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Ring-Closing Metathesis and Glycosylation Reactions: Synthesis and Biophysical Studies of Polyether-Linked Carbohydrate-Based Macrocyclic Nucleosides

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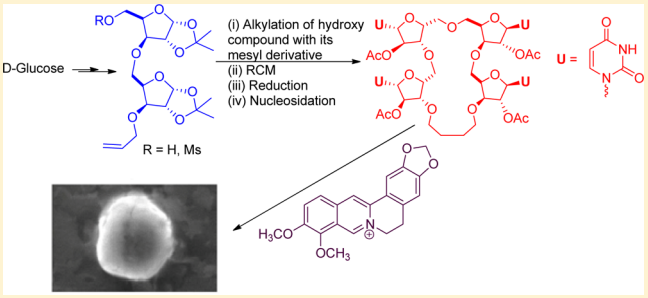
Supporting Information

ABSTRACT: Bis-, tris-, and tetrakisuracil-substituted 12-, 13-, 17-, and 21-membered macrocyclic nucleoside analogues with polyether linkages, including C_2-symmetric molecules, have been synthesized through coupling of two appropriately allylated sugar derivatives, derived from D-glucose, followed by a sequential ring-closing metathesis reaction using Grubbs catalysts, double-bond reduction, and nucleoside base insertion under Vorbrüggen reaction conditions. Spectroscopic studies on the interaction of these nucleoside analogues with small molecules, such as the alkaloids berberine and palmatine and the DNA intercalator ethidium bromide, revealed a change in the absorbance and fluorescence of the small molecules suggesting the potential use of these nucleoside molecules as a carrier of small molecules in biological systems. Circular dichroism studies indicated that the complexes of the nucleosides with small molecules undergo aggregation/self-organization. This has been further evidenced by a SEM experiment showing the binding of berberine with one of the nucleoside derivatives, which confirms the occurrence of secondary structure reorganization.



INTRODUCTION

Carbohydrate-based macrocycles have been found to have versatile applications in biological, pharmaceutical, and medicinal studies, particularly as building blocks in supramolecular chemistry,[1] drug-carrier systems,[2] molecular reactors,[3] artificial receptors[4] in the recognition of ionic and neutral compounds, and agents with antibacterial,[5] antiviral,[6] antifungal,[7] and anticancer functions;[8] hence, interest in these compounds has greatly increased.[9] The incorporation of carbohydrate residues into macrocycles also facilitates modulation of their properties.[10] The importance of these macrocycles has led to the development of efficient and facile synthesis methods involving the intramolecular Diels–Alder reaction,[11] the aldol reaction,[12] the click reaction,[13] and various lactonization reactions[14] for the generation of diverse glycosides, nucleosides, and bioactive compounds. Using a ring-closing-metathesis(RCM)-based strategy[15] on a carbohydrate backbone using a Grubbs catalyst has been considered a useful method for the construction of difficult-to-access cyclic rings of varied sizes present in naturally occurring bioactive compounds,[16] conformationally restricted β-turn mimics,[17] and cyclic nucleosides[18] and nucleotides.[19] However, very few carbohydrate-based macrocyclic nucleosides with polyether linkages (crown-ether-type) are known in the literature.[18c,20] Therefore, we made sustained efforts to build an array of diverse macrocycles and their nucleoside analogues to find out their important properties. The strategy was to synthesize them from easily available carbohydrates via operationally simple routes that are fundamentally different from the methods routinely exploited. We report herein the chemical synthesis of macrocycles of different ring sizes through the application of catalytic olefin metathesis for C–C bond construction in a crucial step followed by functional group transformations and nucleobase incorporation to generate structurally unique nucleoside analogues containing bis-, tris-, and tetrakisuracil moieties. To the best of our knowledge, the nucleosides described herein are the first examples of their kind.

