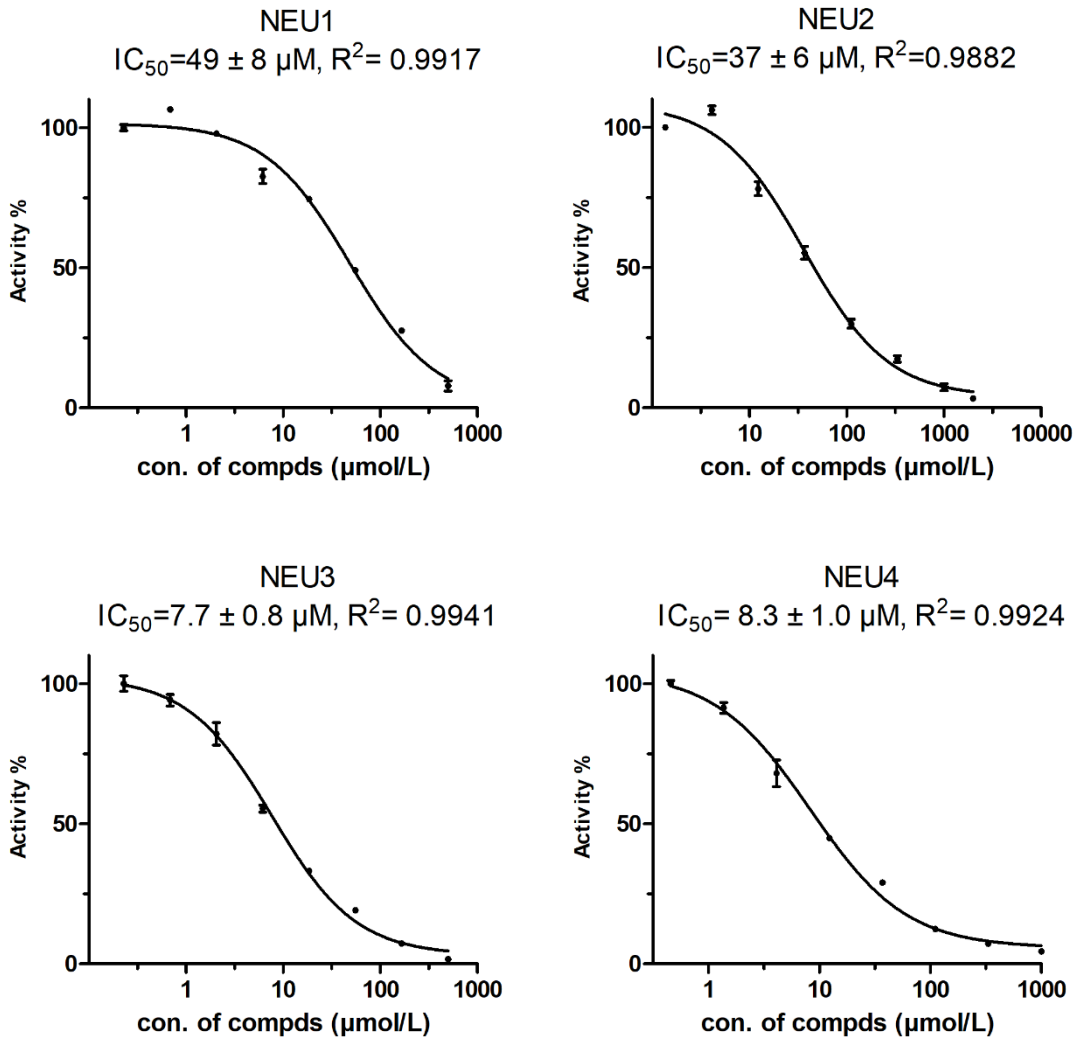

Figure S.4. IC_{50} curves for DANA (**2**)

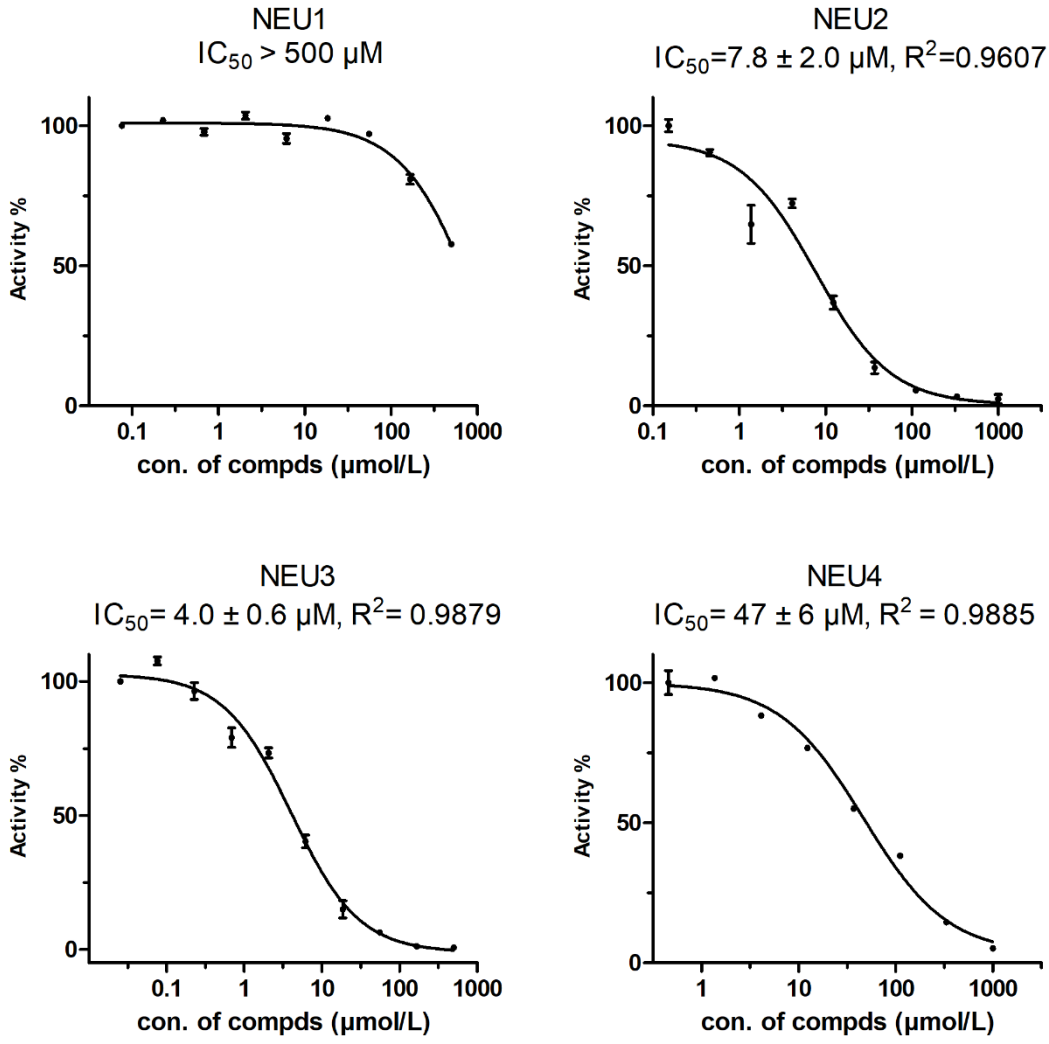

Figure S.5. IC_{50} curves for Zanamivir (**3**)

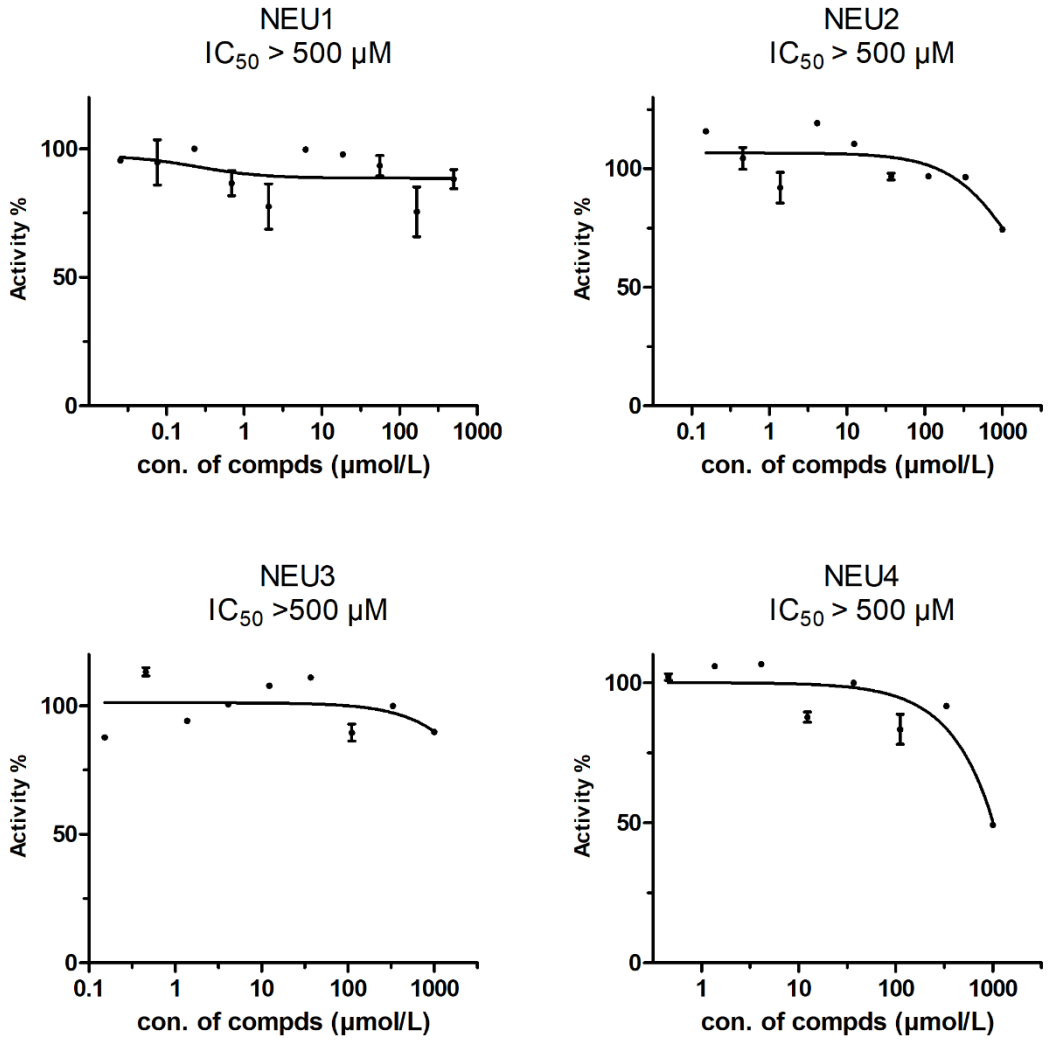

Figure S.6. IC_{50} curves for Oseltamivir (**4**)

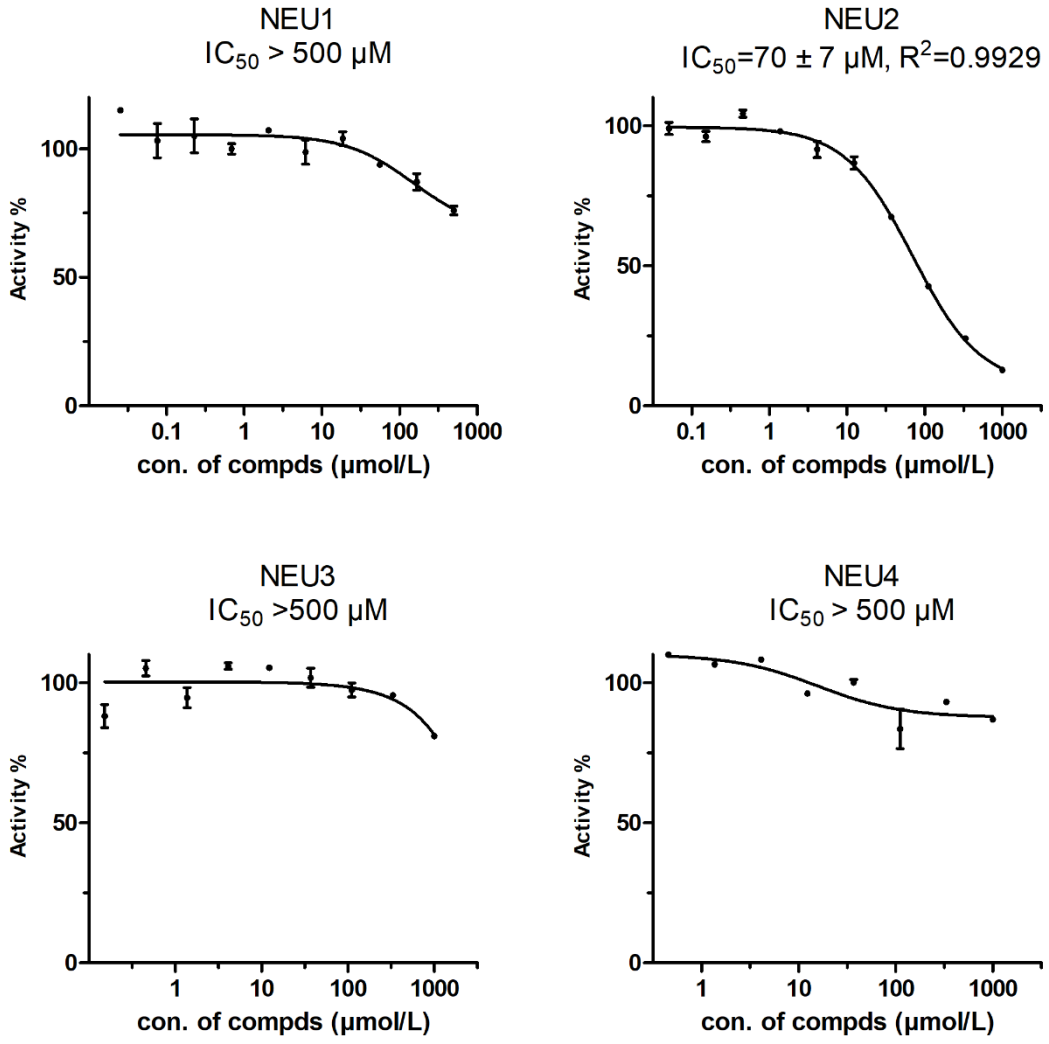

Figure S.7. IC_{50} curves for Peramivir (**5**)

