

Second-generation dimeric inhibitors of chitin synthase

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Abstract—Chitin synthase (CS) is essential for fungal cell wall biosynthesis and is an attractive medicinal target. Expanded results from our efforts to develop mechanism based inhibitors of CS are presented here. Specifically, we describe uridine dimers linked by tartrate amides as potential pyrophosphate mimics.

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1. Introduction and background

Chitin synthase (CS) is the enzyme responsible for the conversion of uridine diphosphoryl-*N*-acetylglucosamine (UDP-GlcNAc) into chitin—polymeric chains of β -1,4-linked *N*-acetylglucosamine (GlcNAc) (Fig. 1). The biosynthesis of chitin is essential for fungal growth and reproduction, and because it is absent in humans CS represents an important antifungal target.¹ In broad terms, three approaches to the development of CS inhibitors warrant consideration: inhibition based on enzyme structure, inhibition based on substrate analogs, and inhibition based on mechanism.

The first of these is not yet a viable option. While the crystal structures of several glycosyltransferases have recently been solved, polymerizing transferases such as CS are large integral membrane proteins and are likely to elude crystallographic characterization for some time to come.² The second option, inhibition by substrate analogs, appears more feasible, but is complicated by the fact that CS (like most transferases) has low affinity for its substrate: K_M values for CS are ~ 1 mM, consistent with very weak substrate binding. While the best inhibitors of CS are the naturally occurring polyoxin and nikkomycins (Chart 1), which are generally regarded as UDP-GlcNAc analogs,³ the rational design of CS inhibitors along these lines has not produced compounds with comparable potency.⁴ Our research has

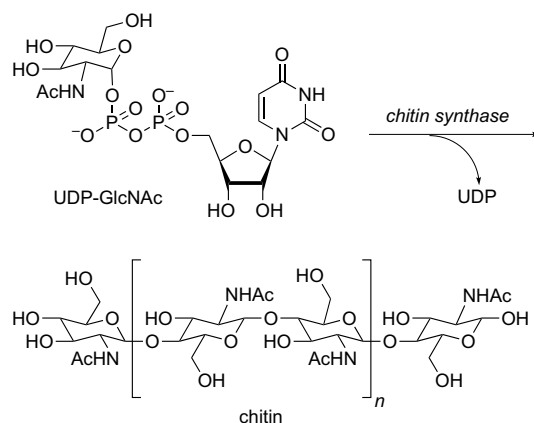


Figure 1. Biosynthesis of chitin.

therefore been focused on a mechanism based approach to the development of CS inhibitors.⁵

Developing mechanism based inhibitors necessarily requires an understanding the mechanism of the enzyme. It was, in fact, the conflicting mechanistic hypotheses in the literature,⁶ combined with the complete absence of experimental mechanistic investigation that provided the initial incentive for our studies of CS. Mechanistic proposals for CS must address the extended structure of the polysaccharide chain, in which adjacent glycosyl units have opposed orientations (Fig. 1). As has been noted,^{6a,b,c} multiple active sites with appropriate proximity and spatial orientation could allow for the sequential transfer of two sugar residues without the need for rotation of the growing chain or the enzyme

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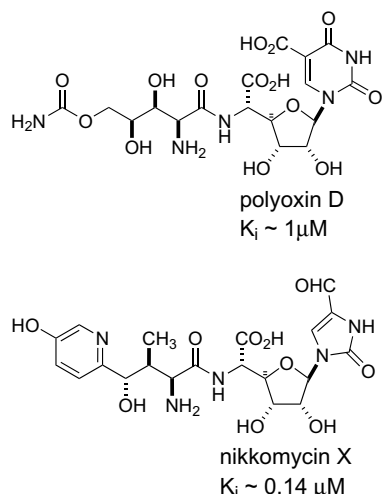


Chart 1. Naturally occurring CS inhibitors.

(Fig. 2). Consequently, even without *specific* structural information, if CS possesses two active sites in close proximity it should be possible to enhance binding affinity through multivalent interactions between multiple UDP-binding motifs.

To this end, we have previously disclosed a series of dimeric uridine compounds with tether-length-dependant

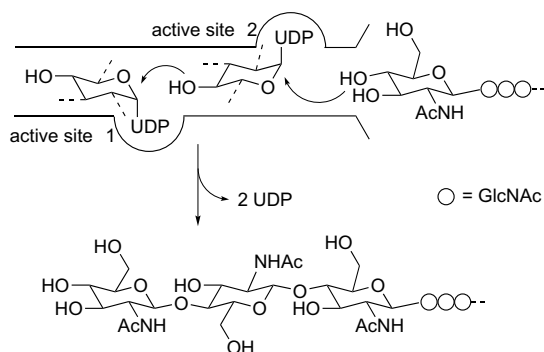


Figure 2. Proposed two site mechanism for CS.

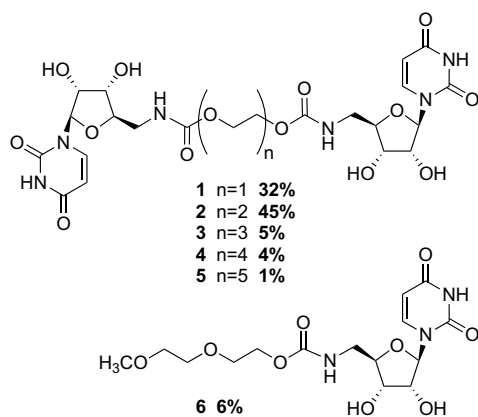


Chart 2. First-generation dimeric inhibitors, monomeric control, and inhibition of CS activity. Percentile values are % inhibition of CS activity at 1 mM inhibitor.

inhibition, providing the first evidence of a two active-site mechanism for CS (Chart 2).^{5c} These data are consistent with the hypothesis that CS operates via a two site mechanism, distinct from that of the more well understood single sugar glycosyltransferases. The best dimeric inhibitor of the first series, compound **2** ($\text{IC}_{50} = 1.1 \text{ mM}$), was an order of magnitude more potent than the corresponding monomeric control (**6**, $\text{IC}_{50} = 11.8 \text{ mM}$). While the difference in inhibitory activity is informative, **2** is still a much weaker inhibitor than any the polyoxins or nikkomycins ($K_i \sim 0.001\text{--}0.1 \text{ mM}$).³ It is anticipated that dimerization of more potent monomeric fragments will lead to significantly enhanced inhibition of CS. This letter describes our most recent efforts in that direction.

2. Design, synthesis, and evaluation of uridine–tartrate monomers of UDP-analogs

The structural resemblance to UDP is regarded as the determining factor of the activities of the polyoxins and nikkomycins.⁷ While these represent ideal monomeric precursors to dimeric inhibitors of CS, dimerization of these compounds is precluded by their lack of availability (due to price and/or synthetic complexity).^{3,8} As an alternative, we have constructed dimeric UDP analogs via the union of uridine and tartaric acid fragments (Chart 3). It was anticipated that the tartrate moiety would provide additional polar interactions similar to the UDP-GlcNAc pyrophosphate (as well as the polyoxamic acid side chain).⁹ Uridine fragments were therefore dimerized through tartrate amide linkages (Chart 4) and evaluated for enhanced chitin synthase inhibition.

Initially, two uridine–tartrate amides (**7**, **8**) were chosen as monomeric substrate analogs (Schemes 1 and 2). The monomers were prepared by the condensation of a protected 5'-aminouridine fragment (**18**) with a protected tartrate monoester. Amine **18** was prepared from the corresponding azido diol (**15**) by sequential TES protection, Boc protection of the uracil imide and hydrogenolysis of the azide.^{10,11} Tartrate acids **19** and **20** were prepared by the mono-saponification of the acetonide protected (*R,R*)- and (*S,S*)-diethyl tartrates.¹² Condensation of amine **18** with acid **19** or **20** provided esters **21** and **22** (Scheme 2).¹³ Treatment of **21** and **22** with

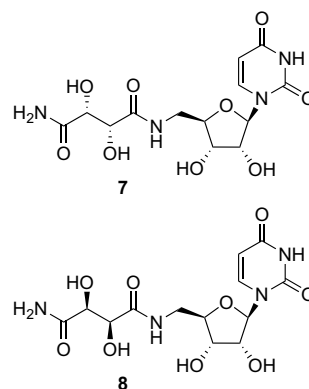


Chart 3. Uridine–tartrate UDP analogs.