Our approach was to place olefin moieties at appropriate places on the backbone of a sugar scaffold, so that they could be subjected to an RCM reaction to generate macrocycles. For this, we decided to couple two fragments of allylated sugar derivatives of varied sizes (e.g., two monosaccharide units, a monosaccharide and a disaccharide unit, or two disaccharide units) to generate the basic macrocycles. Deprotection of acetonide groups followed by appropriate trimming of the furanose moieties could deliver macrocycles of varied sizes containing polyether linkages. It was also expected that the products would undergo nucleosidation at the anomeric centers of the sugar rings to furnish the corresponding macrocyclic

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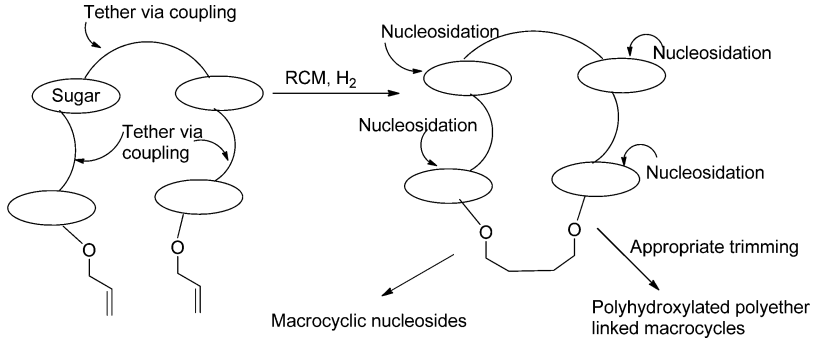
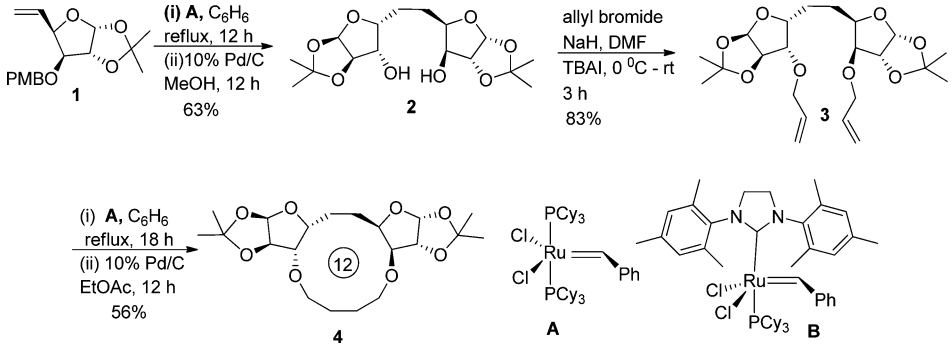


Figure 1. Synthesis strategy for macrocyclic nucleosides built on polyether macrocycles.

Scheme 1. Preparation of C_2-Symmetric 12-Membered Macrocycle 4



nucleoside analogues (Figure 1). These nucleosides could be characterized as important materials with regard to their performance in bioactivity and drug delivery system.[21]

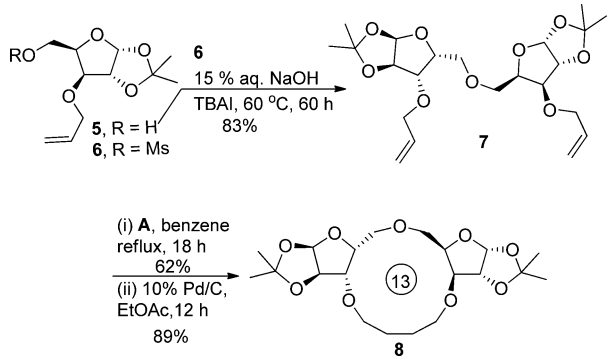
RESULTS AND DISCUSSION

Synthesis of Carbohydrate-Based Macrocycles. A cross metathesis (CM) reaction of D-glucose-derived olefin precursor **1**[22] using first-generation Grubbs catalyst **A** afforded a cis/trans mixture of an alkene. Without further purification, the intermediate was subjected to catalytic hydrogenation followed by hydrogenolysis over 10% Pd/C to provide symmetrical diol **2** in 63% yield. Allylation of **2** using allyl bromide in DMF containing tetrabutylammonium iodide (TBAI) furnished **3** (83%), which gave (upon RCM reaction followed by reduction of the olefin moiety) C_2-symmetric 12-membered macrocycle **4** (Scheme 1). The symmetric nature of **4** was indicated by the presence of a single set of peaks in the ^{1}H and ^{13}C NMR spectra caused by symmetry-related protons and carbon atoms. HRMS (ESI−QToF, positive ion) analysis of **4** showed a molecular ion peak at m/z 423.1980 $(M + Na)^+$ confirming its indicated structure.