K_i curves

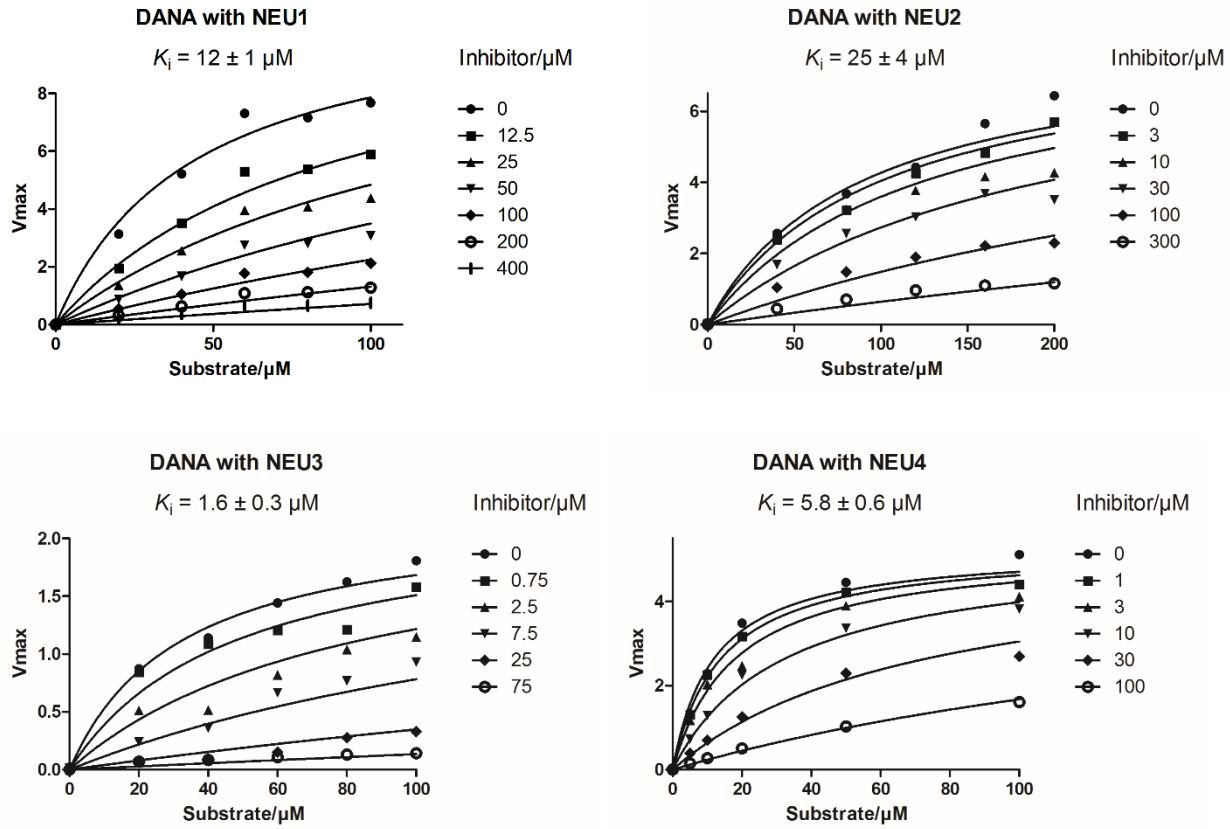

Figure S.8. K_i determination for DANA (**2**)

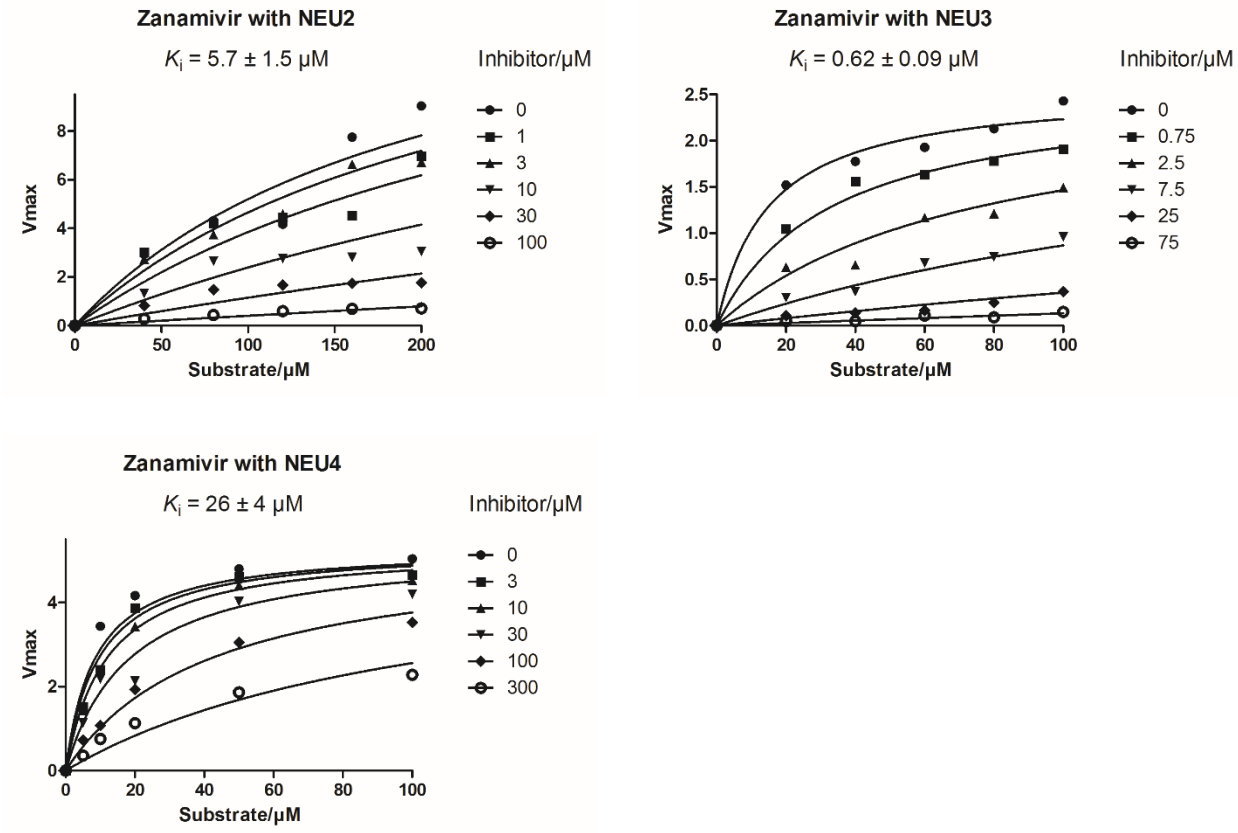

Figure S.9. K_i determination for Zanamivir (**3**)

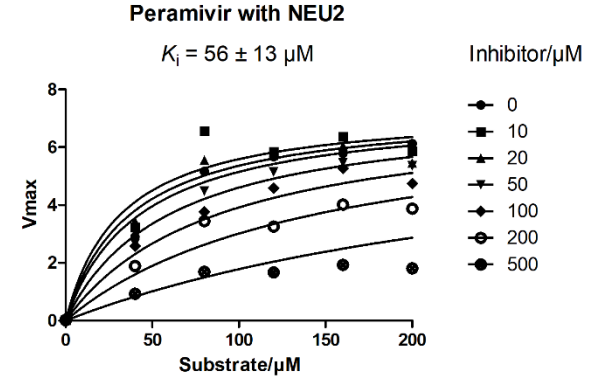

Figure S.10. K_i determination for Peramivir (**5**)

K_m curves

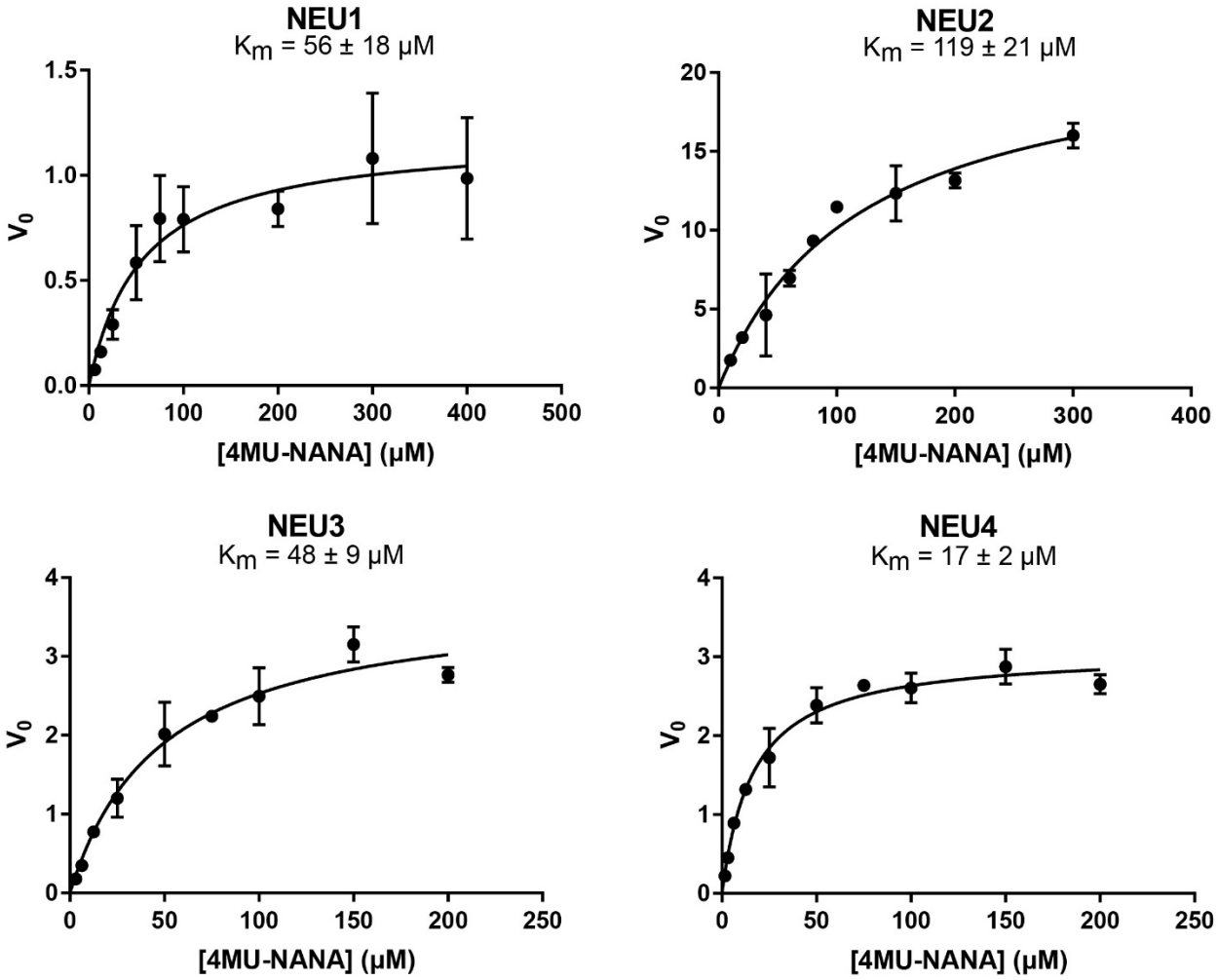

Figure S.11. K_m determination for 4MU-NANA.