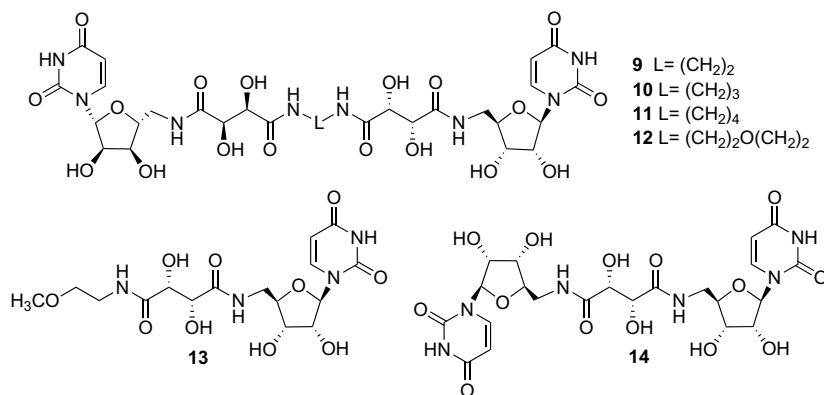
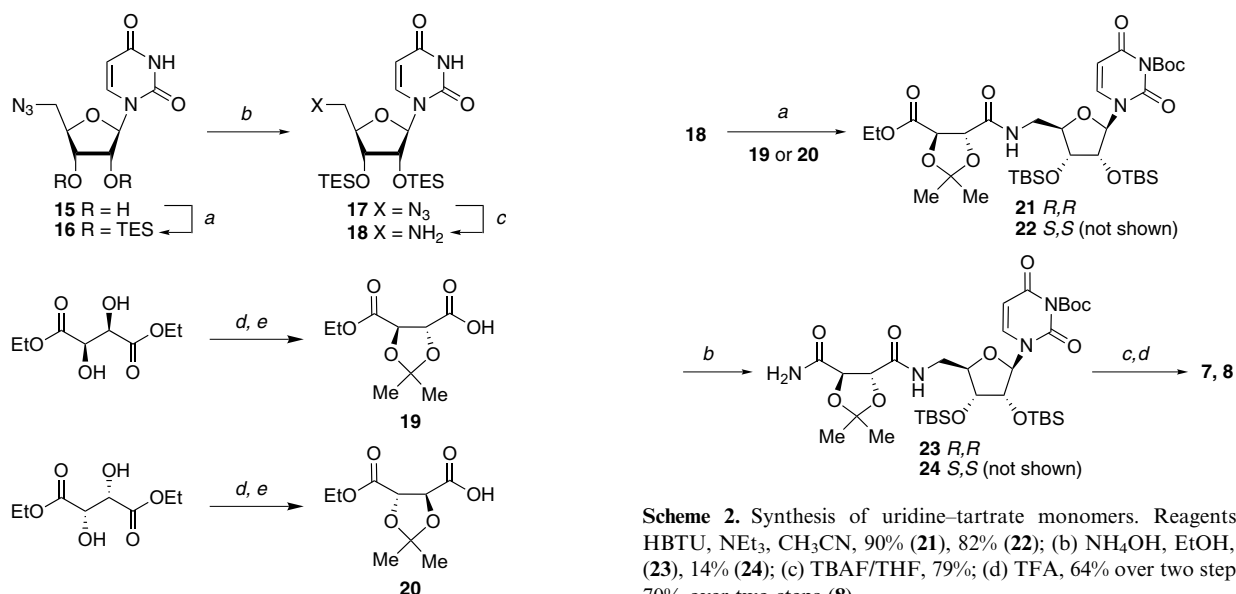


Chart 4. Uridine–tartrate dimers, linked by diamines.



Scheme 2. Synthesis of uridine–tartrate monomers. Reagents: (a) HBTU, NEt₃, CH₃CN, 90% (**21**), 82% (**22**); (b) NH₄OH, EtOH, 58% (**23**), 14% (**24**); (c) TBAF/THF, 79%; (d) TFA, 64% over two steps (**7**), 70% over two steps (**8**).

Scheme 1. Synthesis of uridine and tartrate intermediates. Reagents: (a) TBSCl, imidazole, pyridine, 95%; (b) Boc₂O, DMAP, pyridine, 99%; (c) H₂, Pd/C, MeOH, 94%; (d) dimethoxy-propane, *p*-TsOH, acetone, 99%; (e) KOH, ETOH, 30–50%.

ammonia resulted in the free amide compounds **23** and **24**. Subsequent removal of TBS, Boc, and acetonide groups provided amides **7** and **8**.

Uridine–tartrate amide compounds **7** and **8** exhibited greater CS inhibition (12% and 20% at 1 mM, respectively), than did uridine glycol monomer **6** (6% at 1 mM). The enhanced inhibition upon addition of the tartrate groups provided incentive for the synthesis of related dimers, as dimerization is expected to amplify the increases inhibition exponentially. (In an ideal case, K_i (dimer) = $[K_i$ (monomer)]².)

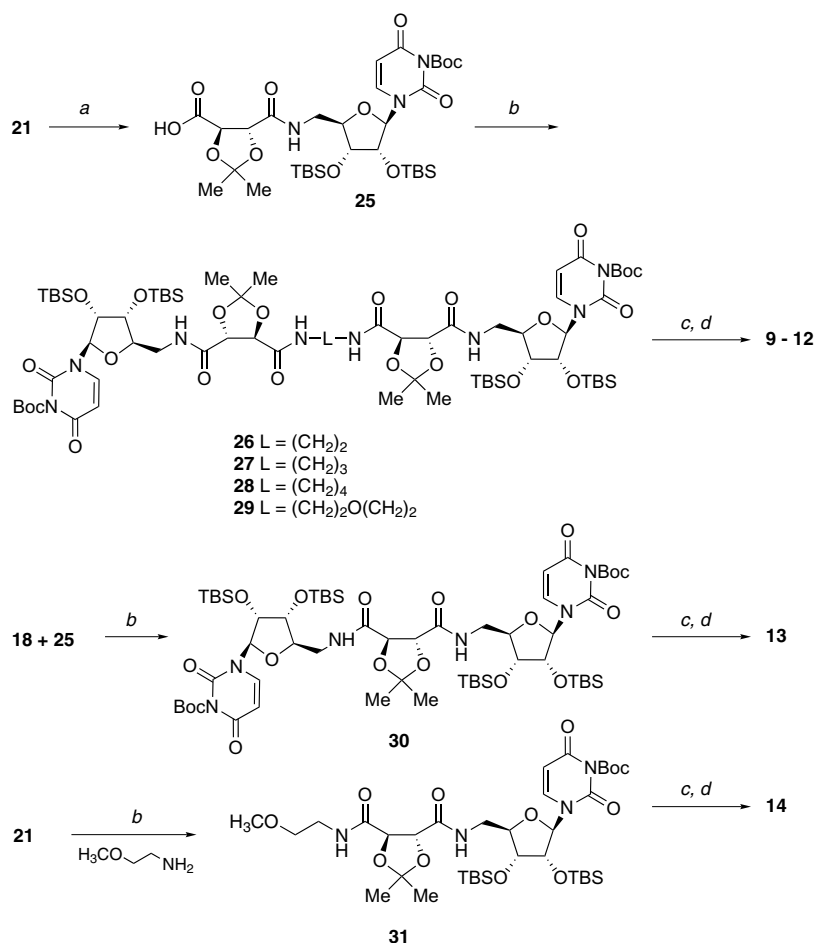
3. Design, and synthesis of uridine–tartrate dimers

Dimeric uridine–tartrate conjugates **9–12** were prepared by coupling **25** with a series of diamine linkers of varying lengths (two to eight atoms; **Chart 3**; **Scheme 3**).¹⁴

Acid **25** was obtained by saponification of ester **21**. Amide bond formations of **25** with commercially available diamines were achieved using HBTU-mediated peptide coupling.¹⁵ The monomeric control compound **14** was prepared by the same method. The shortest dimer (**13**) was created through the coupling of amine **18** with acid **25**. Deprotections of the dimers (and monomer) were performed under the same conditions employed for **7** and **8**, and the final products were purified by both silica and reverse-phase chromatography.¹⁶

4. Evaluation of uridine–tartrate dimers

The uridine–tartrate dimeric compounds were evaluated as inhibitors of chitin synthase (**Fig. 2**). Monomeric control **14** had reduced activity (8%) relative to the uridine–tartaric amide monomer **8** (12%), indicating that addition of the methyl ether served to diminish inhibition. However, dimeric compound **9** is more active than monomer **14**, indicating that there is still a benefit from dimerization. As the length of the diamine linkers



Scheme 3. Synthesis of uridine–tartrate dimers. Reagents: (a) KOH, EtOH, 99%; (b) HBTU, NEt_3 , CH_3CN , 60–80%; (c) TBAF, THF, 90%; (d) TFA, 70–80%.

increases (from **9** to **12**), the inhibitory activities of the compounds decline, becoming essentially non-existent for **11** and **12**. Only the shortest dimer of the series, **13** (uridine–tartrate–uridine), had significant activity toward CS (35%). Not only did dimer **13** have substantially more activity than the monomeric control, it surpassed the uridine–tartrate amides and the longer dimers **9–12** (Fig. 3).