In another approach, mesyl derivative **6**,[15c] derived from 3-*O*-allyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**5**),[23] was treated with its precursor under alkaline conditions in the presence of TBAI to furnish 5,5′-ether-linked diallylated pseudodisaccharide **7**[15c] (83%). The olefin-metathesis reaction of **7** could be carried out with Grubbs catalyst **A**; subsequent hydrogenation over 10% Pd/C provided 13-membered macrocycle **8** in 89% yield, incorporating three oxygen atoms (Scheme 2). The structure of the macrocycle was confirmed by single crystal X-ray analysis (Figure 2, wireframe; Figure S1, ORTEP diagram).

In a further derivation of the above strategy, initial formation of a 3,5′-ether linkage by the treatment of **6** with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose under alkaline conditions could be followed by opening up the acetonide group via acid. Subsequent vicinal diol cleavage with sodium metaperiodate and reduction of the aldehyde group with sodium borohydride generated pseudodisaccharide derivative **9**.[24] This allowed us to perform alkylation of the hydroxyl group of **9** with another molecule of **6** under the same conditions to obtain pseudotrisaccharide derivative **10** (68%). At this stage,

Scheme 2. Synthesis of 13-Membered Macrocycle 8 through a RCM Reaction



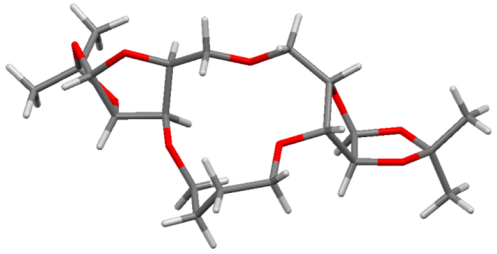


Figure 2. Wireframe representation of the crystal structure of **8**.

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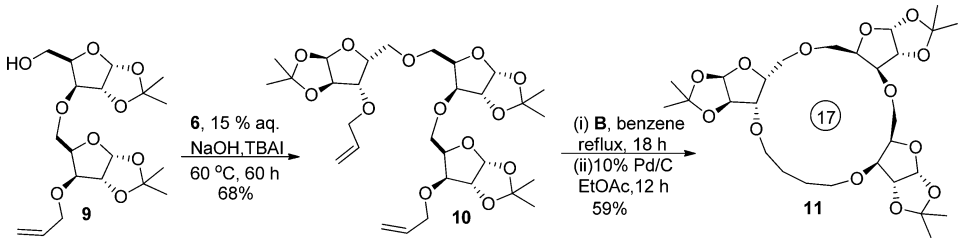
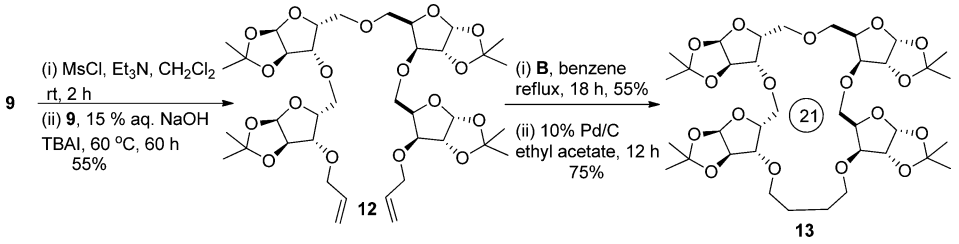
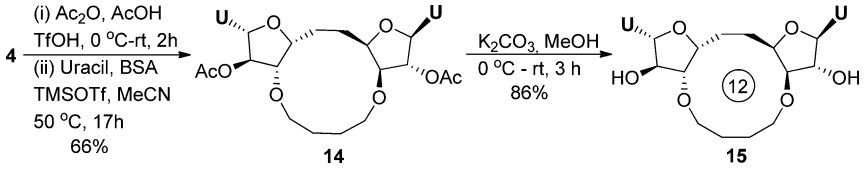
Scheme 3. Synthesis of 17-Membered Polyether-Linked Carbohydrate-Based Macrocycle 11



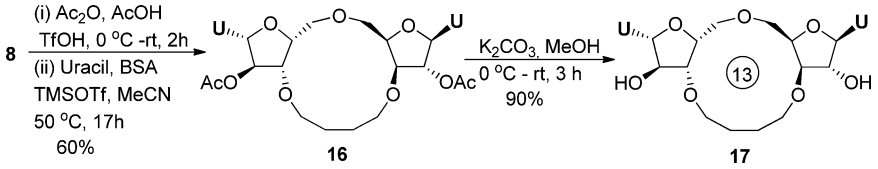
Scheme 4. Synthesis of 21-Membered Polyether-Linked Carbohydrate-Based Macrocycle 13