Analysis of MD simulations

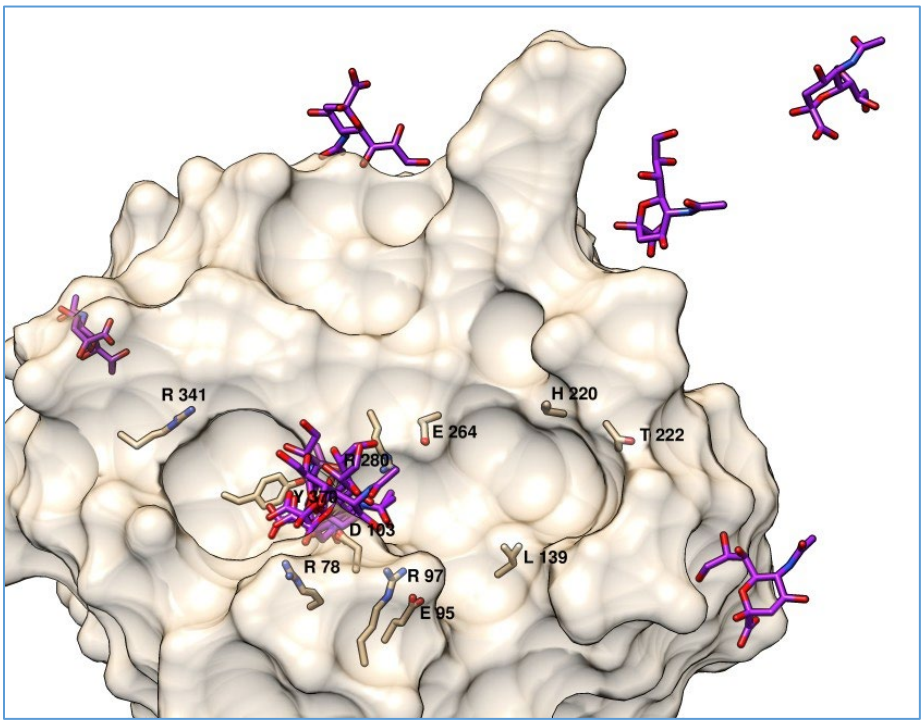

Figure S.12. Average structure of NEU1 with Neu5Ac (**1**) shown as 10 structures from the MD trajectory, taken every 10 ns.

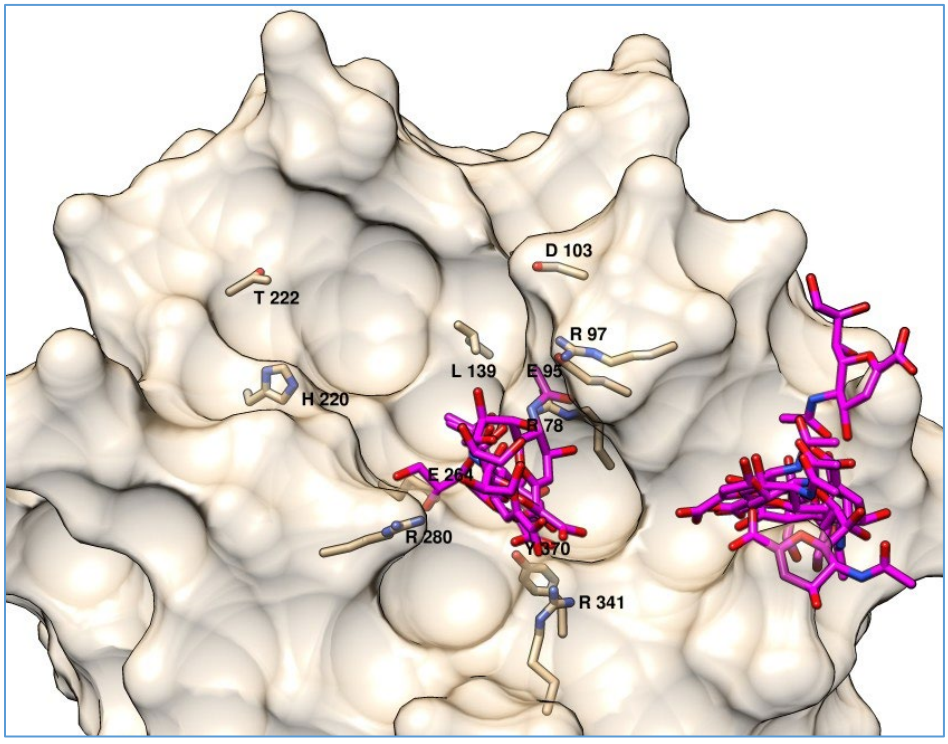

Figure S.13. Average structure of NEU1 with DANA (**2**) shown as 10 structures from the MD trajectory, taken every 10 ns.

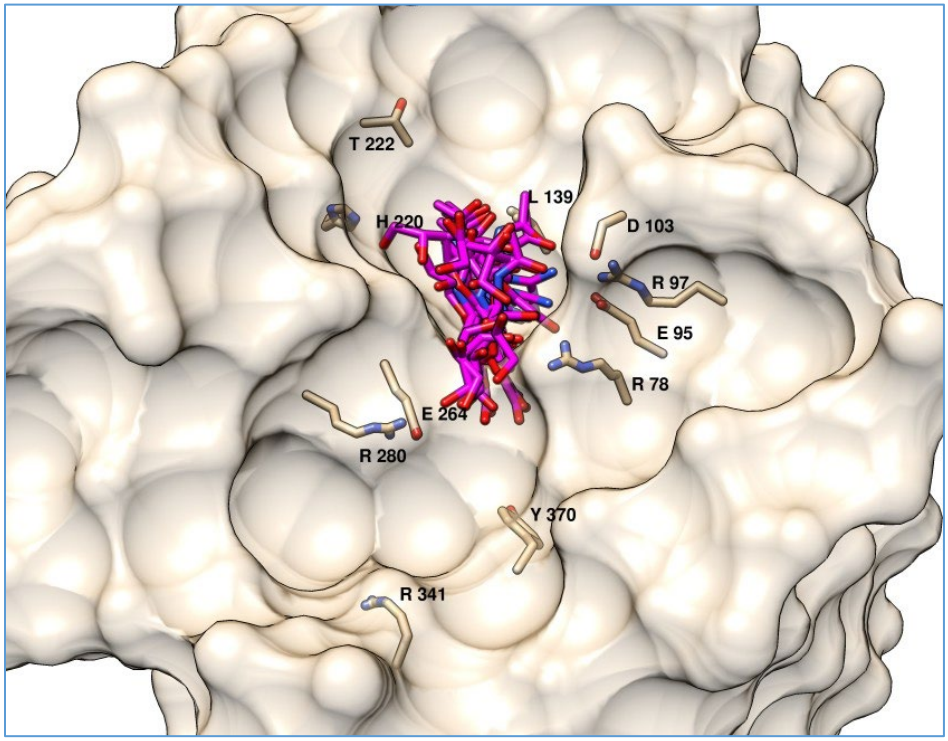

Figure S.14. Average structure of NEU1 with zanamivir (**3**) shown as 10 structures from the MD trajectory, taken every 10 ns.

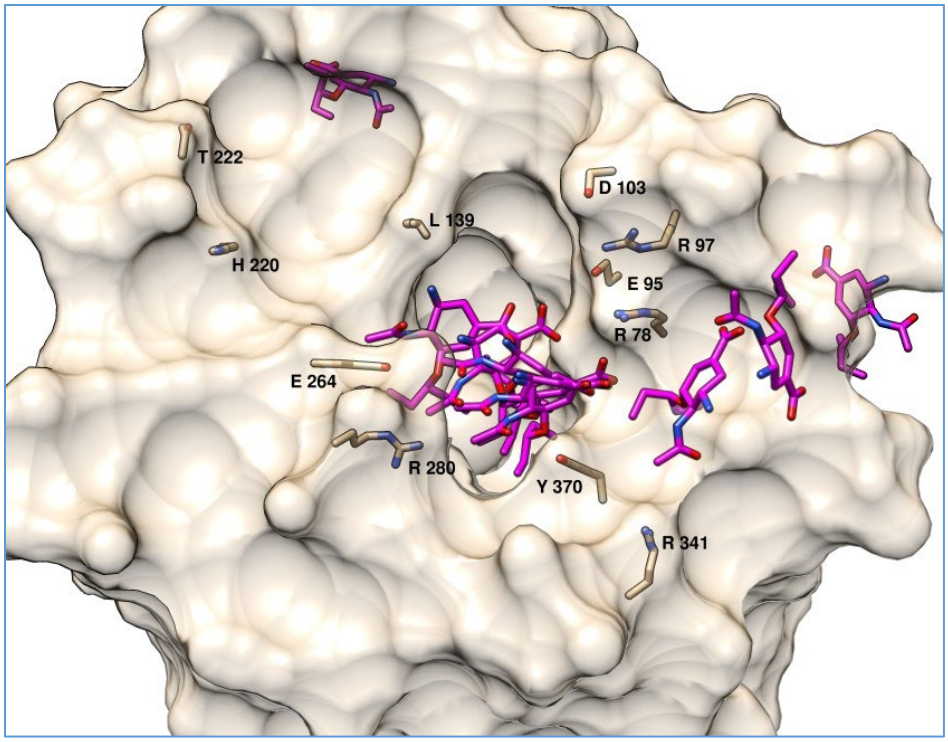

Figure S.15. Average structure of NEU1 with oseltamivir (**4**) shown as 10 structures from the MD trajectory, taken every 10 ns.

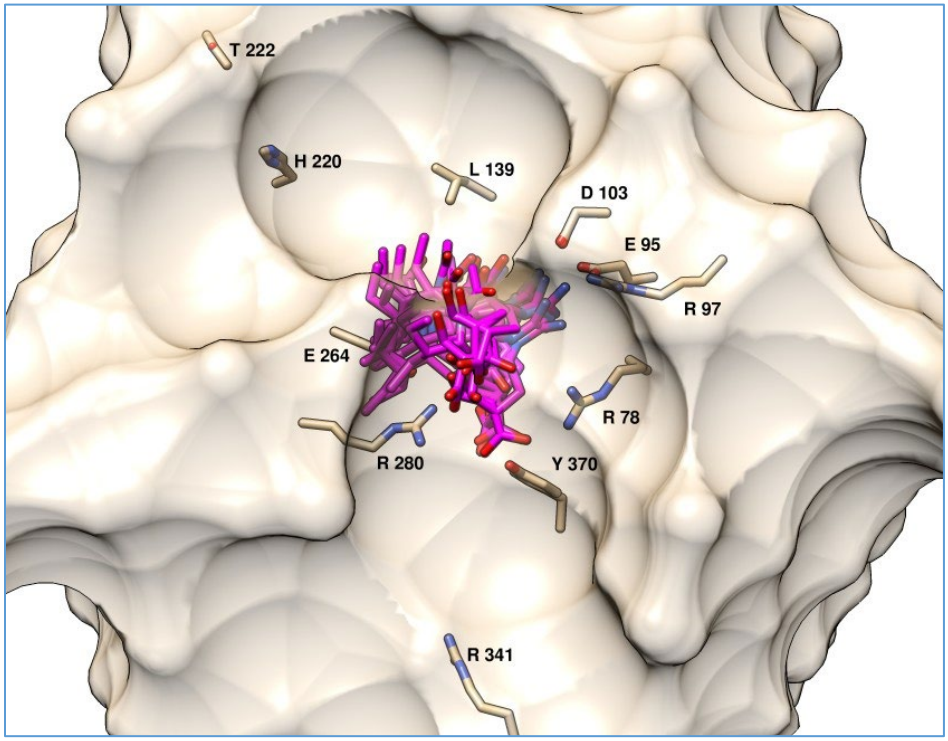

Figure S.16. Average structure of NEU1 with peramivir (**5**) shown as 10 structures from the MD trajectory, taken every 10 ns.