The extended distance between uridine fragments for dimer **13** ($\sim 12\text{\AA}$) is very similar to that of the shortest

uridine carbamate compound (**1**, $\sim 14\text{\AA}$),¹⁷ and they exhibit similar inhibitory activity despite significant differences in the structure of the linker connecting the uridine fragments. While this strengthens the conclusions drawn from previous results, it also indicates that inter-uridine distance is still the primary determinant of inhibitory activity and that the use of tartrate linkers as pyrophosphate analogs provides no benefit.

5. Conclusions

These data reinforce our earlier conclusions regarding the mechanism by which chitin synthase produces chitin: they are consistent with a two site mechanism for CS, and in addition appear to further delineate the distance between two uridine binding sites. While it is unfortunate that the incorporation of tartrate fragments does not significantly enhance the affinity of uridine dimers for CS, this is in keeping with previous findings that the success of tartrate as a pyrophosphate mimic is very case dependent. With regard to the design of future inhibitors, it appears that there is no need to further explore ‘long’ dimers. Instead, work will focus on more rigid dimers,¹⁸ and on identifying linkers that make a positive contribution to the binding affinity.

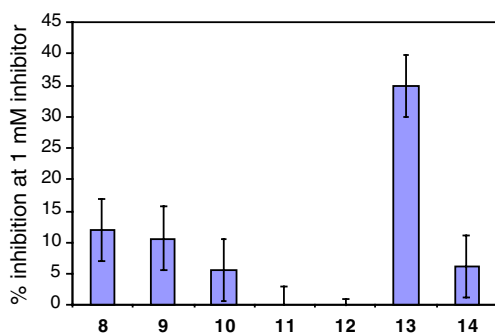


Figure 3. Inhibition of CS by **8–14**.

6. Experimental

6.1. General

All reactions were carried out in oven or flame dried glassware, under an atmosphere of nitrogen, except where noted. THF and CH_2Cl_2 were dried by passage through an activated column of alumina, and pyridine and acetonitrile were distilled from CaH_2 . All amines used in amide bond forming reactions were dried over P_2O_5 in vacuo. All other reagents were used as obtained, unless otherwise stated. Thin layer chromatography was performed on silica gel 60 (F_{254} , 250nm, EM Science) plates and visualized with UV light or stained with KMnO_4 , ninhydrin, or PMA. Flash column chromatography was performed using silica gel (Selecto Scientific, 32–63nm) or reverse phase (EM Science, silica gel 60, RP-18) as indicated. IR spectra were recorded using a Nicolet 550 spectrometer. ^1H NMR data were acquired on a Varian Mercury-400 (400MHz) spectrometer and are reported in parts per million relative to the solvent (CHCl_3 at 7.26ppm, CHD_2OH at 3.30ppm, D_2O at 4.67ppm). Proton decoupled ^{13}C NMR spectra were obtained on a Varian Mercury-400 (100MHz) spectrometer and are reported in parts per million relative to solvent as internal standard (CDCl_3 at 77.0ppm, CD_3OH at 49.0ppm, added CH_3OH at 49.5ppm for D_2O). High resolution mass spectra were obtained on an Ionspec Ultima FTMS (MALDI-FTMS) at The Scripps Research Institute, La Jolla, CA, or the Pasarow Mass Spectrometry Laboratory at the University of California, Los Angeles. Chitin synthase activity assays were performed as previously described.^{5,19}

6.2. Experimental procedures

6.2.1. Fully deprotected uridine tartrate amide 7. The fully protected amide **23** (0.08g, 0.06mmol) was dissolved in THF (1mL) and TBAF (0.13mL of 1M solution in THF, 0.13mmol) was added. After 40min, the reaction was concentrated and purified by silica gel chromatography (5–10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to provide the diol intermediate (0.02g, 79%) as a white solid. ^1H NMR (400MHz, CD_3OD): δ 1.46 (s, 3H), 1.47 (s, 3H), 3.59 (m, 2H), 4.03 (m, 2H), 4.23 (m, 1H), 4.59 (s, 2H), 5.76 (d, 1H, $J = 2.8\text{Hz}$), 5.81 (d, 1H, $J = 5.3\text{Hz}$), 7.75 (d, 1H, $J = 5.3\text{Hz}$). ^{13}C NMR (100MHz, CD_3OD): δ 26.5, 27.7, 41.9, 72.3, 74.5, 78.7, 79.1, 83.8, 87.8, 92.2, 95.6, 102.3, 113.9, 142.8, 148.8, 149.8, 162.1, 172.1, 174.6. FTIR (KBr), cm^{-1} : 3362 (br), 2995 (s), 2934 (s), 1789 (s), 1676 (s), 1536 (s), 1457 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{21}\text{H}_{30}\text{N}_4\text{O}_{11}\text{Na}$ (MNa^+): 537.1828, found 537.1809. TLC (10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$), R_f : 0.56. The resulting diol (0.03g, 0.05mmol) was dissolved in TFA (0.5mL), stirred for 8h, and concentrated. The resulting oil was purified by silica gel chromatography (15% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$) to provide **7** (0.01g, 79%) as a white solid. ^1H NMR (400MHz, D_2O): δ 3.47 (dd, 1H, $J = 2.0, 9.3\text{Hz}$), 3.54 (dd, 1H, $J = 2.8, 8.0\text{Hz}$), 3.99 (d, 2H, $J = 1.5\text{Hz}$), 4.21 (t, 1H, $J = 3.0\text{Hz}$), 4.41 (d, 1H, $J = 0.8\text{Hz}$), 4.43 (d, 1H, $J = 1.3\text{Hz}$), 5.68 (d, 1H, $J = 2.8\text{Hz}$), 5.74 (d, 1H, $J = 5\text{Hz}$), 7.54 (d, 1H, $J = 5\text{Hz}$). ^{13}C NMR (100MHz, D_2O):

δ 40.4, 70.4, 72.3, 72.4, 73.1, 81.9, 90.1, 102.4, 142.0, 152.0, 166.8, 174.1, 176.8. FTIR (KBr), cm^{-1} : 3375 (br), 1688 (s), 1548 (s), 1474 (s).

6.2.2. Fully deprotected uridine tartrate amide 8. Fully protected amide **24** (0.08g, 0.10mmol) was dissolved in THF (1mL) TBAF (0.22mL of 1M solution in THF, 0.22mmol) was added. After 20min, the reaction was concentrated and purified by silica gel chromatography (5–10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to provide the intermediate diol (0.04g, 76%) as a white solid. ^1H NMR (400MHz, CD_3OD): δ 1.48 (s, 6H), 1.57 (s, 9H), 3.56 (dd, 1H, $J = 2.5, 10\text{Hz}$), 3.64 (dd, 1H, $J = 3.3, 7.5\text{Hz}$), 4.04 (m, 2H), 4.25 (t, 1H, $J = 3.1\text{Hz}$), 4.59 (m, 2H), 5.77 (d, 1H, $J = 3\text{Hz}$), 5.82 (d, 1H, $J = 5\text{Hz}$), 7.75 (d, 1H, $J = 5\text{Hz}$). ^{13}C NMR (100MHz, CD_3OD): δ 26.6, 27.7, 41.7, 72.3, 74.4, 78.9, 79.1, 83.7, 87.8, 92.2, 102.4, 113.9, 142.9, 148.8, 149.8, 162.1, 172.0, 174.6. FTIR (KBr), cm^{-1} : 3356 (br), 2995 (s), 2944 (s), 1789 (s), 1676 (s), 1557 (s), 1458 (s), 1384 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{21}\text{H}_{30}\text{N}_4\text{O}_{11}\text{Na}$ (MNa^+): 537.1818, found 537.1808. TLC (15% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$), R_f : 0.51. The resulting diol (0.04g, 0.05mmol) was dissolved in TFA (0.5mL), stirred for 8h, and concentrated. The resulting oil was purified by silica gel chromatography (8% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$) to provide **8** (0.02g, 96%) as a white solid. ^1H NMR (400MHz, D_2O): δ 3.49 (s, 2H), 3.98 (s, 2H), 4.20 (s, 1H), 4.40 (s, 1H), 4.42 (s, 1H), 5.65 (d, 1H, $J = 4.4\text{Hz}$), 5.72 (d, 1H, $J = 8.0\text{Hz}$), 7.55 (d, 1H, $J = 6.8\text{Hz}$). ^{13}C NMR (100MHz, D_2O): δ 41.2, 71.1, 72.9, 72.9, 73.7, 82.6, 90.8, 102.8, 142.6, 151.9, 166.5, 174.5, 177.3. FTIR (KBr), cm^{-1} : 3388 (br), 1684 (s), 1553 (s), 1492 (s), 1422 (s), 1387 (s), 1265 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{14}\text{H}_{18}\text{N}_4\text{O}_9$ (MH^+): 375.1146, found 375.1148. TLC (20% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$), R_f : 0.35.