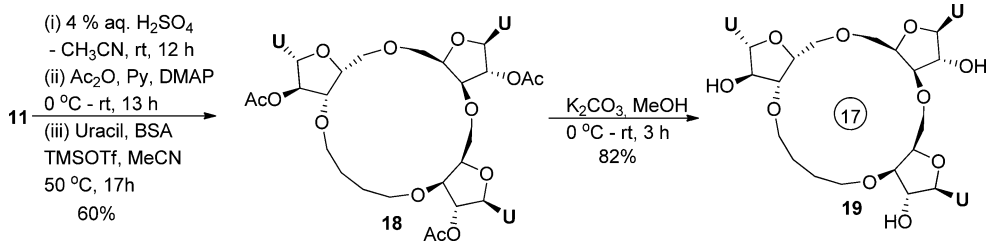
Scheme 5. Nucleosidation of 4 to 12-Membered Macrocyclic Nucleoside Derivative 15



Scheme 6. Nucleosidation of 8 to 13-Membered Macrocyclic Nucleoside Derivative 17



Scheme 7. Conversion of 11 to 17-Membered Macrocyclic Nucleoside Derivative 19



intramolecular cyclization of **10** was carried out by using Grubbs catalyst **B** (second generation), and this was followed by catalytic hydrogenation to smoothly convert the intermediate to 17-membered macrocycle **13** in 59% yield (Scheme 3). The asymmetrical nature of **11** was indicated by the presence of appropriate signals for all the protons and carbons in the ^{1}H and ^{13}C NMR spectra (i.e., six singlets caused by the six methyl groups of the three isopropylidene groups and the appropriate signals for H1 and H2 of the three sugar moieties).

Finally, the reaction of **9** with its mesyl derivative produced pseudotetrasaccharide derivative **12** (55%), which could be made to undergo an intramolecular RCM reaction using Grubbs catalyst **B** (second generation). Subsequent catalytic hydrogenation afforded 21-membered macrocycle **13** in 75% yield (Scheme 4). The NMR spectra of the macrocycle showed, as anticipated, peaks for only 28 protons and 18 carbon atoms; this coresponds to half the number of nuclei and is due to the C_2-symmetric nature of the molecule.

Synthesis of Macrocyclic Bisuracil Nucleoside Derivatives. The stage was now set to form the macrocyclic bioactive nucleoside derivatives.[25] Thus, the opening of the acetonide groups of **4** followed by peracetylation of the generated hydroxyl groups produced an intermediate mixture of anomers, which was subjected without further purification to nucleosidation with uracil base in the presence of *N,O*-bis(trimethylsilyl)acetamide (BSA) and TMSOTf in CH_3CN under Vorbrüggen reaction conditions[26] to furnish nucleoside derivative **14** (66%, Scheme 5). Deacetylation of **14** using

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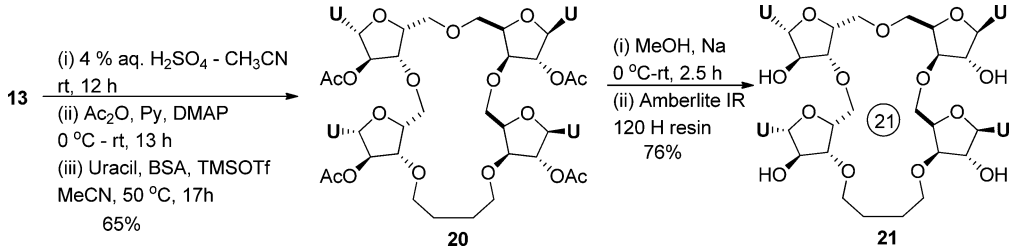
Scheme 8. Conversion of 13 to Tetrakis- Nucleoside Derivative 21 Appended to a 21-Membered Macrocycle