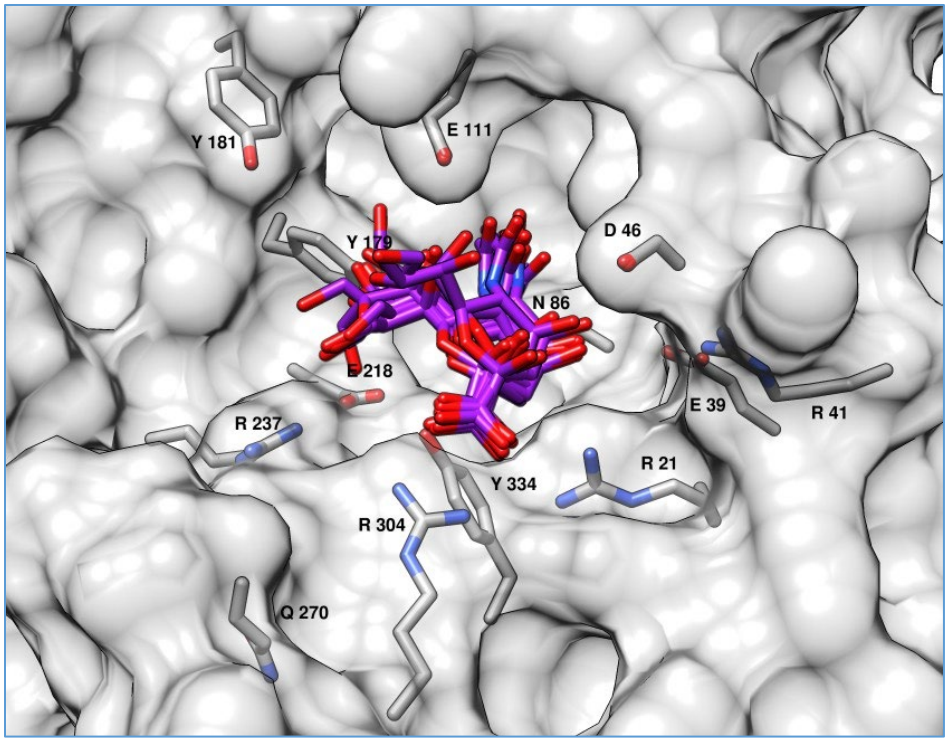

Figure S.17. Average structure of NEU2 with Neu5Ac (**1**) shown as 10 structures from the MD trajectory, taken every 10 ns.

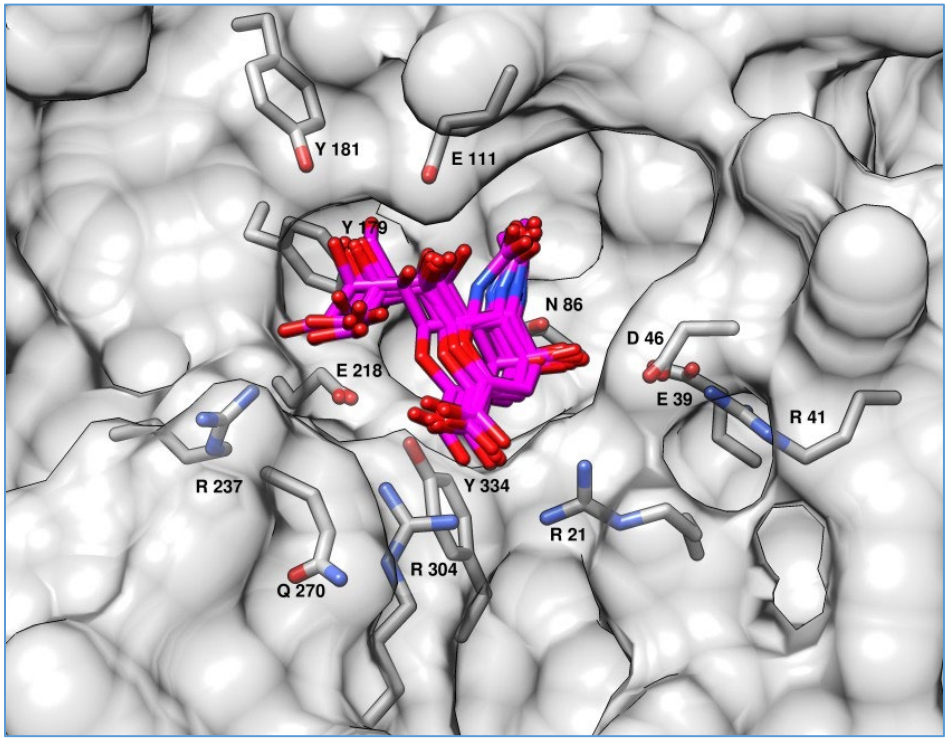

Figure S.18. Average structure of NEU2 with DANA (**2**) shown as 10 structures from the MD trajectory, taken every 10 ns.

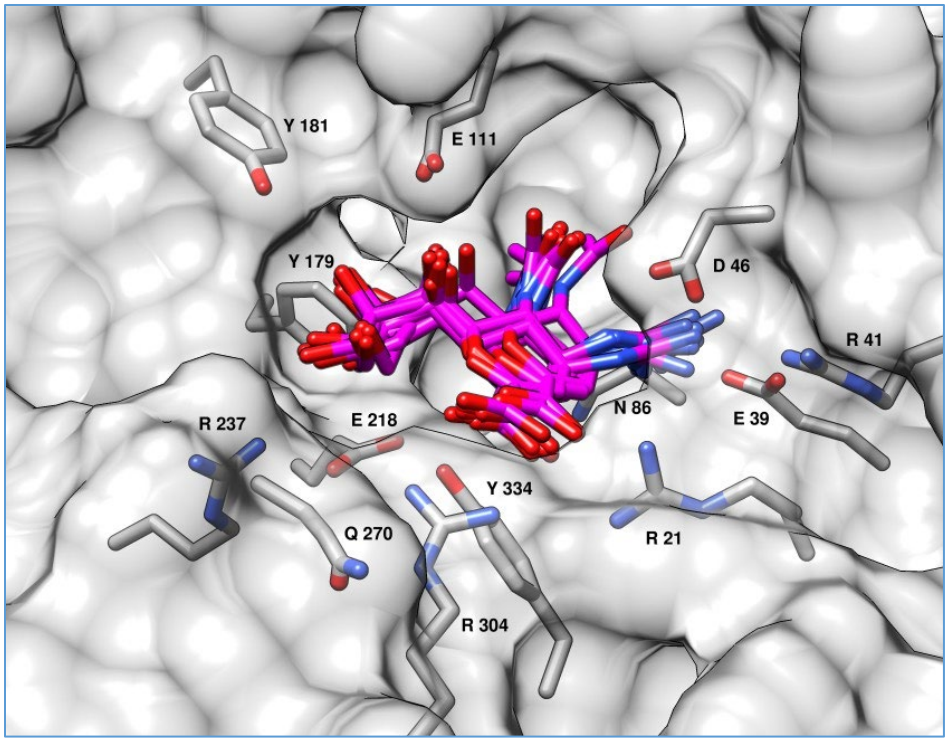

Figure S.19. Average structure of NEU2 with zanamivir (**3**) shown as 10 structures from the MD trajectory, taken every 10 ns.

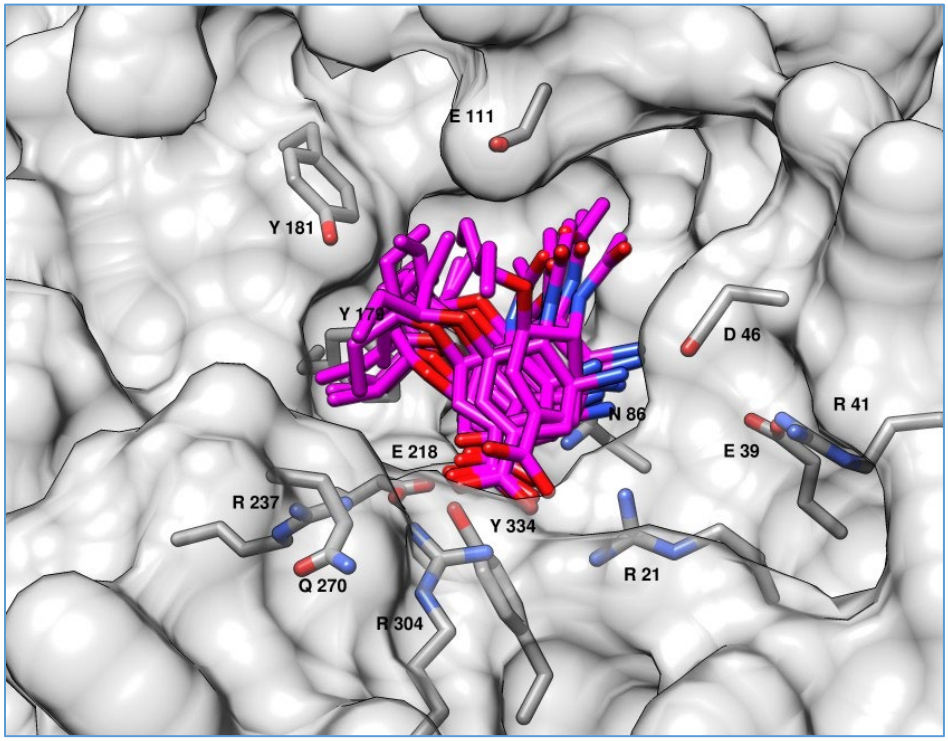

Figure S.20. Average structure of NEU2 with oseltamivir (**4**) shown as 10 structures from the MD trajectory, taken every 10 ns.

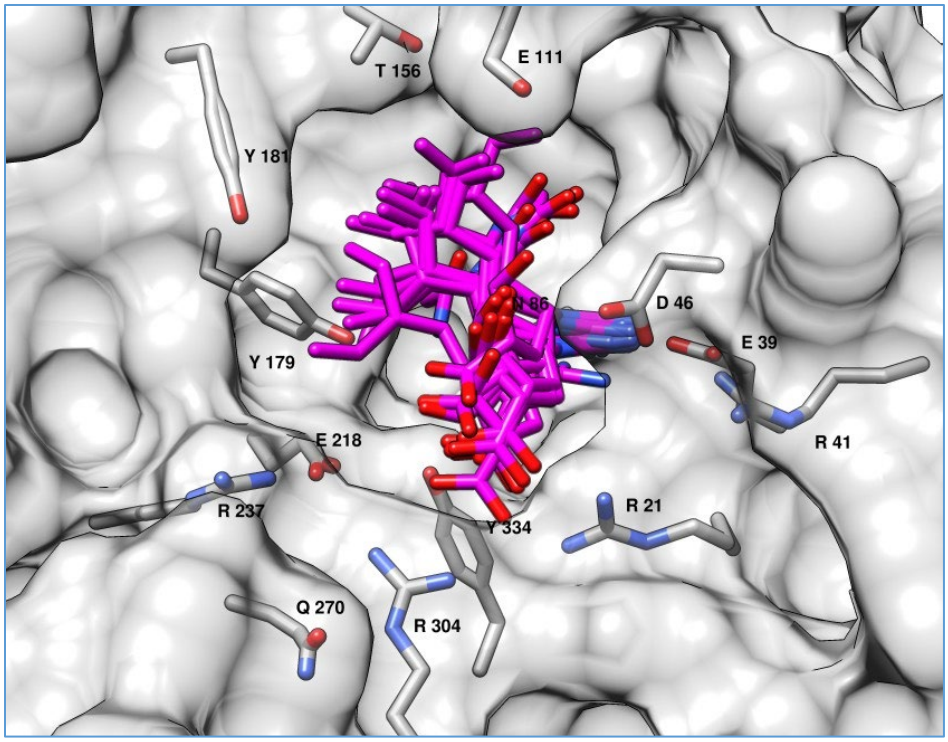

Figure S.21. Average structure of NEU2 with peramivir (**5**) shown as 10 structures from the MD trajectory, taken every 10 ns.

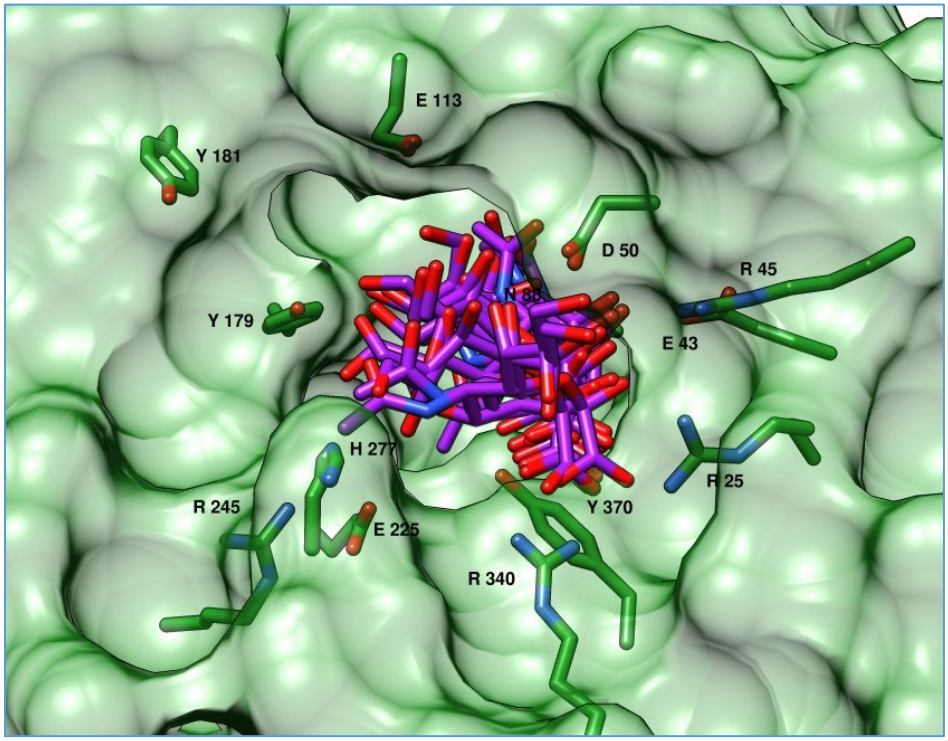

Figure S.22. Average structure of NEU3 with Neu5Ac (**1**) shown as 10 structures from the MD trajectory, taken every 10 ns.

