

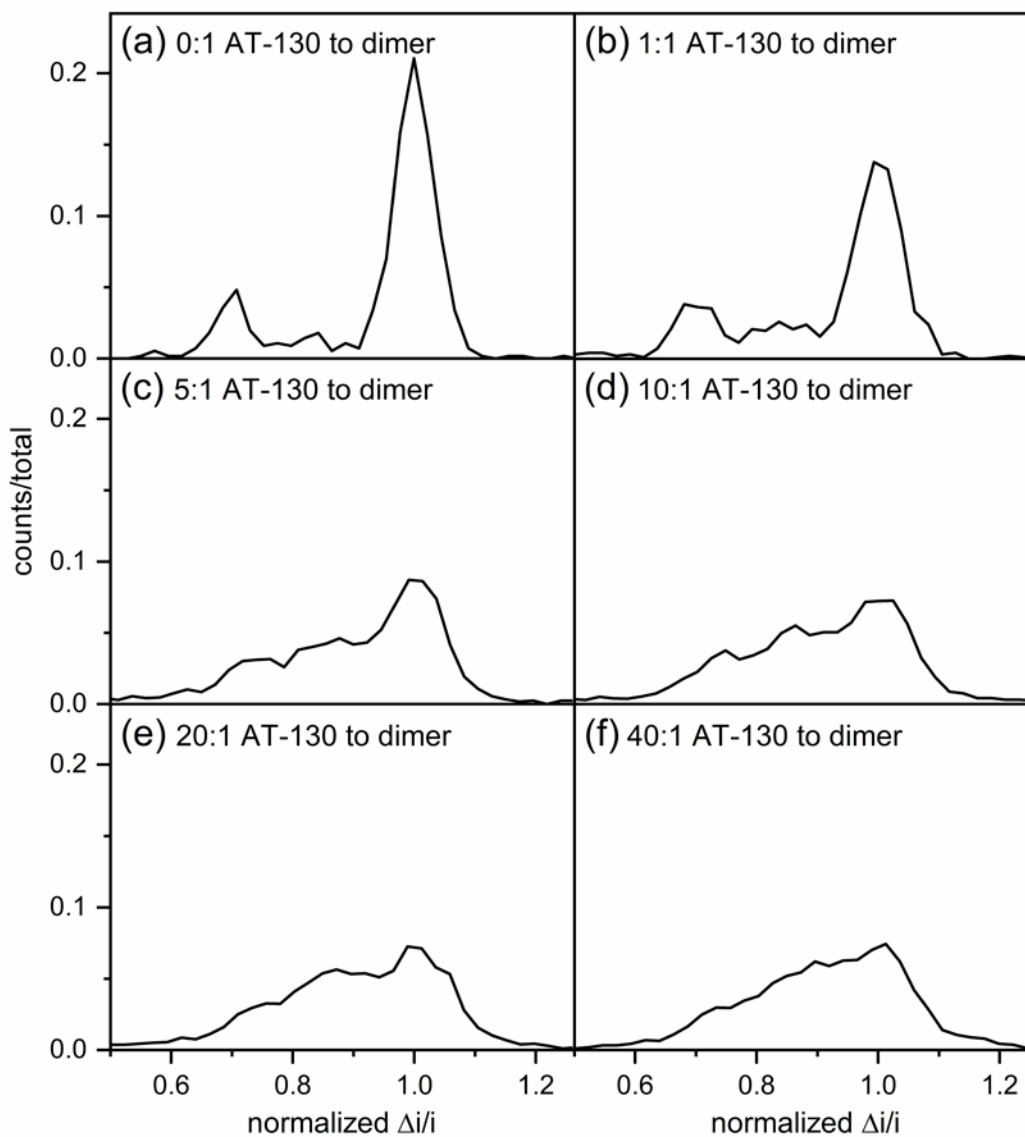
## Supporting Information

Evolution of Intermediates during Capsid Assembly of Hepatitis B Virus with  
Phenylpropenamide-based Antivirals

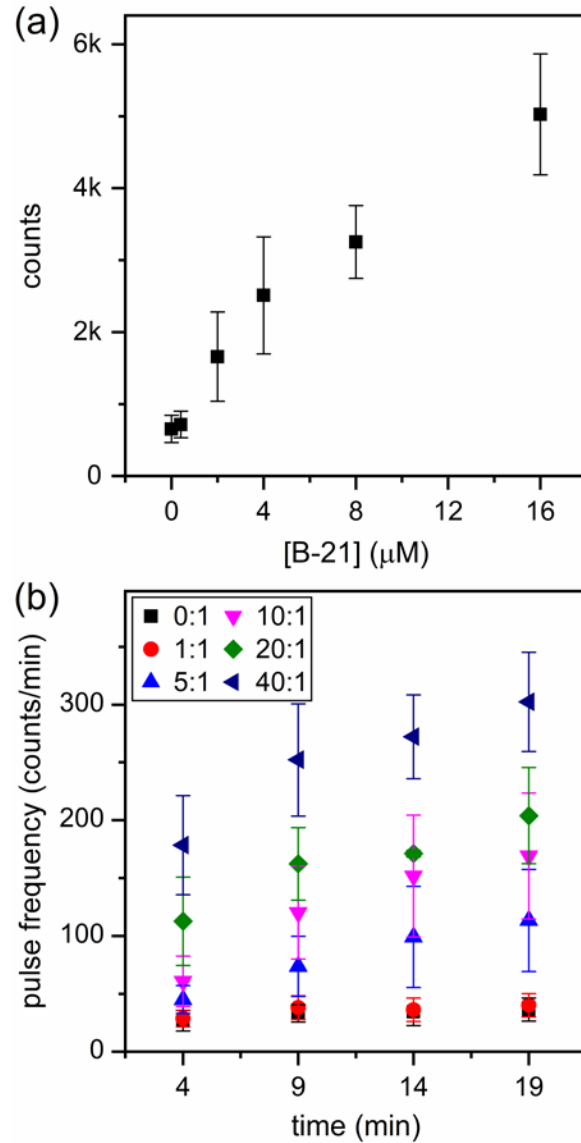
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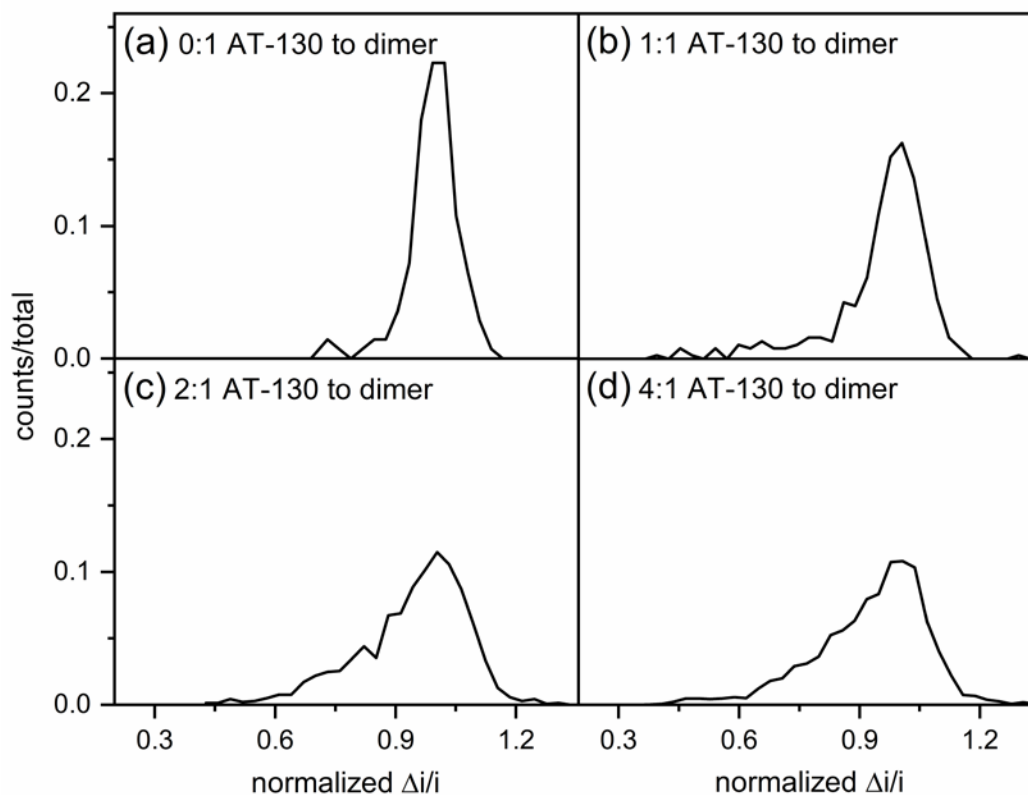