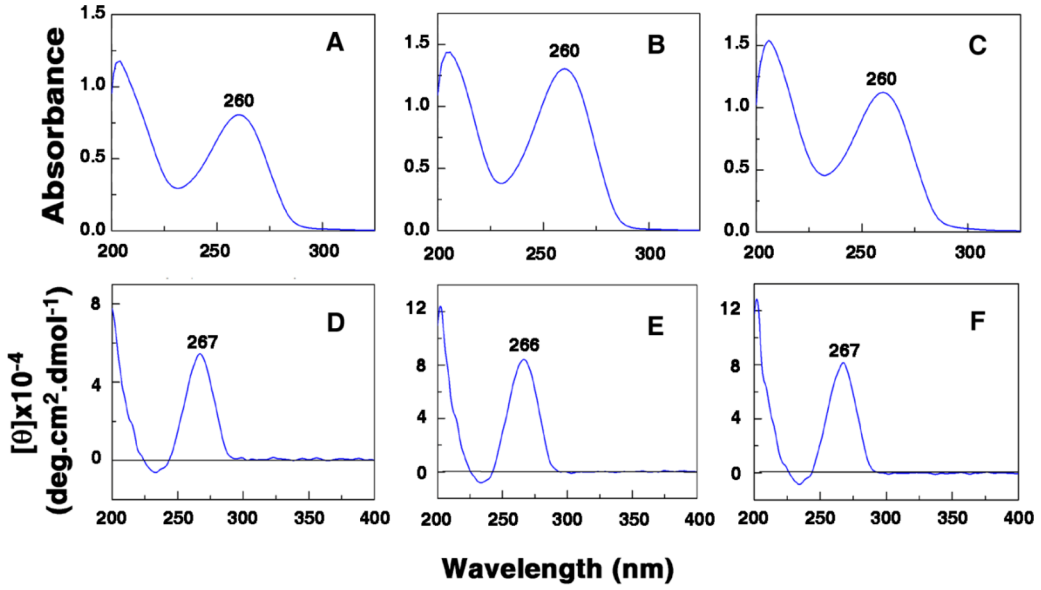


Figure 3. Top row: absorption spectra of a 30 μM solution of **16** (A), **18** (B), **20** (C). Bottom row: CD spectra of a 60 μM solution of **16** (D), **18** (E), and **20** (F). All experiments were carried out in 100% methanol.

K_2CO_3 in MeOH afforded fully deprotected 12-membered macrocyclic bisuracil nucleoside derivative **15** (86%), maintaining the C_2-symmetric nature of the precursor.

Similar acetonide deprotection of **8** followed by peracetylation and nucleosidation at the anomeric centers afforded nucleoside derivative **16** (60%). This was deacetylated to 13-membered macrocyclic bisuracil nucleoside analogue **17** in 90% yield (Scheme 6).

Synthesis of a Macrocyclic Trisuracil Nucleoside Derivative. Removal of acetonide protection of **11** with 4% H_2SO_4 in aqueous acetonitrile followed by acetylation and subsequent Lewis acid catalyzed installation of the uracil base on the anomeric carbons of the furanoside moieties under similar reaction conditions afforded 17-membered macrocyclic trinucleoside **18** (60%). Deprotection of **18** using K_2CO_3 in MeOH furnished the corresponding trisuracil nucleoside analogue **19** in 82% yield (Scheme 7).

Synthesis of a Macrocyclic Tetrakisuracil Nucleoside Derivative. In the same vein, cyclic pseudotetrasaccharide **13** was converted to 21-membered macrocyclic tetrauracil nucleoside derivative **20** in 65% yield via acetonide deprotection, acetylation, and subsequent nucleosidation (Scheme 8). However, attempted deprotection of the acetyl groups of **20** using various reagents such as (i) K_2CO_3/MeOH,[27] (ii) Et_3N/MeOH-H_2O,[28] (iii) NH_4OH/MeOH,[29] and (iv) dibutyltin oxide/MeOH[30] failed to furnish the targeted nucleoside; instead these all gave an intractable mixture, from which the pure product could not be isolated by chromatography. Nevertheless, deprotection was achieved by treating the compound in methanol with metallic sodium followed by neutralization of alkali with Amberlite IR120 H resin furnishing macrocyclic tetrakisuracil nucleoside **21** (76%).

Biophysical Studies of the Macrocyclic Nucleosides. *Absorption and Circular Dichroism Spectral Studies.* Macrocyclic nucleoside derivatives **14**–**21** exhibited characteristic UV spectra with absorbance maxima around 260 nm. Representative absorption patterns of **16**, **18**, and **20** are presented in Figure 3 (top row). The circular dichroism (CD) pattern of these nucleosides (Figure 3, bottom row) revealed spectra in the range of 200–400 nm with a positive maximum around 265 nm and a small negative trough in the 230 nm region followed by a peak near 205 nm, indicating an asymmetric arrangement of the nucleobases. The absorption and CD spectra of **14**, **15**, **17**, **19**, and **21** have been given in Figure S2.