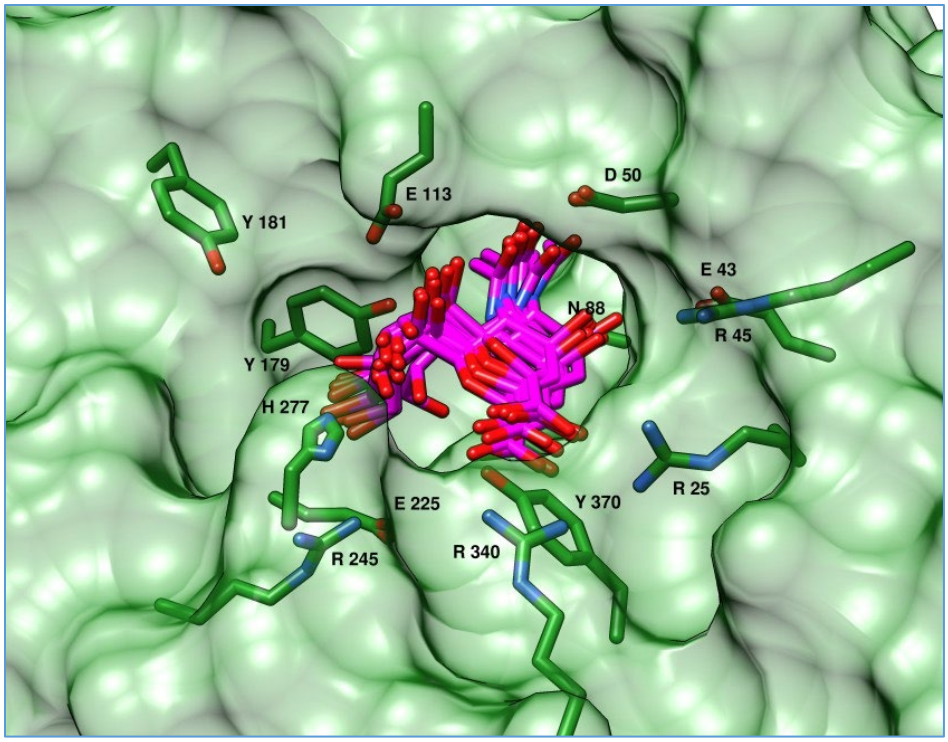

Figure S.23. Average structure of NEU3 with DANA (**2**) shown as 10 structures from the MD trajectory, taken every 10 ns.

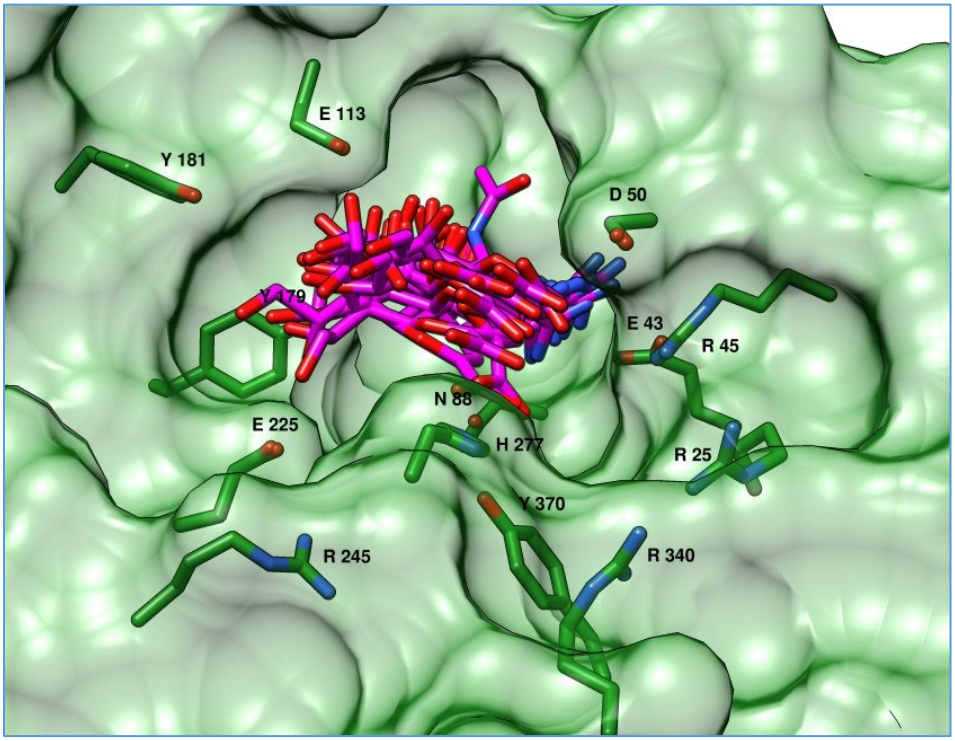

Figure S.24. Average structure of NEU3 with zanamivir (**3**) shown as 10 structures from the MD trajectory, taken every 10 ns.

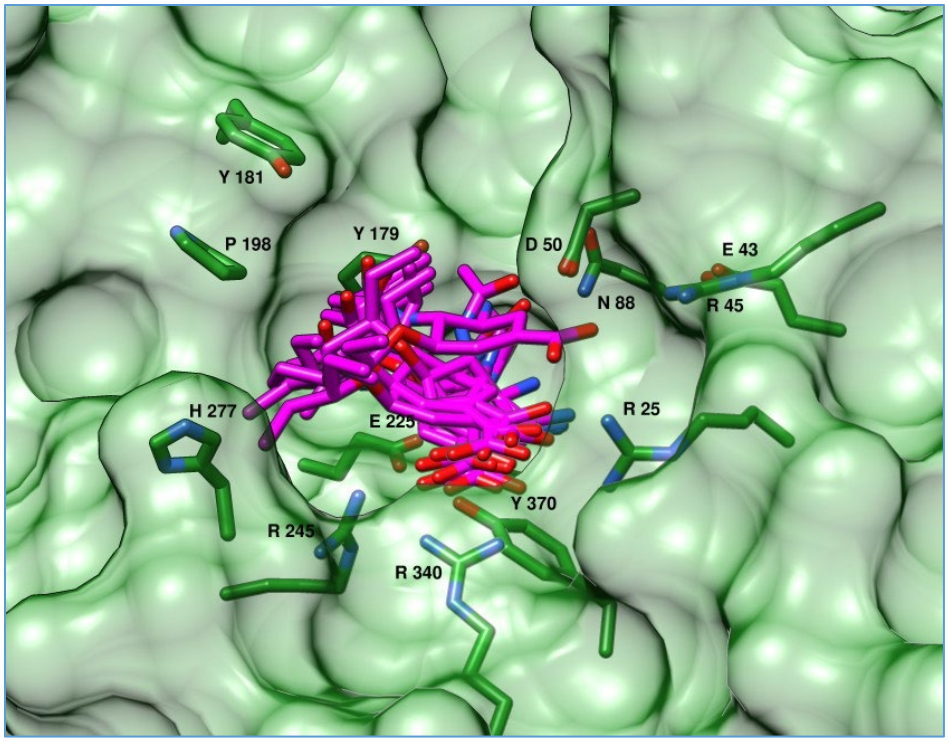

Figure S.25. Average structure of NEU3 with oseltamivir (**4**) shown as 10 structures from the MD trajectory, taken every 10 ns.

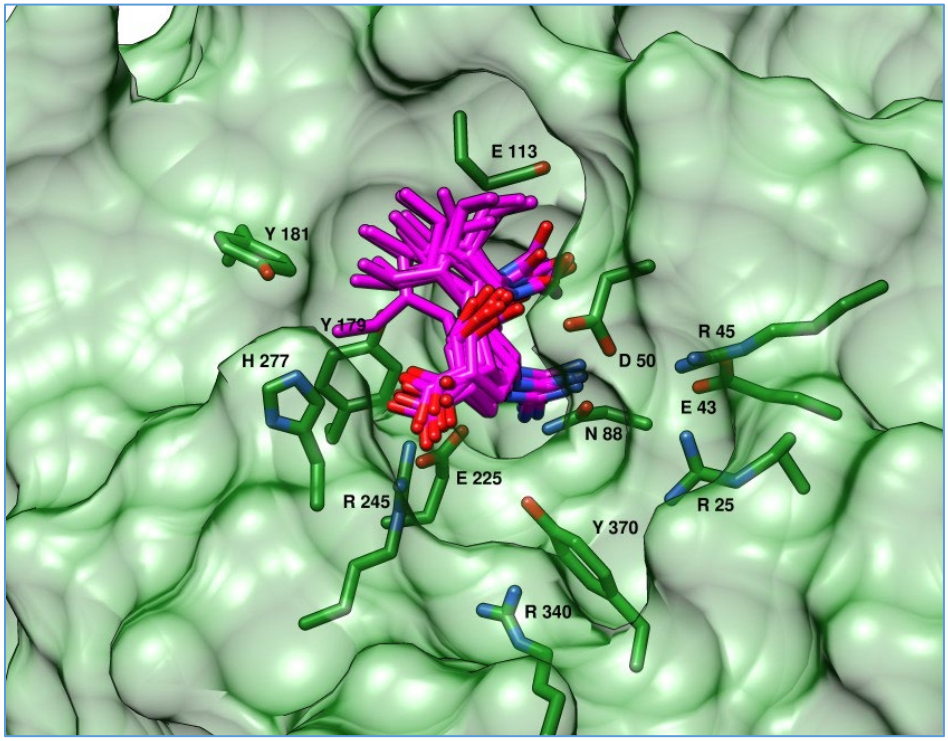

Figure S.26. Average structure of NEU3 with peramivir (**5**) shown as 10 structures from the MD trajectory, taken every 10 ns.

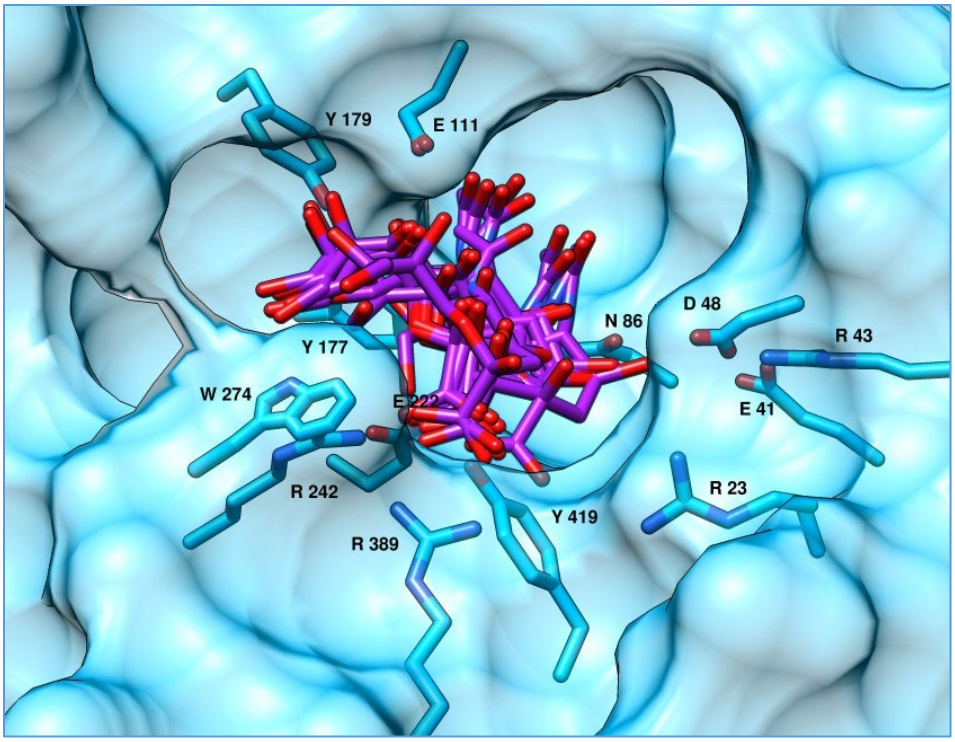

Figure S.27. Average structure of NEU4 with Neu5Ac (**1**) shown as 10 structures from the MD trajectory, taken every 10 ns.