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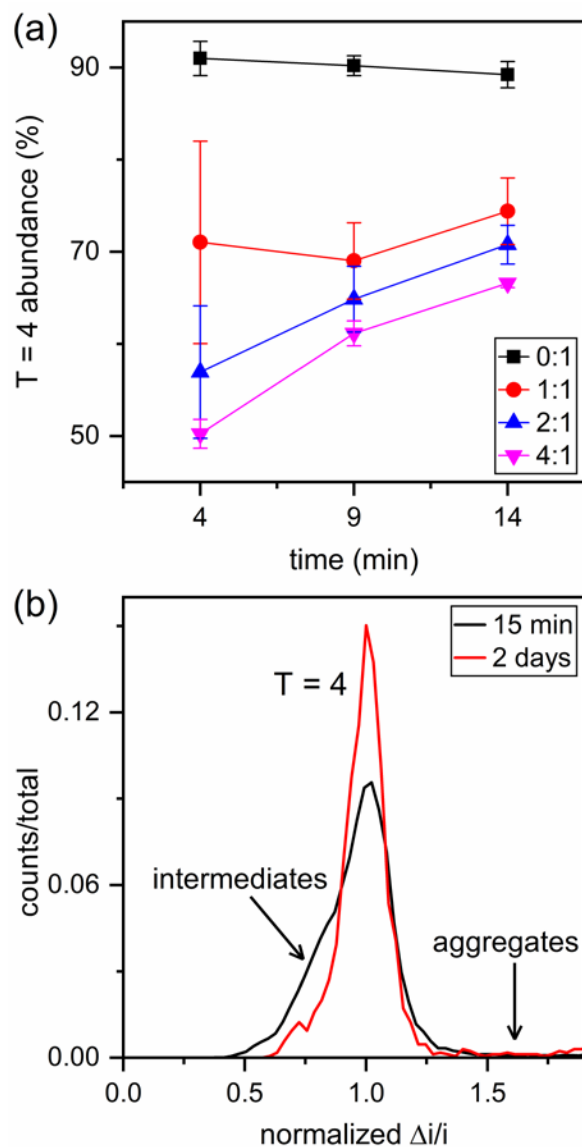
**Figure S1. Accumulation of assembly intermediates with AT-130 in 1 M NaCl.** Pulse-amplitude histograms of the reaction products for molar ratios of AT-130 to Cp149 dimer of (a) 0:1, (b) 1:1, (c) 5:1, (d) 10:1, (e) 20:1, and (f) 40:1 assembled in 1 M NaCl. AT-130 had a similar effect on HBV assembly as B-21. Final dimer concentration was 0.4  $\mu$ M, and assembly was monitored for 20 min. Pulse amplitudes are normalized to a T = 4 capsid.