Binding Studies with Small Molecules. The ability of macrocyclic nucleosides **16**–**21** to bind to small molecules, such as DNA-binding alkaloids berberine (BER) and palmatine (PAL) and the classical DNA intercalator ethidium bromide (EB), was studied using absorbance and fluorescence spectroscopy. It was observed that in the presence of the nucleosides **16**–**19**, and **21** the absorbance of the alkaloids showed a hypochromic effect with an enhancement of fluorescence intensity; there was also an increase of the absorbance and fluorescence of these alkaloids in the presence of the nucleoside **20** (Figure S3A,B). However, the changes to the spectra were smaller for ethidium bromide than for the alkaloids.

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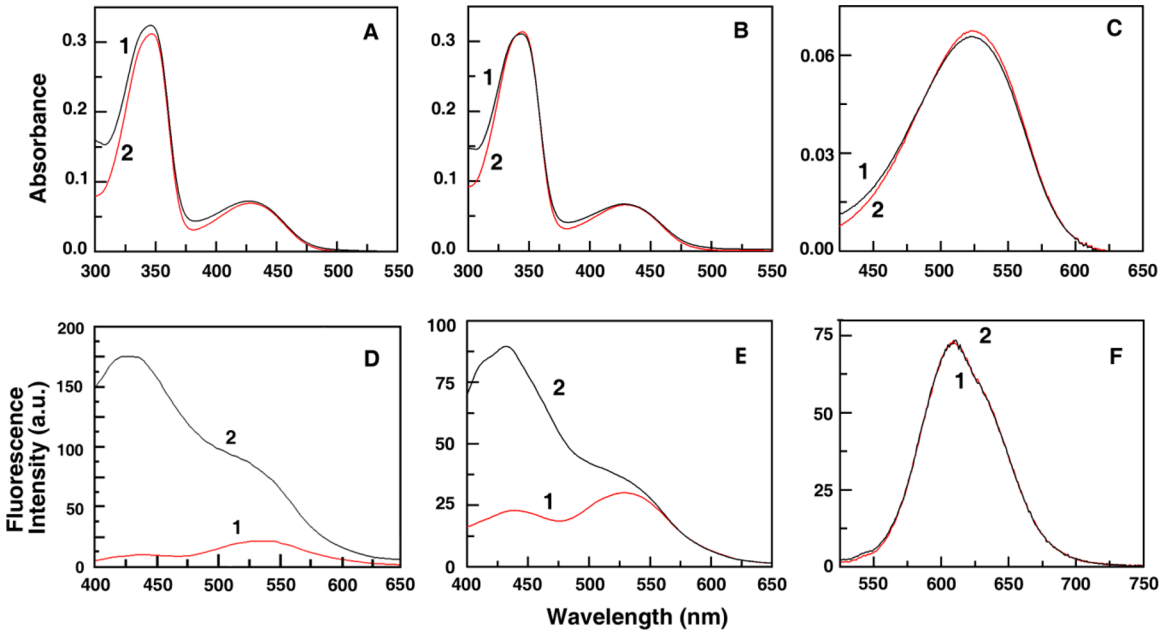

Figure 4. Top row: absorption spectra of berberine (A), palmatine (B), or ethidium bromide (C). Curve 1, 13 μM solution alone; curve 2, 18 μM solution in the presence of **21**. Bottom row: fluorescence emission spectra of berberine (D), palmatine (E), or ethidium bromide (F). Curve 1, 10 μM solution; curve 2, 18 μM solution in the presence of **21**. All experiments were carried out in 30% methanol in water.