6.2.3. General procedure for deprotection of compounds 26–31 to provide 9–14. The dimers were dissolved in THF and TBAF (1M in THF, 1.1 equiv per TBS group) was added. After approximately 90min, the reactions were concentrated and purified by silica gel chromatography (8% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$) to provide an intermediate diol, which was then stirred in neat TFA for 24h. The sample was then concentrated and purified by silica gel chromatography (12% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$) followed by reverse-phase chromatography (to provide white solids) prior to use in CS assays.

6.2.4. Dimer 9. White solid, 0.05g, 43% over two steps. ^1H NMR (400MHz, D_2O): δ 3.30 (s, 4H), 3.52 (q, 4H, $J = 3, 12.6\text{Hz}$), 3.99 (m, 4H), 4.20 (m, 2H), 4.42 (m, 4H), 5.66 (d, 2H, $J = 2.8\text{Hz}$), 5.74 (d, 2H, $J = 5.0\text{Hz}$), 7.54 (d, 2H, $J = 5\text{Hz}$). ^{13}C NMR (100MHz, D_2O): δ 39.2, 40.9, 70.9, 72.9, 73.0, 73.6, 82.5, 90.7, 102.9, 142.6, 151.8, 166.4, 174.4, 174.5. FTIR (KBr), cm^{-1} : 3449 (br), 1649 (s), 1544 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{28}\text{H}_{38}\text{N}_8\text{O}_{18}\text{Na}$ (MNa^+) 797.2196, found 797.2213. TLC (20% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$), R_f : 0.34.

6.2.5. Dimer 10. White solid, 0.06g, 37% over two steps. ^1H NMR (400MHz, D_2O): δ 1.63 (s, 2H), 3.19 (m, 4H), 3.52 (m, 4H), 4.01 (m, 4H), 4.23 (m, 2H), 4.43 (s, 2H),

4.45 (s, 2H), 4.50 (s, 4H), 5.69 (d, 2H, $J = 2.8$ Hz), 5.77 (d, 2H, $J = 5.0$ Hz), 7.57 (d, 2H, $J = 5.0$ Hz). ^{13}C NMR (100 MHz, D_2O): δ 28.9, 36.9, 39.2, 40.9, 70.9, 73.0, 73.1, 73.6, 82.6, 90.6, 102.9, 142.7, 152.2, 166.8, 174.2, 174.7. FTIR (KBr), cm^{-1} : 3445 (br), 1676 (s), 1562 (s), 1475 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{29}\text{H}_{40}\text{N}_8\text{O}_{18}\text{Na}$ (MNa^+) 811.2353, found 811.2387. TLC (20% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$), R_f : 0.40.

6.2.6. Dimer 11. White solid, 0.06 g, 30% over two steps. ^1H NMR (400 MHz, D_2O): δ 1.40 (s, 4H), 3.12 (m, 4H), 3.48 (m, 4H), 3.97 (m, 4H), 4.19 (t, 2H, $J = 2.8$ Hz), 4.38 (s, 2H), 4.40 (s, 2H), 5.65 (d, 2H, $J = 2.8$ Hz), 5.73 (d, 2H, $J = 5.0$ Hz), 7.54 (d, 2H, $J = 5.0$ Hz). ^{13}C NMR (100 MHz, D_2O): δ 26.6, 39.4, 40.9, 70.9, 73.0, 73.1, 73.6, 82.6, 90.6, 102.9, 142.7, 151.9, 166.4, 174.0, 174.7. FTIR (KBr), cm^{-1} : 3371 (br), 1667 (s), 1553 (s), 1475 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{30}\text{H}_{43}\text{N}_8\text{O}_{18}$ (MH^+) 803.2690, found 803.2693. TLC (18% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$), R_f : 0.13.

6.2.7. Dimer 12. White solid, 0.08 g, 38% over two steps. Dimer 12 was purified by reverse-phase silica gel chromatography (0–10% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$) before use in chitin synthase assays. ^1H NMR (400 MHz, D_2O): δ 3.30 (s, 4H), 3.48 (m, 8H), 3.97 (m, 4H), 4.19 (m, 2H), 4.41 (s, 4H), 5.66 (d, 2H, $J = 2.8$ Hz), 5.73 (d, 2H, $J = 5.0$ Hz), 7.54 (d, 2H, $J = 5.0$ Hz). ^{13}C NMR (100 MHz, D_2O): δ 39.6, 41.1, 69.4, 71.1, 73.1, 73.7, 82.7, 90.7, 102.9, 142.7, 151.9, 166.4, 174.3, 174.7. FTIR (film), cm^{-1} : 3365 (br), 1681 (s), 1541 (s), 1475 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{30}\text{H}_{42}\text{N}_8\text{O}_{19}\text{Na}$ (MNa^+) 841.2458, found 841.2466. TLC (20% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$), R_f : 0.38.

6.2.8. Dimer 13. White solid, 0.17 g, 61% over two steps. ^1H NMR (400 MHz, D_2O): δ 3.39 (dd, 2H, $J = 2.3, 8.3$ Hz), 3.52 (dd, 2H, $J = 3.0, 6.5$ Hz), 3.93 (m, 4H), 4.13 (t, 2H, $J = 3.0$ Hz), 4.41 (s, 2H), 5.62 (d, 1H, $J = 2.3$ Hz), 5.66 (d, 1H, $J = 5.0$ Hz), 7.47 (d, 1H, $J = 5.0$ Hz). ^{13}C NMR (100 MHz, D_2O): δ 40.7, 70.9, 73.1, 73.6, 82.5, 90.5, 102.8, 142.6, 151.7, 166.7, 174.5. FTIR (KBr), cm^{-1} : 3441 (br), 1702 (s), 1553 (s), 1466 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{22}\text{H}_{28}\text{N}_6\text{O}_{14}\text{Na}$ (MNa^+) 623.1566, found 623.1542.

6.2.9. Monomer 14. White solid, 0.23 g, 61% over two steps. ^1H NMR (400 MHz, D_2O): δ 3.24 (s, 3H), 3.42 (m, 6H), 3.99 (m, 2H), 4.20 (m, 1H), 4.42 (s, 1H), 4.44 (s, 1H), 5.67 (d, 1H, $J = 1.5$ Hz), 5.74 (d, 1H, $J = 5.0$ Hz), 7.55 (d, 1H, $J = 5.0$ Hz). ^{13}C NMR (100 MHz, D_2O): δ 39.3, 40.9, 58.6, 70.9, 71.0, 73.0, 73.1, 73.6, 82.6, 90.6, 102.9, 142.7, 151.9, 166.4, 174.3, 174.6. FTIR (KBr), cm^{-1} : 3353 (br), 1676 (s), 1545 (s), 1475 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_{10}$ (MNa^+) 433.1565, found 433.1576. TLC (20% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$), R_f : 0.38.