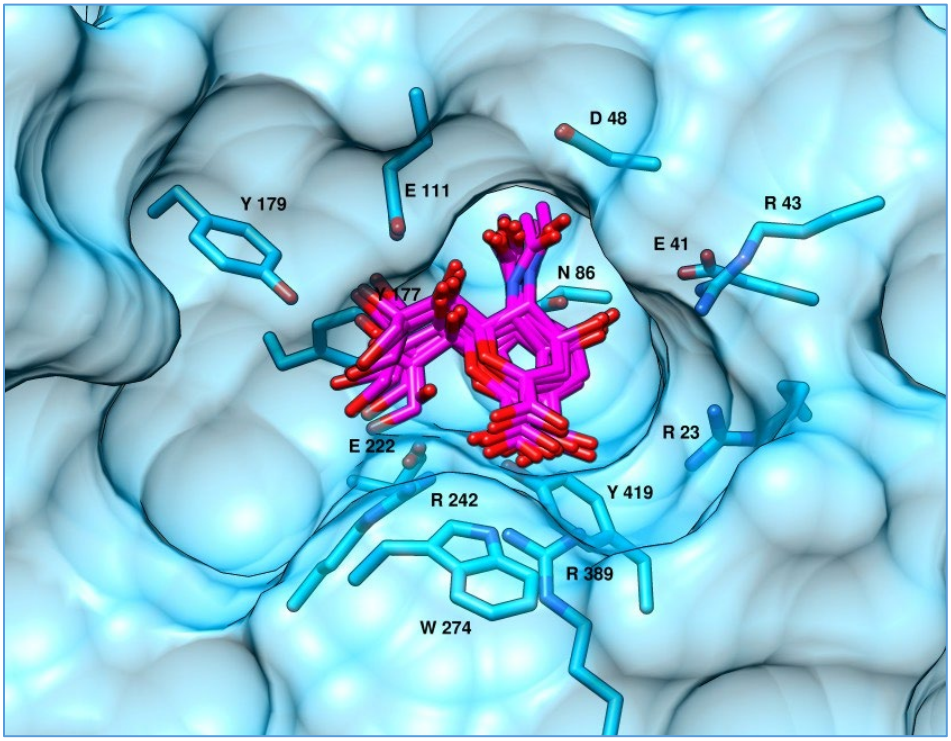

Figure S.28. Average structure of NEU4 with DANA (**2**) shown as 10 structures from the MD trajectory, taken every 10 ns.

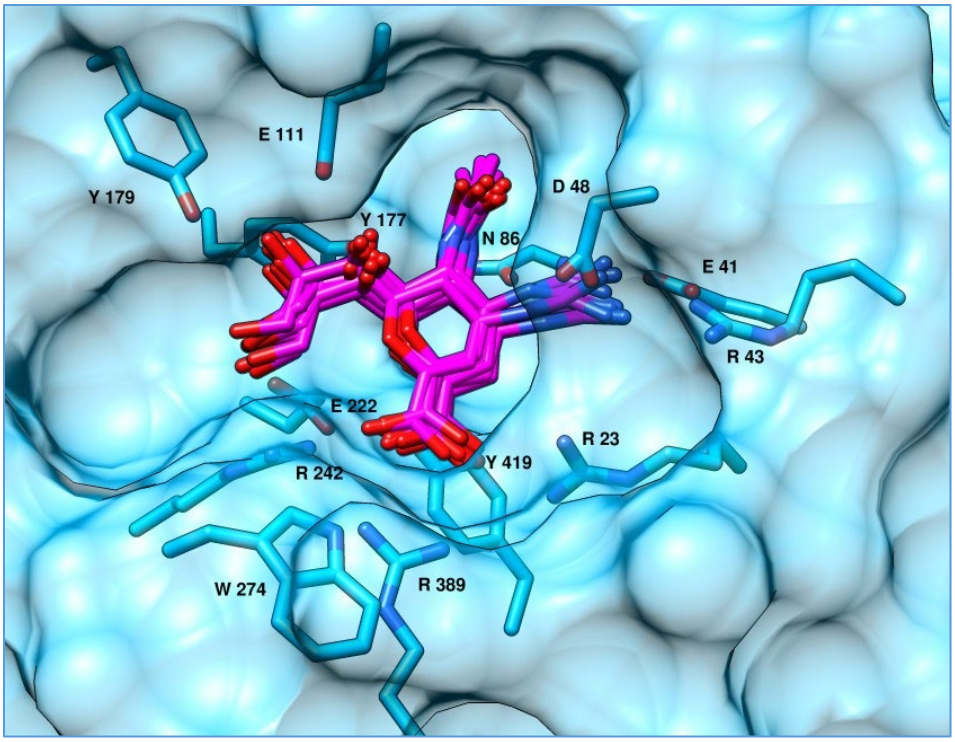

Figure S.29. Average structure of NEU4 with zanamivir (**3**) shown as 10 structures from the MD trajectory, taken every 10 ns.

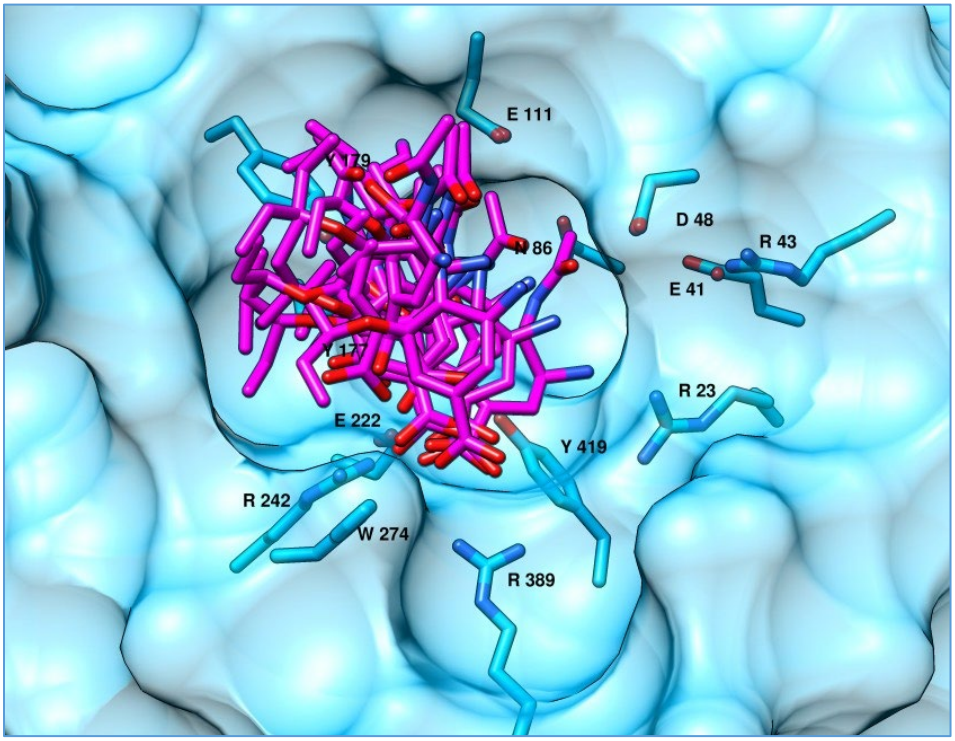

Figure S.30. Average structure of NEU4 with oseltamivir (**4**) shown as 10 structures from the MD trajectory, taken every 10 ns.

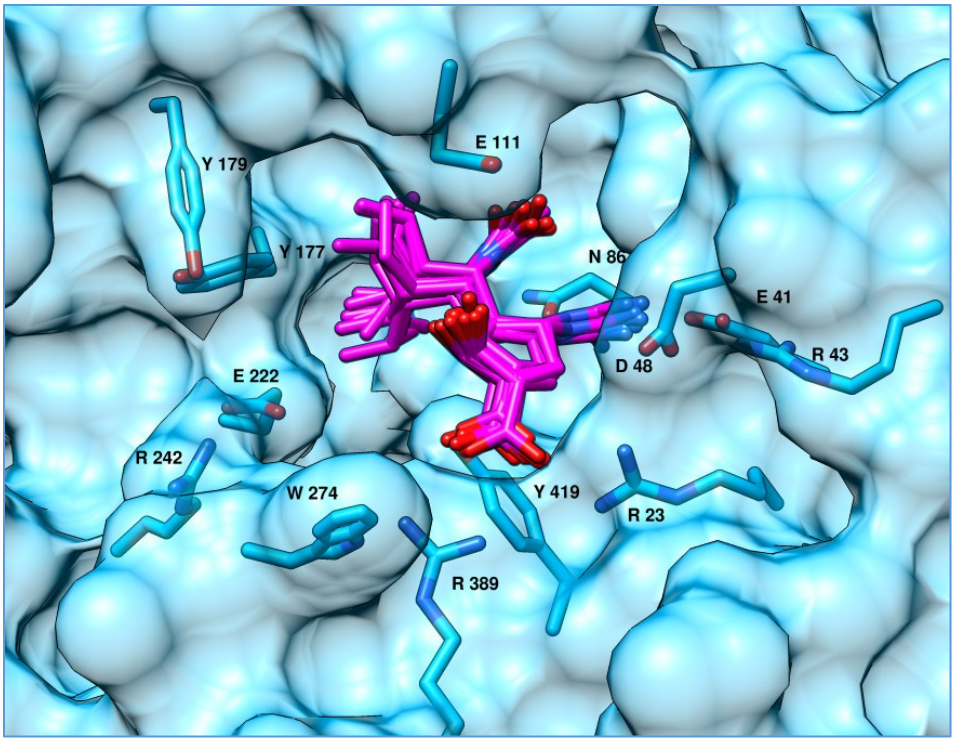

Figure S.31. Average structure of NEU4 with peramivir (**5**) shown as 10 structures from the MD trajectory, taken every 10 ns.

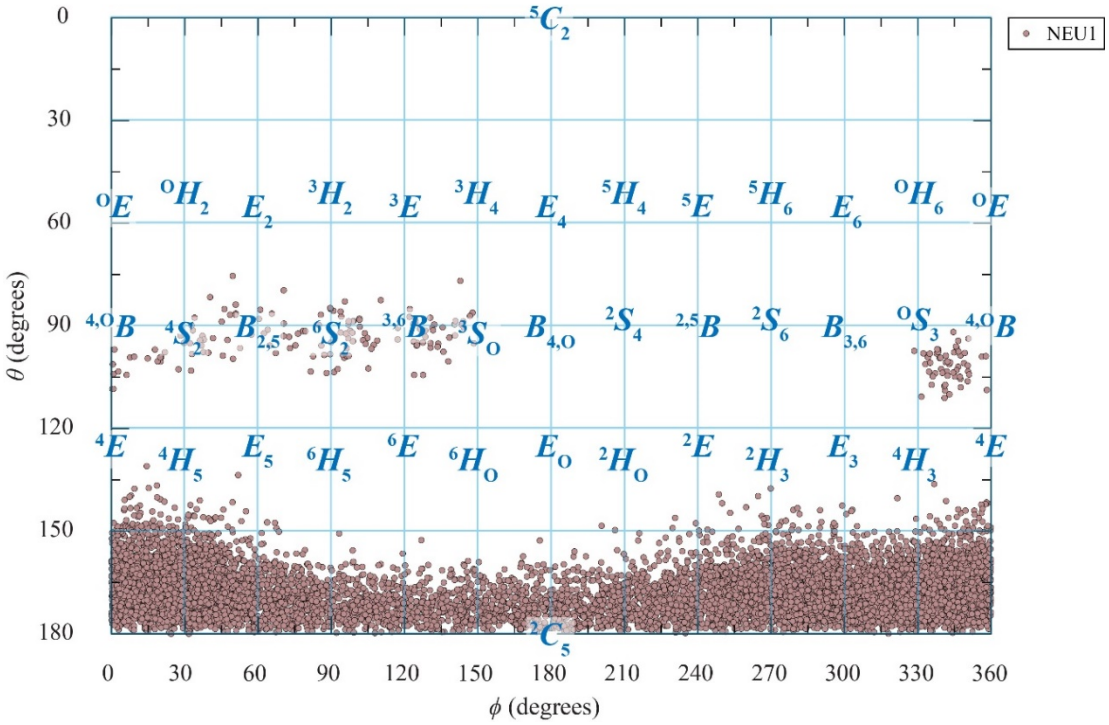

Figure S.32. Ring conformation[2] of Neu5Ac (1) during MD simulation with NEU1.