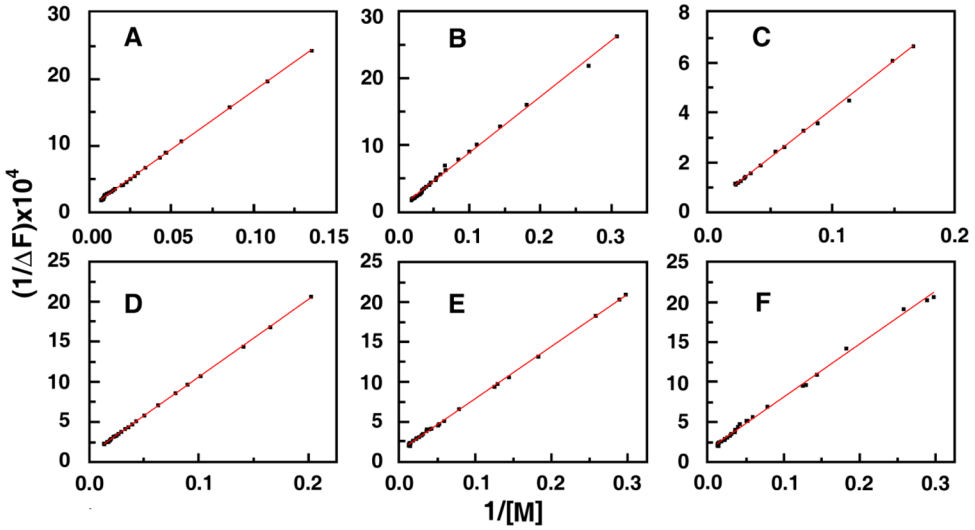

Figure 5. Benesi–Hildebrand plots of the complexes between berberine and macrocyclic nucleosides **16** (A), **17** (B), **18** (C), **19**(D), **20** (E), and **21** (F).

Representative absorption and fluorescence spectral patterns of **21** with BER, PAL, and EB are shown (Figure 4). These results suggest the potential use of these macrocyclic nucleoside molecules as carriers of small-molecule ligands in biological systems.

The binding affinity of BER to macrocyclic nucleosides **16**–**21** was established from the fluorescence spectral titration data, which were analyzed by a Benesi–Hildebrand plot[31]. The binding constants were determined using the relation

$$\frac{1}{\Delta F} = \frac{1}{\Delta F_{max}} + \frac{1}{K_{BH}(\Delta F_{max})} \times \frac{1}{[M]}$$

where K_{BH} is the Benesi–Hildebrand binding constant, [M] is the concentration of the macrocyclic nucleosides, and F is the fluorescence intensity. A plot of $1/\Delta F$ against $1/[M]$ gave a straight line in all cases (Figure 5).

The respective binding constants for berberine bound to macrocyclic nucleosides **16**–**21** were found to be 3.62×10^3, 4.86×10^3, 6.41×10^3, 8.92×10^3, 2.06×10^4, 2.29×10^4 M^{-1}. It was observed that an increase in the ring size of the macrocycles led to the enhancement of the binding affinity of the alkaloids to the nucleoside. More prominent increases in the affinity were observed for 21-membered macrocyclic nucleosides **20** and **21**. Another significant observation derived from the analysis is that the binding constants of the hydroxylated macrocyclic nucleosides were higher than those of their acetyl derivatives (i.e., **17** > **16**, **19** > **18**, **21** > **20**). This is probably due to better stacking interactions between the bases of the deprotected macrocyclic nucleosides and the

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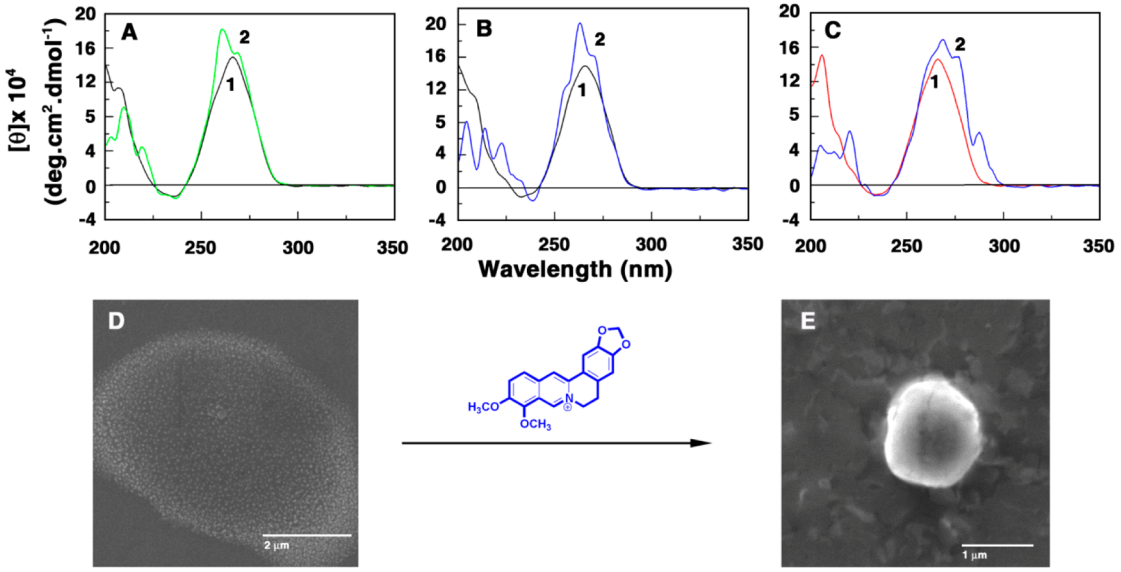