6.2.10. TBS protected azidouridine 16. Compound 15 (1.13 g, 4.2 mmol)⁵ was dried by co-evaporation with THF, dissolved in dry pyridine (5 mL) under N_2 , and imidazole (1.70 g, 25.2 mmol) was added. After cooling to 0 °C, TBSCl (3.80 g, 25.2 mmol) was added, and the

reaction allowed to stir for 16 h, concentrated, and purified by silica gel chromatography (40% EtOAc/hexanes) to afford 16 (1.97 g, 95%), as a white foam. ^1H NMR (400 MHz, CDCl_3): δ 0.10 (m, 12H), 0.89 (s, 9H), 0.90 (s, 9H), 3.61 (dd, 1H, $J = 2, 8.3$ Hz), 3.82 (dd, 1H, $J = 2, 8.5$ Hz), 3.96 (m, 1H), 4.13 (m, 1H), 4.21 (t, 1H, $J = 2$ Hz), 5.65 (d, 1H, $J = 1.8$ Hz), 5.77 (d, 1H, 5Hz), 7.70 (d, 1H, $J = 5$ Hz), 9.70 (s, 1H). ^{13}C NMR (100 MHz, CDCl_3): δ -4.8, -4.7, -4.3, -4.1, 18.1, 18.1, 25.8, 51.0, 71.0, 75.0, 81.2, 91.1, 102.1, 140.1, 150.0, 163.3. FTIR (film) cm^{-1} : 2855–3187 (br), 2121 (s), 1693 (br), 1474 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{21}\text{H}_{39}\text{N}_5\text{O}_5\text{Si}_2\text{Na}$ ($\text{M}+\text{Na}^+$) 520.2388, found 520.2409. TLC (40% EtOAc/hexanes), R_f : 0.40.

6.2.11. Fully protected azidouridine 17. The TBS-protected azide 16 (8.05 g, 16.2 mmol) was dried by co-evaporation with tetrahydrofuran and dissolved in dry pyridine (20 mL) under N_2 . DMAP (0.40 g, 3.24 mmol), the mixture was cooled to 0 °C, and Boc_2O (10.6 g, 48.6 mmol) was added. The reaction was allowed to stir for 16 h at room temperature, concentrated, and purified by silica gel chromatography (35–40% EtOAc/hexanes) to provide 17 (9.60 g, 99%) as a white foam. ^1H NMR (400 MHz, CDCl_3): δ 0.08 (m, 12H), 0.88 (s, 9H), 0.90 (s, 9H), 1.59 (s, 9H), 3.58 (dd, 1H, $J = 2.2, 8.3$ Hz), 3.75 (dd, 1H, $J = 2.3, 7.5$ Hz), 3.95 (m, 1H), 4.11 (m, 1H), 4.17 (m, 1H), 5.72 (d, 1H, $J = 2.3$ Hz), 5.78 (d, 1H, 5Hz), 7.62 (d, 1H, 5.2Hz). ^{13}C NMR (100 MHz, CDCl_3): δ -4.8, -4.7, -4.6, -4.2, 18.0, 18.0, 25.8, 27.4, 51.3, 71.4, 74.8, 82.0, 86.5, 90.2, 101.8, 139.1, 147.3, 147.9, 160. FTIR (film), cm^{-1} : 3091 (s), 2942 (s), 2855 (s), 2121 (s), 1789 (s), 1728 (s), 1693 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{26}\text{H}_{47}\text{N}_5\text{O}_7\text{Si}_2\text{Na}$ (MNa^+) 620.2883, found 620.2912. TLC (40% EtOAc/hexanes), R_f : 0.67.

6.2.12. Fully protected aminouridine 18. The fully protected azide 17 (1.99 g, 3.33 mmol) was dissolved in MeOH (10 mL) and 10% Pd/C (0.08 g) was added. The solution was flushed with H_2 (3x) and allowed to stir under H_2 for 4 h. The reaction was then concentrated and purified by silica gel chromatography (8–15% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$) to afford 18 (1.70 g, 94%) as a white solid. Extended reaction times provided unwanted by-products resulting from Boc migration from the uracil ring to the primary amine. ^1H NMR (400 MHz, CD_3OD): δ 0.07 (m, 12H), 0.88 (s, 9H), 0.90 (s, 9H), 1.60 (s, 9H), 2.89 (dd, 1H, $J = 3.3, 7.5$ Hz), 3.05 (dd, 1H, $J = 2.0, 7.5$ Hz), 3.93 (m, 1H), 4.02 (m, 1H), 4.23 (t, 1H, $J = 2.7$ Hz), 5.75 (m, 2H), 7.811 (d, 1H, $J = 5.3$ Hz). ^{13}C NMR (100 MHz, CD_3OD): δ -4.7, -4.5, -4.5, -4.3, 18.0, 18.1, 25.8, 26.0, 28.0, 42.1, 72.2, 73.8, 84.2, 86.8, 92.0, 101.8, 141.2, 147.3, 148.2, 160.0. FTIR (KBr), cm^{-1} : 2935 (s), 2861 (s), 1797 (s), 1731 (s), 1681 (s), 1458 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{26}\text{H}_{50}\text{N}_3\text{O}_7\text{Si}_2\text{Na}$ (MH^+) 572.3182, found 572.3174. TLC (15% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$), R_f : 0.49.

6.2.13. L-(–)-Diethyltartrate acetonide. L-(+)-Diethyltartrate (30.40 g, 147.60 mmol) was dissolved in dry benzene (80 mL). Dimethoxypropane (145.00 mL, 1.18 mol) and *p*-TsOH (2.79 g, 14.80 mmol) were added and the

reaction was heated to reflux (90°C) with a Dean–Stark apparatus. After 1.5h removal of CH₃OH/C₆H₆ (21 mL) was complete and the reaction was concentrated. The resulting oily solid was dissolved in Et₂O (500 mL) and washed with H₂O (1 × 400 mL), NaHCO₃ (1 × 400 mL), and brine (1 × 400 mL), then dried (Na₂SO₄), and concentrated to yield the acetonide of L-(–)-diethyltartrate. (36.00 g, 99%) as a thick brown liquid, pure by ¹H NMR. ¹H NMR (400 MHz, CDCl₃): δ 1.30 (t, 3H, J = 6.8 Hz), 1.47 (s, 6H), 4.27 (m, 2H), 4.77 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 14.1, 26.3, 61.7, 113.5, 169.3. FTIR (film), cm^{–1}: 2991 (s), 2951 (s), 1765 (s), 1469 (s), 1381 (s), 1221 (br), 1108 (s), 1036 (s), 852 (s). HRMS (MALDI-FTMS), *m/z*: calcd for C₁₁H₁₈O₆Na (MNa)⁺ 269.0996, found 269.0992. TLC (10% MeOH/CH₂Cl₂), *R*_f: 0.9.

6.2.14. Tartrate monoester 19. KOH (2.50 g, 44.6 mmol) was dissolved in EtOH (70 mL) and added dropwise (30 min) to a solution of diester intermediate **19** (10.98 g, 44.6 mmol) in EtOH (70 mL). After 2 h, it was diluted with Et₂O (800 mL), and extracted with H₂O (2 × 400 mL). The aqueous layer was washed with Et₂O (3 × 500 mL) to provide unreacted starting material. The aqueous layer was acidified with 2 M HCl, extracted with Et₂O (3 × 400 mL), dried (Na₂SO₄), and concentrated to yield crude monoester **19** (5.10 g, 50%) as a thick black liquid, pure by ¹H NMR. ¹H NMR (400 MHz, CDCl₃): δ 1.32 (t, 3H, J = 6.8 Hz), 1.49 (s, 3H), 1.51 (s, 3H), 4.29 (q, 2H, J = 7.2, 14.4 Hz), 4.79 (d, 1H, J = 5.6 Hz), 4.86 (d, 1H, J = 5.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 14.2, 26.4, 26.4, 62.2, 76.5, 77.1, 114.0, 169.4, 173.7. FTIR (film), cm^{–1}: 3519 (br), 3004 (s), 2951 (s), 1746 (s), 1641 (s), 1396 (s), 1238 (br), 1125 (s). HRMS (MALDI-FTMS), *m/z*: C₉H₁₈N₁O₆ (MNH₄)⁺ 236.1136, found 236.1134. TLC (10% MeOH/CH₂Cl₂), *R*_f: 0.10.