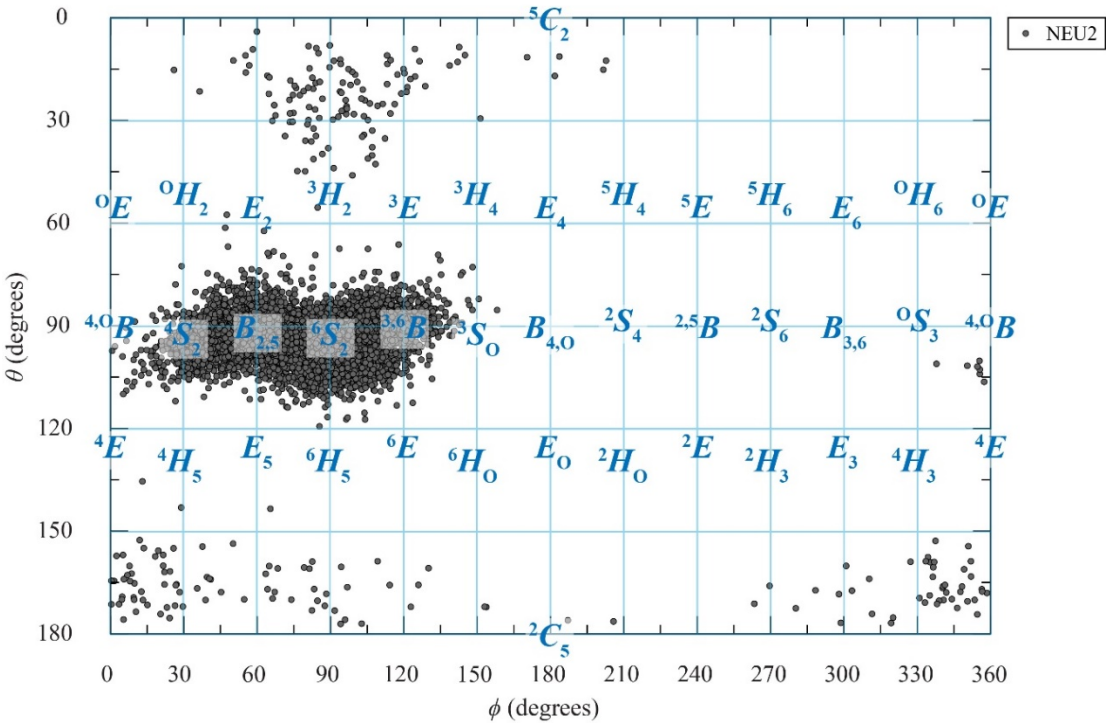

Figure S.33. Ring conformation of Neu5Ac (**1**) during MD simulation with NEU2.

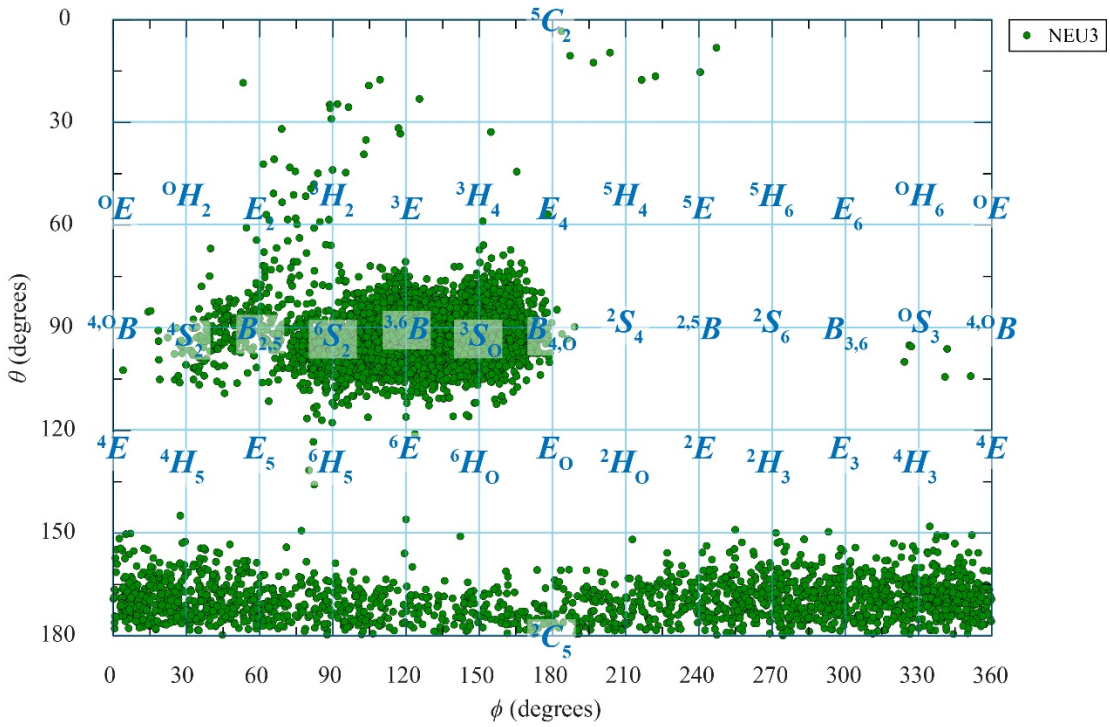

Figure S.34. Ring conformation of Neu5Ac (**1**) during MD simulation with NEU3.

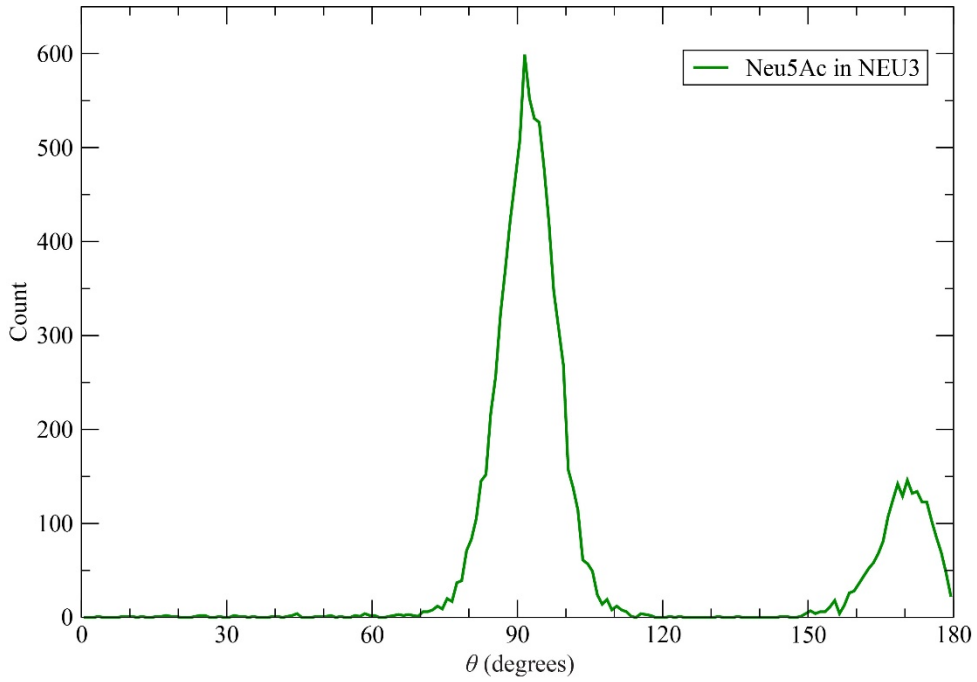

Figure S.35. Histogram of θ for Neu5Ac (**1**) during MD simulation with NEU3. 80.4% of the population is between 0° and 135° and 19.6% between 136° and 180°.

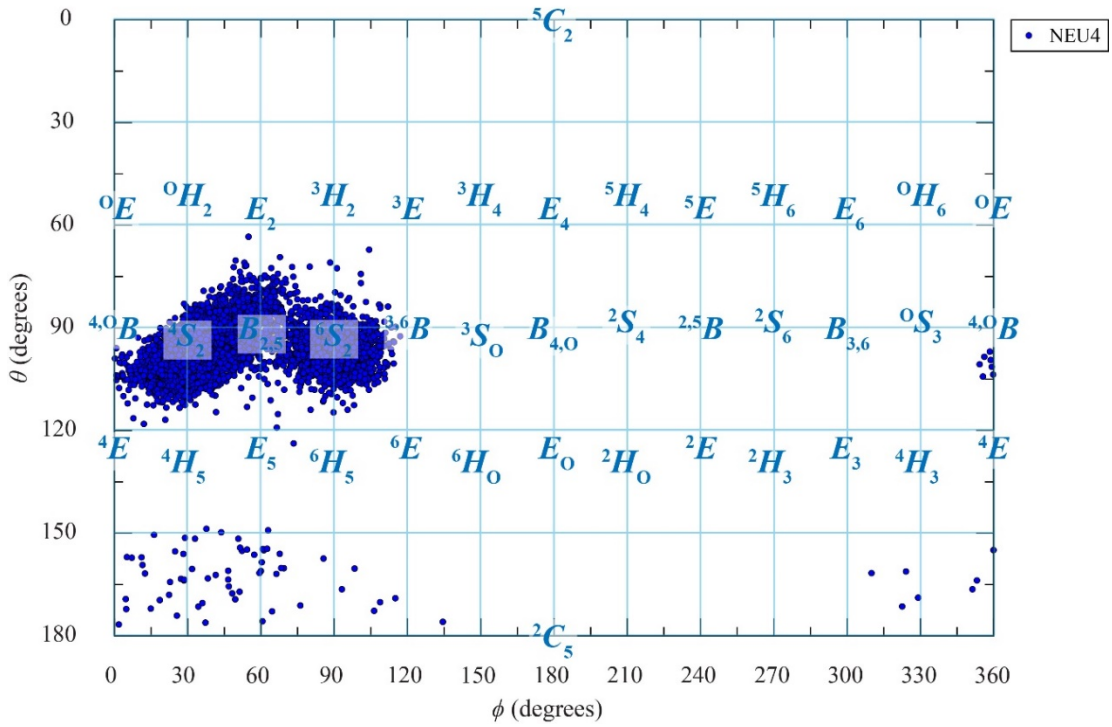

Figure S.36. Ring conformation of Neu5Ac (**1**) during MD simulation with NEU4.

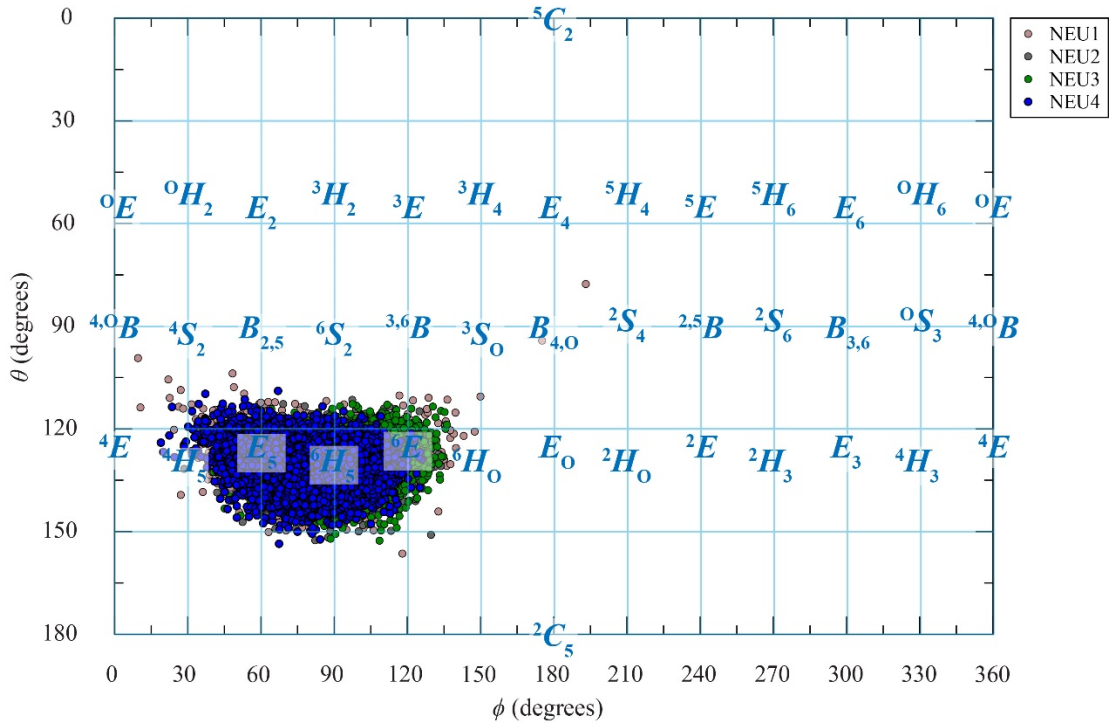

Figure S.37. Ring conformation of DANA (**2**) in MD simulations with NEU1 (tan), NEU2 (grey) NEU3 (green), NEU4 (blue).