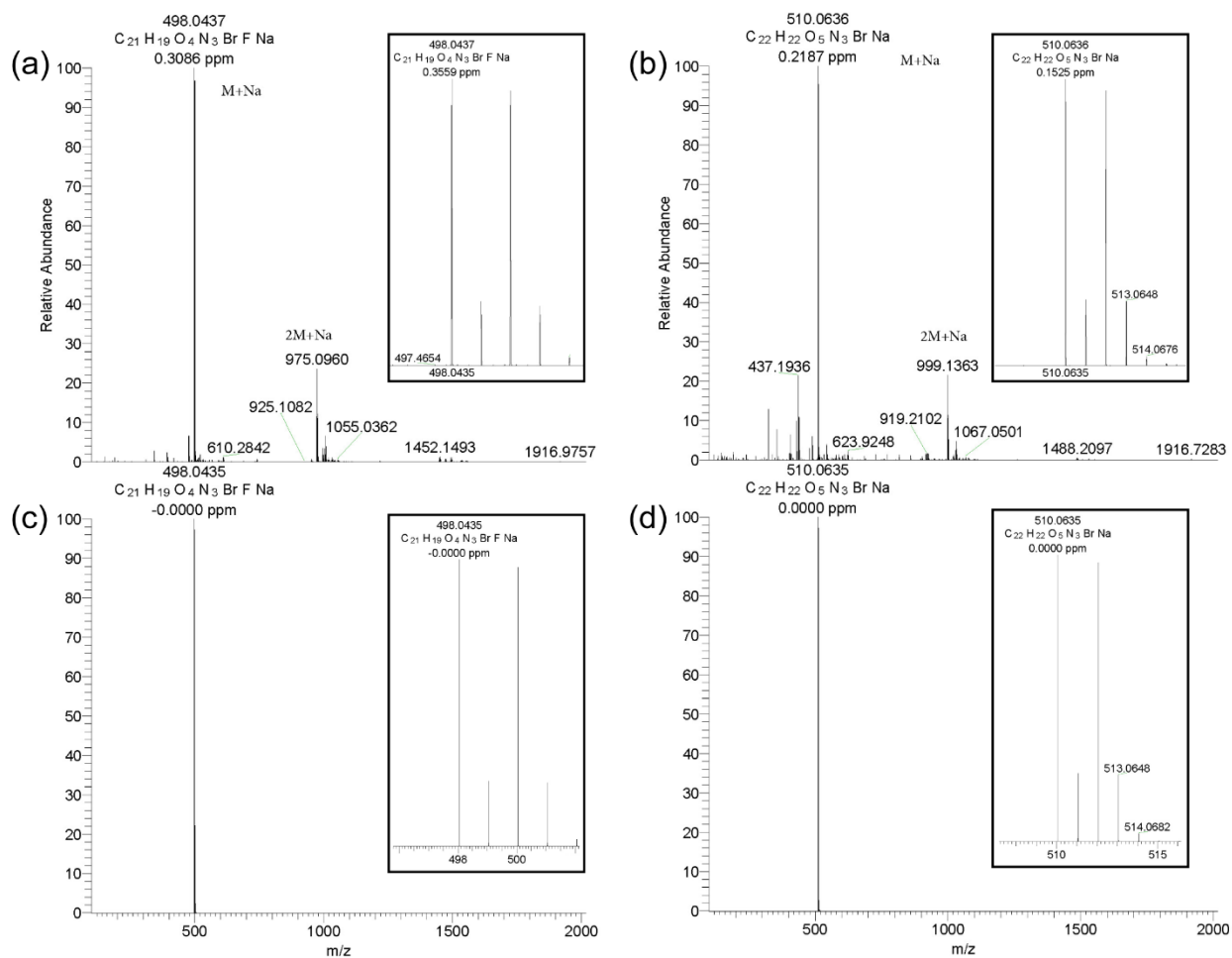
**Figure S2. Increased rate of assembly with B-21 in 1 M NaCl.** (a) Change in the number of counts with B-21 concentration integrated over the 20 min of the resistive-pulse measurements (initial 1.5–21.5 min of assembly). With a molar ratio of 40:1 B-21 to Cp149 dimer, the extent of HBV assembly increased by  $\sim 6.5$ -fold. (b) Increase in pulse frequency over the initial 1.5–16.5 min of the assembly reaction.