6.2.15. D-(–)-Diethyltartrate acetonide. D-(–)-Diethyltartrate (30.40 g, 147.60 mmol) was dissolved in dry benzene (80 mL). Dimethoxypropane (145.00 mL, 1.18 mol) and *p*-TsOH (2.79 g, 14.80 mmol) were added and the reaction was heated to reflux (90°C) in a flask equipped with a Dean–Stark apparatus. After 1.5h removal of CH₃OH/C₆H₆ (21 mL) was complete and the reaction was concentrated. The resulting oily solid was dissolved in Et₂O (1 × 500 mL) and washed with H₂O (1 × 400 mL), NaHCO₃ (1 × 400 mL), and brine (1 × 400 mL), dried (Na₂SO₄), and concentrated to yield the acetonide of D-diethyl tartrate (36.00 g, 99%) as a thick brown liquid, pure by ¹H NMR. ¹H NMR (400 MHz, CDCl₃): δ 1.33 (t, 6H, J = 7.2 Hz), 1.51 (s, 6H), 4.29 (q, 4H, J = 7.6, 14.2 Hz), 4.78 (s, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 14.1, 26.4, 61.8, 113.5, 169.3. FTIR (film), cm^{–1}: 2959 (br), 2871 (s), 1757 (s), 1669 (s), 1461 (s), 1381 (s), 1213 (s), 1117 (s). HRMS (MALDI-FTMS), *m/z*: calcd for C₁₁H₁₈O₆Na (MNa)⁺, 269.0996, found 269.0998. TLC (40% EtOAc/hexanes), *R*_f: 0.80.]

6.2.16. Tartrate monoester 20. KOH (2.50 g, 44.6 mmol) was dissolved in EtOH (70 mL) and added dropwise (30 min) to a solution of diester intermediate of **20**

(10.98 g, 44.6 mmol) in EtOH (70 mL). After 2 h, it was diluted with Et₂O (800 mL), and extracted with H₂O (2 × 400 mL). The aqueous layer was washed with Et₂O (3 × 500 mL) to provide unreacted starting material. The aqueous layer was acidified with 2 M HCl, extracted with Et₂O (3 × 400 mL), dried (Na₂SO₄), and concentrated to yield monoester **20** (5.10 g, 50%) as a thick black liquid, pure by ¹H NMR. ¹H NMR (400 MHz, CDCl₃): δ 1.33 (t, 3H, J = 7.2 Hz), 1.50 (s, 3H), 1.52 (s, 3H), 4.29 (q, 2H, J = 7.2, 14.4 Hz), 4.79 (d, 1H, J = 5.6 Hz), 4.87 (d, 1H, J = 5.2 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 14.2, 26.4, 26.4, 62.2, 114.1, 169.3, 174.2. FTIR (film) cm^{–1}: 3431 (br), 1741 (s), 1645 (s), 1389 (s), 1213 (s), 1108 (s). HRMS (MALDI-FTMS), *m/z*: calcd for C₉H₁₄O₆Na (MNa)⁺ 241.0688, found 241.0690. TLC (10% MeOH/CH₂Cl₂), *R*_f: 0.10.

6.2.17. Fully protected uridine tartrate monoester 21. The acid **19** and amine **18** were each dried by co-evaporation with THF. The acid (0.78 g, 3.38 mmol) was dissolved in dry CH₃CN (12 mL) under N₂. HBTU (1.28 g, 3.38 mmol), the amine (1.61 g, 2.80 mmol), and NEt₃ (0.48 mL, 3.38 mmol) were added. The reaction was stirred for 16 h, concentrated, and then partitioned between EtOAc (400 mL) and brine (400 mL). The organic layer was washed with NaHCO₃ (1 × 400 mL) and H₂O (1 × 400 mL), then dried (Na₂SO₄), and concentrated. The resulting solid was purified by silica gel chromatography (3% MeOH/CH₂Cl₂) to yield **21** (1.83 g, 90%) as a white foam. ¹H NMR (400 MHz, CDCl₃): δ 0.04 (m, 12H), 0.85 (s, 9H), 0.89 (s, 9H), 1.3 (t, 3H, J = 7.7, 7.7 Hz), 1.46 (s, 3H), 1.47 (s, 3H), 1.57 (s, 9H), 3.53 (m, 2H), 3.87 (t, 1H, J = 3.3, 4.3 Hz), 4.06 (m, 1H), 4.27 (q, 2H, J = 8, 17 Hz), 4.49 (t, 1H, J = 5.7 Hz), 4.70 (q, 2H, J = 6.2, 17 Hz), 5.49 (d, 1H, J = 6.7 Hz), 5.76 (d, 1H, J = 9 Hz), 7.01 (t, 1H, J = 6.7 Hz), 7.29 (d, 1H, J = 9 Hz). ¹³C NMR (100 MHz, CDCl₃): δ –4.6, –4.3, –4.1, 18.9, 19.0, 26.4, 26.5, 26.5, 26.5, 26.8, 27.7, 42.3, 53.2, 68.8, 74.4, 75.2, 78.5, 79.3, 86.0, 87.7, 90.6, 102.7, 114.5, 142.9, 148.6, 149.8, 171.6, 171.8. FTIR (film) cm^{–1}: 3380 (br), 2943 (s), 2847 (s), 1798 (s), 1728 (s), 1693 (s), 1536 (s), 1449 (s), 1379 (s), 1256 (s). HRMS (MALDI-FTMS), *m/z*: calcd for C₃₀H₅₃N₃O₁₀Si₂Na (MNa⁺–Boc) 694.3167, found 694.3143. TLC (10% MeOH/CH₂Cl₂), *R*_f: 0.87.

6.2.18. Fully protected uridine tartrate monoester 22. Acid **20** and amine **18** were each dried by co-evaporation with THF. The acid (1.20 g, 5.40 mmol) was dissolved in dry CH₃CN (15 mL) under N₂. HBTU (2.20 g, 5.88 mmol), the amine (2.82 g, 4.90 mmol), and NEt₃ (0.82 mL, 5.88 mmol) were added. The reaction was stirred for 15 h, concentrated, and partitioned between EtOAc (400 mL) and brine (400 mL). The organic layer was washed with NaHCO₃ (1 × 400 mL) and H₂O (1 × 400 mL), then dried (Na₂SO₄), and concentrated. The resulting solid was purified by silica gel chromatography (35–40% EtOAc/hexanes) to yield **22** (3.05 g, 82%) as a white foam. ¹H NMR (400 MHz, CDCl₃): δ –0.03 (s, 3H), 0.00 (s, 3H), 0.05 (s, 6H), 0.82 (s, 6H), 0.86 (s, 6H), 1.27 (t, 3H, J = 4.5 Hz), 1.43 (s, 3H), 1.44 (s, 3H), 1.54 (s, 9H), 3.88 (q, 1H, J = 1.5, 2.5 Hz), 4.04 (t, 1H, J = 2 Hz), 4.23 (q, 2H, J = 4.4, 8.9 Hz), 4.4 (t, 1H,

$J = 3.4$ Hz), 4.64 (d, 1H, $J = 3.8$ Hz), 4.70 (d, 1H, $J = 3.5$ Hz), 5.48 (d, 1H, $J = 3.8$ Hz), 5.72 (d, 1H, $J = 5$ Hz), 7.08 (t, 1H, $J = 3.8$ Hz), 7.28 (d, 1H, $J = 5$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ -4.9, -4.7, -4.6, -4.4, 14.1, 17.9, 18.0, 25.7, 25.8, 26.2, 26.6, 27.4, 40.9, 61.8, 73.0, 77.5, 77.7, 84.0, 86.6, 92.9, 101.9, 113.2, 141.2, 147.1, 147.9, 159.9, 169.6, 169.6. FTIR (film) cm^{-1} : 3406 (br), 2493 (s), 2873 (s), 1728 (s), 1693 (s), 1632 (s), 1527 (s), 1387 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{30}\text{H}_{53}\text{N}_3\text{O}_{10}\text{Si}_2\text{Na}$ ($\text{MNa}^+ - \text{Boc}$) 694.3162, found 694.3155. TLC (15% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$), R_f : 0.91.

6.2.19. Uridine tartrate amide 23. Compound **21** (0.20 g, 0.26 mmol) was dissolved in EtOH (1 mL). Ammonium hydroxide (0.13 mL of a 37% solution, 1.32 mmol) was added, and the reaction was allowed to stir 30 h then concentrated. Purification by silica gel chromatography (3% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) provided amide **23** (0.11 g, 58%) as a white solid. ^1H NMR (400 MHz, CD_3OD): δ -0.01 (s, 3H), 0.08 (s, 3H), 0.12 (s, 3H), 0.13 (s, 3H), 0.88 (s, 9H), 0.94 (s, 9H), 1.47 (s, 6H), 1.56 (s, 9H), 3.53 (m, 2H), 4.086 (m, 1H), 4.13 (m, 1H), 4.34 (m, 1H), 4.58 (q, 2H, $J = 4.0$, 3.8 Hz), 5.86 (m, 2H), 7.80 (d, 1H, $J = 5.3$ Hz). ^{13}C NMR (100 MHz, CD_3OD): δ -4.6, -4.3, -4.1, 18.9, 19.0, 26.4, 26.5, 26.5, 26.6, 27.8, 42.3, 74.4, 75.2, 78.8, 79.1, 86.1, 87.7, 90.5, 102.7, 113.9, 142.8, 148.6, 149.8, 161.8, 172.1, 174.5. FTIR (film), cm^{-1} : 3353 (br), 2934 (s), 2864 (s), 1789 (s), 1693 (s), 1527 (s), 1457 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{33}\text{H}_{58}\text{N}_4\text{O}_{11}\text{Si}_2\text{Na}$ (MNa^+) 765.3543, found 765.3538. TLC (10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$), R_f : 0.56.