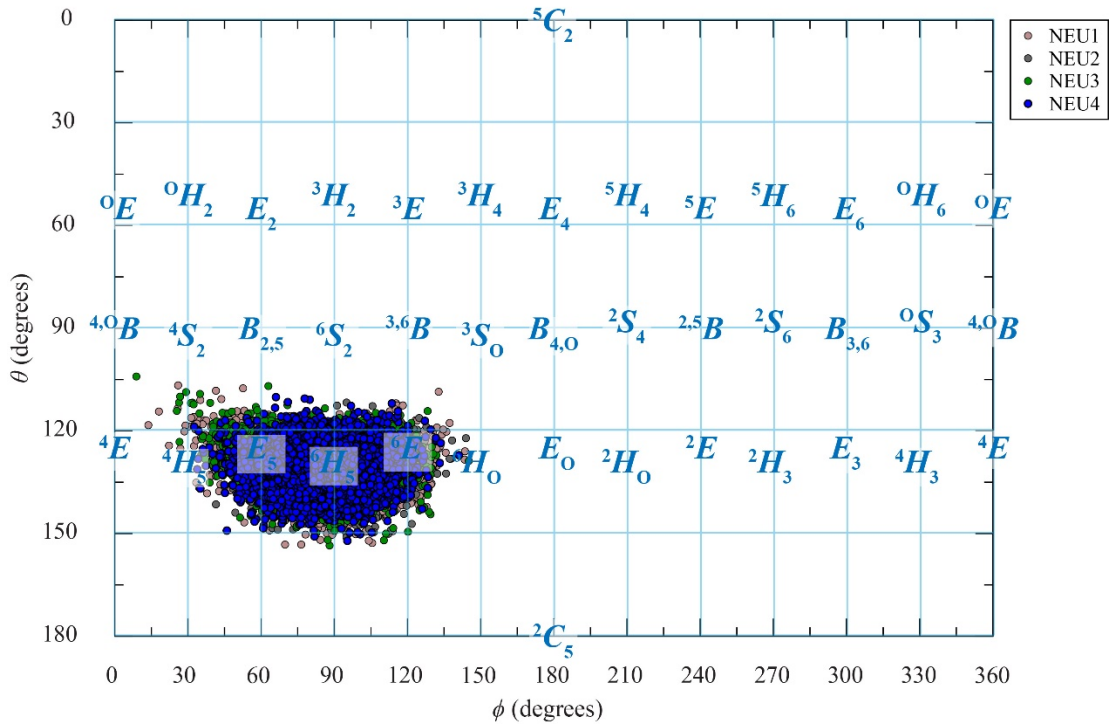

Figure S.38. Ring conformation of zanamivir (**3**) in MD simulations with NEU1 (tan), NEU2 (grey) NEU3 (green), NEU4 (blue).

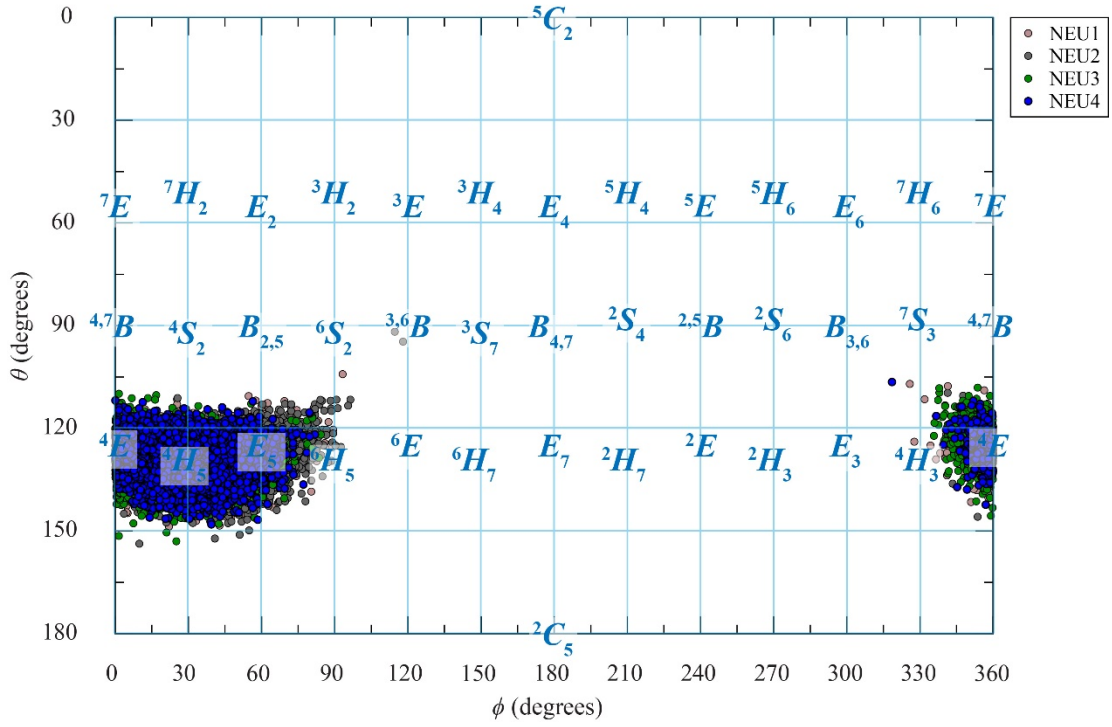

Figure S.39. Ring conformation of oseltamivir (**4**) in MD simulations with NEU1 (tan), NEU2 (grey) NEU3 (green), NEU4 (blue).

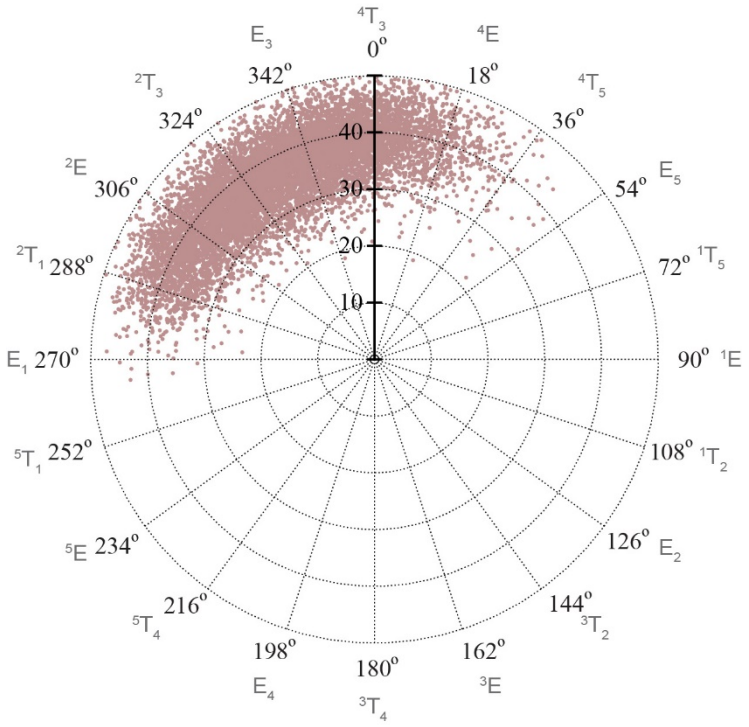

Figure S.40. Ring conformation of peramivir (**5**) during MD simulation with NEU1.

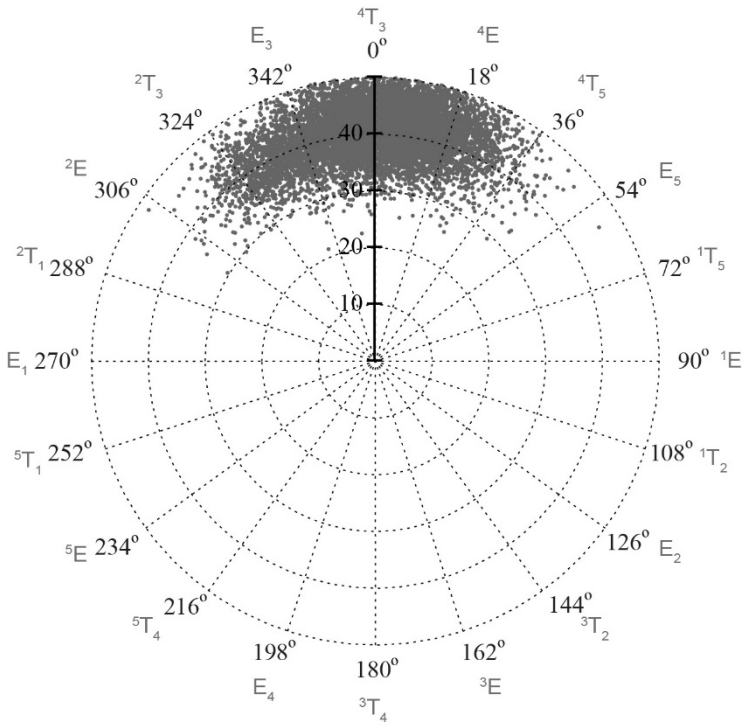

Figure S.41. Ring conformation of peramivir (**5**) during MD simulation with NEU2.

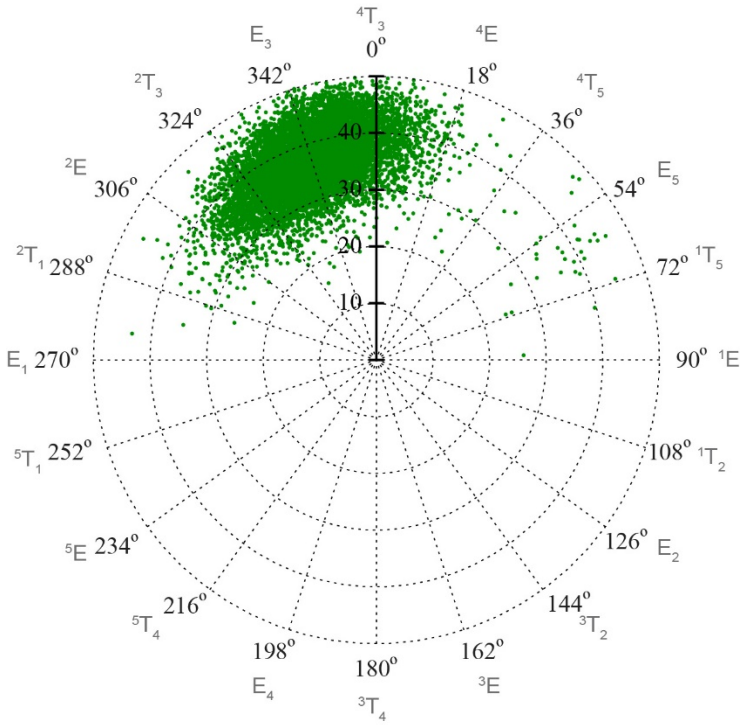

Figure S.42. Ring conformation of peramivir (**5**) during MD simulation with NEU3.

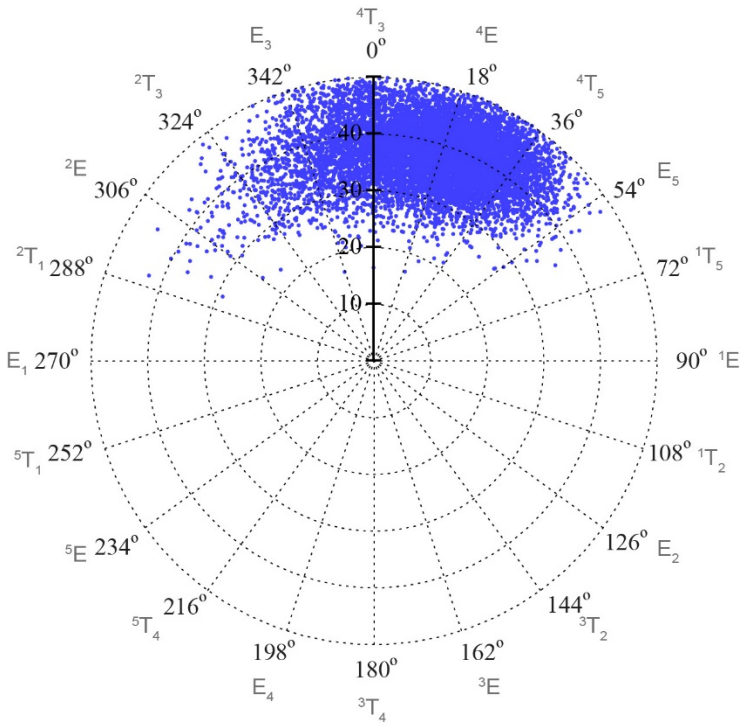

Figure S.43. Ring conformation of peramivir (**5**) during MD simulation with NEU4.

References

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2. IUPAC-IUB Joint Commission on Biochemical Nomenclature. Conformational Nomenclature for Five and Six-Membered Ring Forms of Monosaccharides and Their Derivatives. *Eur J Biochem.* 1980;111(2): 295–298.