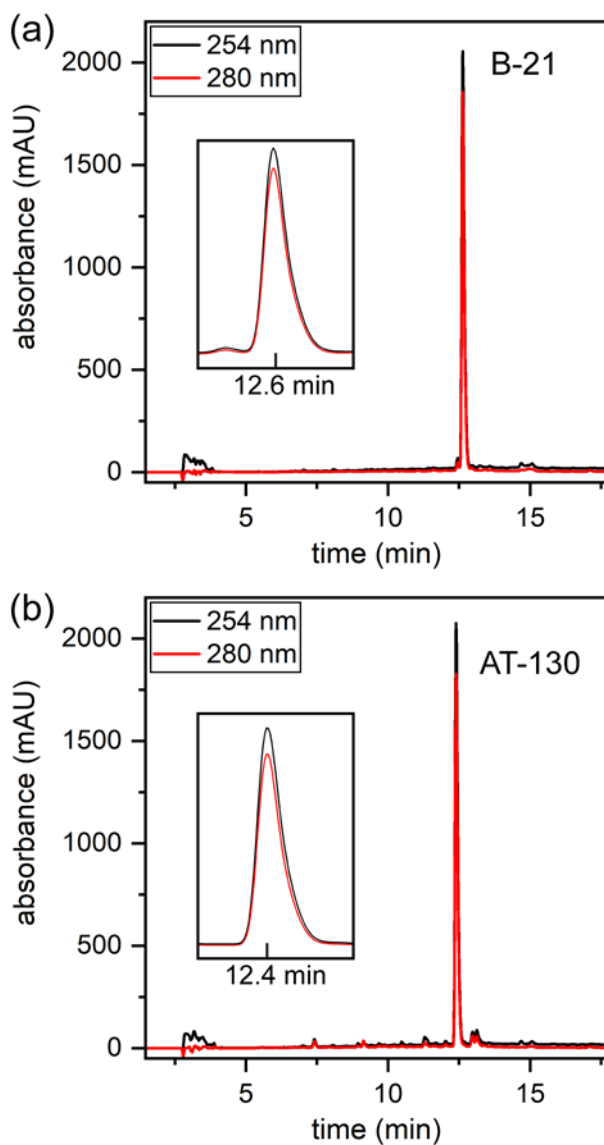
**Figure S3. Accumulation of assembly intermediates with AT-130 in 0.5 M NaCl.** Pulse-amplitude histograms of the reaction products for molar ratios of AT-130 to Cp149 dimer of (a) 0:1, (b) 1:1, (c) 2:1, and (d) 4:1 assembled in 0.5 M NaCl. Similar to what observed at 1 M NaCl, AT-130 had the same effect on HBV assembly as B-21. The final dimer concentration was 1.5  $\mu\text{M}$ , and HBV assembly was monitored for 15 min. Pulse amplitudes are normalized to a  $T = 4$  capsid.



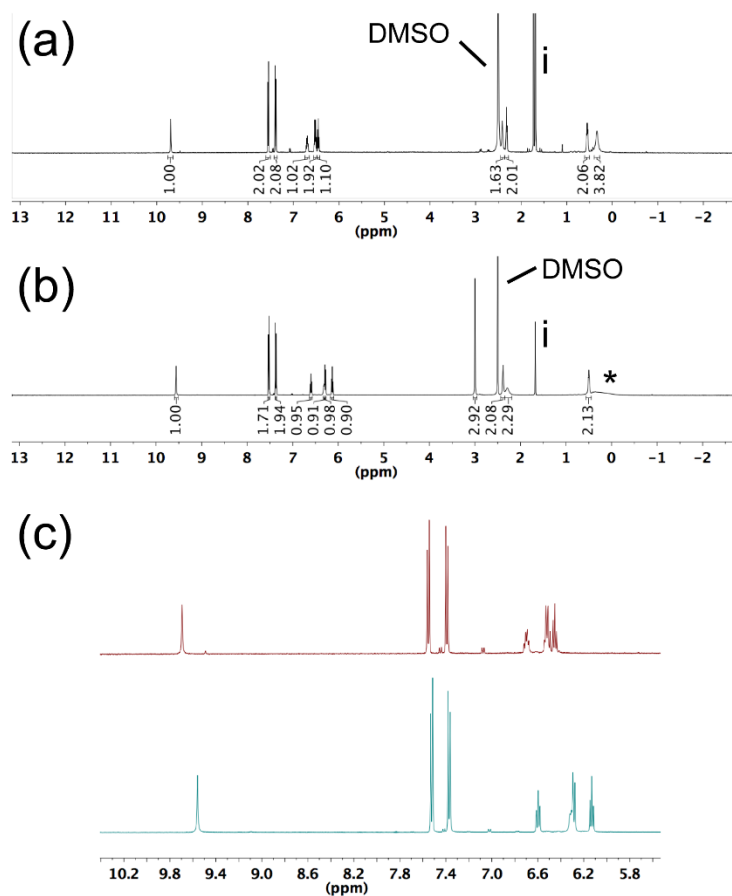
**Figure S4. Time evolution of assembly reactions with B-21 in 0.5 M NaCl.** (a) Variation of the abundance of T = 4 capsids over the initial 1.5–16.5 min of assembly reaction for different B-21 to Cp149 dimer ratios in 0.5 M NaCl. (b) Pulse-amplitude histograms of assembly products for a 4:1 ratio of B-21 to Cp149 dimer in 0.5 M NaCl after the initial 1.5–16.5 min and 2 days of reaction. In 0.5 M NaCl, most of the intermediates rearrange and form T = 4 capsids. Pulse amplitudes are normalized to a T = 4 capsid.