6.2.20. Uridine tartrate amide 24. Compound **22** (0.62 g, 0.80 mmol) was dissolved in EtOH (1 mL). Ammonium hydroxide (0.77 mL of a 37% solution, 8.0 mmol) was added, and the reaction was allowed to stir 15 h then concentrated. Purification by silica gel chromatography (3% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) provided amide **24** (0.08 g, 14%) as a white solid. ^1H NMR (400 MHz, CD_3OD): δ -0.01 (s, 3H), 0.07 (s, 3H), 0.12 (s, 3H), 0.14 (s, 3H), 0.87 (s, 9H), 0.94 (s, 9H), 1.47 (s, 3H), 1.48 (s, 3H), 1.56 (s, 9H), 3.50 (dd, 1H, $J = 6.2$, 13 Hz), 3.58 (dd, 1H, $J = 7.2$, 16 Hz), 4.08 (t, 1H, $J = 6.2$ Hz), 4.14 (d, 1H, $J = 3.3$ Hz), 4.35 (m, 1H), 4.56 (q, 2H, $J = 6.8$, 14 Hz), 5.85 (m, 2H), 7.80 (d, 1H, $J = 9.3$ Hz). ^{13}C NMR (100 MHz, CD_3OD): δ -4.6, -4.2, -4.1, 18.8, 18.9, 26.5, 26.7, 27.8, 28.7, 42.2, 74.1, 75.1, 78.9, 79.1, 86.0, 87.6, 90.4, 102.7, 113.9, 142.9, 148.6, 149.7, 161.8, 172.0, 174.3. FTIR (KBr), cm^{-1} : 3371 (br), 2943 (s), 2864 (s), 1789 (s), 1684 (s), 1545 (s), 1466 (s). HRMS (MALDI-FTMS), m/z : $\text{C}_{28}\text{H}_{50}\text{N}_4\text{O}_9\text{Si}_2\text{Na}$ ($\text{MNa}^+ - \text{Boc}$) $^+$ 665.3009, found 665.2983. TLC (10% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$), R_f : 0.60.

6.2.21. Fully protected uridine tartrate 25. Compound **21** (0.50 g, 0.65 mmol) was dissolved in EtOH (5 mL) and potassium hydroxide (0.04 g, 0.69 mmol) was added. After 110 min, the reaction was concentrated and purified by silica gel chromatography (8–15% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to provide acid **25** (0.41 g, 85%) as a white foam. ^1H NMR (400 MHz, CD_3OD): δ 0.00 (s, 3H), 0.09 (s, 3H), 0.13 (s, 3H), 0.13 (s, 3H), 0.88 (s, 9H), 0.94 (s, 9H), 1.44 (s, 3H), 1.47 (s, 3H), 1.57 (s, 9H),

3.54 (m, 2H), 4.09 (t, 1H, $J = 4.0$, 4.3 Hz), 4.13 (d, 1H, $J = 2.8$ Hz), 4.34 (m, 1H), 4.53 (d, 1H, $J = 3.8$ Hz), 4.66 (d, 1H, $J = 3.8$ Hz), 5.87 (m, 2H), 7.78 (d, 1H, $J = 5$ Hz), 8.48 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ -0.46, -4.2, -4.1, 18.9, 19.0, 42.3, 68.8, 74.4, 75.2, 79.1, 85.8, 87.7, 90.6, 102.8, 113.6, 142.8, 148.6, 149.7, 161.8, 172.8, 174.7. FTIR (KBr), cm^{-1} : 3398 (Br), 2944 (s), 2861 (s), 1805 (s), 1739 (s), 1681 (s), 1458 (s), 1367 (s), 1268 (s). HRMS (MALDI-FTMS), m/z : Calcd for $\text{C}_{28}\text{H}_{49}\text{N}_3\text{O}_{10}\text{Si}_2\text{Na}$ ($\text{MNa} - \text{Boc}$) $^+$, 666.2854, found 666.2826. TLC (15% $\text{MeOH}/\text{CH}_2\text{Cl}_2$), R_f : 0.30.

6.2.22. General procedure for dimerization of 26–29. Acid **21** was azeotropically dried with THF and dissolved in a minimum amount of dry CH_3CN . HBTU (1.50 equiv) was added and the reactions were allowed to stir for 10 min. The appropriate diamine (0.50 equiv) and NEt_3 (1.00 equiv) were added and the reactions were allowed to stir for 15–24 h. The mixtures were partitioned between EtOAc (400 mL) and brine (400 mL), the organic layers were washed with NaHCO_3 (1 $\times$ 400 mL) and H_2O (1 $\times$ 400 mL), dried (Na_2SO_4), and concentrated. The resulting solids were purified by silica gel chromatography (2–5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to yield white solids.

6.2.23. Dimer 26. White solid, 0.19 g, 63%. ^1H NMR (400 MHz, CDCl_3): δ -0.02 (s, 6H), 0.02 (s, 6H), 0.07 (s, 12H), 0.84 (s, 12H), 0.88 (s, 12H), 1.46 (s, 3H), 1.48 (s, 3H), 1.56 (s, 18H), 3.33 (q, 4H, $J = 3.9$, 7.8 Hz), 3.52 (m, 2H), 3.59 (m, 2H), 3.91 (t, 2H, $J = 2.3$ Hz), 4.06 (t, 2H, $J = 2.5$ Hz), 4.29 (t, 2H, $J = 3.0$ Hz), 5.62 (d, 2H, $J = 3.8$ Hz), 5.77 (d, 2H, $J = 5.0$ Hz), 7.33 (t, 2H, $J = 3.8$ Hz), 7.45 (d, 2H, $J = 5$ Hz), 7.56 (t, 2H, $J = 3.5$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ -4.8, -4.6, -4.5, -4.3, 18.0, 18.1, 25.8, 25.8, 26.1, 26.1, 27.5, 38.6, 41.0, 72.9, 73.8, 83.8, 86.6, 91.5, 102.0, 112.4, 140.8, 147.3, 148.1, 160.1, 169.8, 170.2. FTIR (film), cm^{-1} : 3345 (br), 3109 (w), 2934 (s), 2847 (s), 1789 (s), 1728 (s), 1684 (s), 1545 (s), 1379 (s), 1274 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{30}\text{H}_{53}\text{N}_3\text{O}_{10}\text{Si}_2\text{Na}$ ($\text{MNa}^+ - 2\text{Boc}$) $^+$ 1333.66814, found 1333.6315. TLC (15% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$) R_f : 0.91.

6.2.24. Dimer 27. White solid, 0.30 g, 46%. ^1H NMR (400 MHz, CDCl_3): δ -0.04 (s, 6H), 0.00 (s, 6H), 0.05 (s, 12H), 0.82 (s, 12H), 0.87 (s, 2H), 1.44 (s, 6H), 1.46 (s, 6H), 1.54 (s, 18H), 3.32 (m, 4H), 3.48 (m, 2H), 3.58 (m, 2H), 3.90 (s, 2H), 4.04 (m, 2H), 4.26 (t, 2H, $J = 2.8$ Hz), 5.62 (d, 2H, $J = 3.8$ Hz), 5.75 (d, 2H, $J = 5.1$ Hz), 7.34 (t, 2H, $J = 3.8$ Hz), 7.45 (d, 2H, $J = 5$ Hz), 7.58 (t, 2H, $J = 3.5$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ -4.8, -4.6, -4.5, -4.4, 17.9, 18.0, 25.8, 26.1, 27.4, 29.6, 35.8, 38.6, 41.0, 72.8, 73.8, 77.1, 77.5, 83.8, 86.5, 91.3, 102.0, 112.3, 140.8, 147.2, 148.0, 160.0, 169.5, 170.1. FTIR (film) cm^{-1} : 3353 (br), 2855 (s), 1807 (s), 1728 (s), 1693 (s), 1553 (s), 1457 (s), 1274 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{30}\text{H}_{53}\text{N}_3\text{O}_{10}\text{Si}_2\text{Na}$ ($\text{MNa}^+ - 2\text{Boc}$) $^+$ 1347.6438, found 1347.6403. TLC (15% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$), R_f : 0.92.

6.2.25. Dimer 28. White solid, 0.22 g, 29%. ^1H NMR (400 MHz, CDCl_3): δ 0.00 (s, 6H), 0.04 (s, 6H), 0.09 (s, 12H), 0.86 (s, 12H), 0.90 (s, 12H), 1.49 (s, 12H), 1.58

(s, 18H), 3.28 (m, 4H), 3.37 (m, 4H), 3.53 (m, 4H), 3.63 (m, 4H), 3.91 (s, 2H), 4.08 (m, 2H), 4.28 (m, 2H), 4.52 (s, 4H), 5.66 (d, 2H, $J = 3.8$ Hz), 5.78 (d, 2H, $J = 5.3$ Hz), 6.97 (t, 2H, $J = 3.8$ Hz), 7.48 (d, 2H, $J = 5.3$ Hz), 7.64 (t, 2H, $J = 3.5$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ -4.8, -4.7, -4.5, -4.4, 17.9, 18.0, 25.7, 26.1, 26.9, 27.4, 38.7, 40.9, 72.8, 73.9, 77.0, 77.4, 83.8, 86.5, 91.0, 101.9, 112.2, 140.7, 147.2, 148.0, 160.0, 169.4, 169.7. FTIR (KBr), cm^{-1} : 3362 (br), 2834 (s), 1807 (s), 1728 (s), 1702 (s), 1536 (s), 1492 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{30}\text{H}_{53}\text{N}_3\text{O}_{10}\text{Si}_2\text{Na}$ ($\text{MNa}^+ - 2\text{Boc}$) $^+$ 1361.6594, found 1361.6630. TLC (10% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$), R_f : 0.69.

6.2.26. Dimer 29. White solid, 0.53 g, 62%. ^1H NMR (400 MHz, CDCl_3): δ 0.00 (s, 6H), 0.04 (s, 6H), 0.09 (s, 12H), 0.86 (s, 12H), 0.90 (s, 12H), 1.48 (s, 12H), 1.58 (s, 18H), 3.56 (m, 12H), 3.91 (t, 2H, $J = 5.2$ Hz), 4.08 (m, 2H), 4.30 (t, 2H, $J = 6.3$ Hz), 4.55 (s, 4H), 5.64 (d, 2H, $J = 6.3$ Hz), 5.79 (d, 2H, $J = 9.3$ Hz), 7.20 (t, 2H, $J = 6.0$ Hz), 7.47 (d, 2H, $J = 9.3$ Hz), 7.59 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3): δ -4.9, -4.7, -4.5, -4.4, 17.9, 18.0, 25.8, 25.9, 26.1, 27.4, 39.0, 40.9, 69.2, 72.8, 73.8, 77.1, 77.5, 83.8, 86.5, 91.2, 101.9, 112.4, 140.7, 147.2, 148.0, 160.0, 169.5, 169.8. FTIR (KBr), cm^{-1} : 3362 (br), 2934 (s), 1798 (s), 1719 (s), 1693 (s), 1536 (s), 1449 (s), 1397 (s), 1265 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{30}\text{H}_{53}\text{N}_3\text{O}_{10}\text{Si}_2\text{Na}$ ($\text{MNa}^+ - 2\text{Boc}$) $^+$ 1377.6544, found 1377.6492. TLC (15% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$), R_f : 0.86.

6.2.27. Dimer 30. Acid **21** (0.37 g, 0.50 mmol) and amine (0.34 g, 0.60 mmol) **18** were azeotropically dried with THF and then combined and dissolved in dry CH_3CN (3 mL). HATU (0.28 g, 0.75 mmol) and NEt_3 (0.10 mL, 0.75 mmol) were added and the reaction was allowed to stir. After 19 h the mixture was partitioned between EtOAc (400 mL) and brine (400 mL), the organic layer was washed with NaHCO_3 (1 $\times$ 400 mL) and H_2O (1 $\times$ 400 mL), then dried (Na_2SO_4), and concentrated. The resulting solid was purified by silica gel chromatography (5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to yield **30** (0.82 g, 77%) as a white solid. ^1H NMR (400 MHz, CDCl_3): δ -0.03 (s, 6H), 0.02 (s, 6H), 0.06 (s, 12H), 0.83 (s, 12H), 0.87 (s, 12H), 1.44 (s, 6H), 1.54 (s, 18H), 3.51 (m, 4H), 3.91 (t, 2H, $J = 2.3$ Hz), 4.04 (t, 2H, $J = 2.5$ Hz), 4.37 (t, 2H, $J = 3.3$ Hz), 4.50 (s, 2H), 5.54 (d, 2H, $J = 3.3$ Hz), 5.74 (d, 2H, $J = 5.3$ Hz), 7.34 (d, 2H, $J = 3.5$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ -4.8, -4.7, -4.5, -4.4, 17.9, 18.0, 25.7, 25.8, 26.0, 27.4, 27.5, 38.6, 41.0, 73.0, 73.4, 77.3, 83.5, 86.5, 92.6, 102.0, 112.4, 141.0, 147.2, 148.0, 159.9, 169.6. FTIR (film) cm^{-1} : 3380 (br), 3091 (w), 2951 (s), 2864 (s), 1798 (s), 1728 (s), 1684 (s), 1536 (s), 1387 (s), 1274 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{30}\text{H}_{53}\text{N}_3\text{O}_{10}\text{Si}_2\text{Na}$ ($\text{MNa}^+ - 2\text{Boc}$) $^+$ 1119.5328, found 1119.5328. TLC (15% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$), R_f : 0.94.

6.2.28. Monomer 31. Acid **21** (0.99 g, 1.34 mmol) was azeotropically dried with THF and dissolved in dry CH_3CN (3 mL). HATU (0.76 g, 2.01 mmol) was added, and the reaction was stirred for 10 min. The amine (0.13 mL, 1.47 mmol) and NEt_3 (0.28 mL, 2.01 mmol) were added and the reaction was allowed to stir. After

16 h the mixture was partitioned between EtOAc (400 mL) and brine (400 mL), the organic layer was washed with NaHCO_3 (1 $\times$ 400 mL) and H_2O (1400 mL), dried (Na_2SO_4), and concentrated. The resulting solid was purified by silica gel chromatography (5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to yield **31** (0.82 g, 77%) as a white solid. ^1H NMR (400 MHz, CDCl_3): δ -0.04 (s, 3H), 0.00 (s, 3H), 0.05 (s, 6H), 0.82 (s, 6H), 0.86 (s, 6H), 1.44 (s, 3H), 1.45 (s, 3H), 1.54 (s, 9H), 3.32 (s, 3H), 3.44 (m, 5H), 3.64 (m, 1H), 3.91 (m, 1H), 4.04 (m, 1H), 4.22 (t, 1H, $J = 3.0$ Hz), 4.51 (s, 2H), 5.65 (d, 1H, $J = 3.8$ Hz), 5.75 (d, 1H, $J = 5.0$ Hz), 7.10 (m, 1H), 7.46 (d, 1H, $J = 5.0$ Hz), 7.63 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3): δ -4.9, -4.7, -4.5, -4.4, 17.9, 18.0, 25.7, 25.8, 25.9, 26.0, 26.1, 27.4, 39.0, 40.9, 58.7, 70.6, 72.7, 73.9, 77.0, 77.5, 83.9, 86.4, 90.8, 102.0, 112.3, 140.5, 147.2, 148.1, 160.0, 169.2, 169.9. FTIR (film), cm^{-1} : 3353 (br), 2951 (s), 2855 (s), 1798 (s), 1728 (s), 1693 (s), 1545 (s), 1466 (s), 1387 (s), 1265 (s). HRMS (MALDI-FTMS), m/z : calcd for $\text{C}_{30}\text{H}_{53}\text{N}_3\text{O}_{10}\text{Si}_2\text{Na}$ ($\text{MNa}^+ - \text{Boc}$) $^+$ 723.3427, found 723.3435. TLC (10% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$), R_f : 0.81.

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