**Figure S5. High resolution mass spectra of (a) B-21 and (b) AT-130.** Panels (c) and (d) correspond to the theoretical estimation of the m/z values of B-21 and AT-130, respectively, based on molecular structures of the compounds (**Figure 1**). The insets show the peaks that correspond to the molecular ion plus a sodium ion. The mass spectra show an excellent agreement between the expected and measured m/z values. The data were collected with an LTQ-Orbitrap mass analyzer with positive electrospray ionization. Stock solutions of B-21 and AT-130 dissolved in DMSO were diluted 10-fold with a 1:1 solution of chloroform/methanol with 5 mM sodium acetate and then injected into the mass analyzer with a continuous flow of methanol.



**Figure S6. High performance liquid chromatograms of (a) B-21 and (b) AT-130.** The insets show the peaks that correspond to B-21 and AT-130. UV absorbance was measured at 250 and 280 nm. Both samples eluted as a single major peak, which confirmed the purity of the samples (e.g., 95% for B-21 and 92% for AT-130). The reversed-phase HPLC was run on a C18 column, and the injected samples were 400  $\mu$ M each in DMSO. The mobile phase was a water/acetonitrile gradient that ranged from 1:9 to 9:1 over 10 min and had 0.1% trifluoroacetic acid (TFA) added. The flow rate was 1 mL/min.



**Figure S7.**  $^1\text{H-NMR}$  of **B-21** and **AT-130**. (a) **B-21** ( $\text{DMSO-}d_6$ )  $\delta$  9.69 (s, 1H, NH), 7.55 (m, 2H), 7.39 (m, 2H), 6.70 (m, 1H), 6.51 (m, 2H), 6.45 (td,  $J = 7.5$  Hz, 1.1 Hz, 1H), 2.41 (s, 2H), 2.32 (t,  $J = 5.6$  Hz, 2H), 0.55 (m, 2H), and 0.34 (s, 4H), and (b) **AT-130** ( $\text{DMSO-}d_6$ ):  $\delta$  9.56 (s, 1H, NH), 7.52 (d,  $J = 8.8$  Hz, 2H), 7.37 (d,  $J = 8.8$  Hz, 2H), 6.60 (td,  $J = 7.9$ , 1.7 Hz, 1H), 6.31 (d,  $J = 7.4$  Hz, 1H), 6.29 (d,  $J = 8.4$  Hz, 1H), 6.13 (t,  $J = 7.5$  Hz, 1H), 3.00 (s, 3H,  $\text{OCH}_3$ ), 2.38 (t,  $J = 5.6$  Hz, 2H), 0.50 (p,  $J = 5.8$  Hz, 2H). The peak at 0.4 ppm (notated with an asterisk) corresponds to 2 peaks representing 4 H atoms. In both panel (a) and (b), the peak at 1.6 ppm (indicated with i) corresponds to an impurity introduced by human error. (c) Overlay of the aromatic regions of the compounds.