Figure 6. Top row: CD spectra of a 60 μM solution of **20** alone (A, B, and C; curve 1) and in the presence of a 40 μM of solution berberine (A, curve 2), palmatine (B, curve 2) and ethidium bromide (C, curve 2). Bottom row: SEM pictures of **20** (D) and **20**-berberine complex (E). The experiments were carried out in 30% methanol in water.

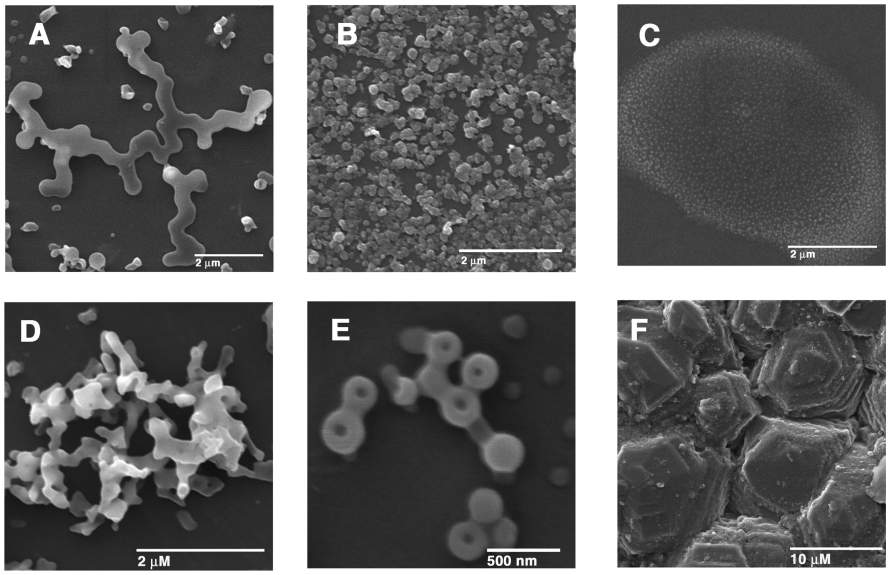

Figure 7. SEM images of nucleosides at different temperatures. Top row at 25 °C: **16** (A), **18** (B), **20** (C). Bottom row at ∼4 °C: **16** (D), **18** (E), **20** (F).

isoquinoline moiety of the berberine alkaloid. The binding of the nucleosides was also investigated by CD spectral experiments. Binding of BER, PAL, and EB led to a strong enhancement in the CD of the nucleoside, eventually leading to splitting of the pattern (Figure 6). The phenomenon was probed by scanning electron microscopy (SEM) imaging the representative case of **20** with berberine, where it was revealed that some secondary structure reorganization was occurring to a single particle.

Temperature-Dependent Aggregation Study by SEM. At low temperature (∼4 °C) the transparent solution of the nucleosides **16**, **18**, and **20** turned into a turbid white aggregate as shown in the photograph (Figure S4). The appearance of the turbidity was anticipated and was attributed to the aggregation of the bis-, tris-, or tetrakisuracil derivatives. Electrospray ionization mass spectra of the aggregates derived from **16** and **18** showed molecular ion peaks at m/z 1239 $(2\ M + Na)^+$ and 1775 $(2\ M + Na)^+$, respectively, indicating the formation of dimers by base pairing between two uracil moieties through intermolecular H-bonding. The dimers could be a symmetric pairing of two 4-carbonyl-N3 molecules, a symmetric pairing of two 2-carbonyl-N3 molecules, or an asymmetric pairing between a 2-carbonyl-N3 and a 4-carbonyl-N3, all of which are present in a wide variety of nucleic acid structures[32] (Figure S5). Base pairing could also be occuring through a stacking interaction between the uracil bases. The mass spectrum of the aggregate obtained from **20** did not, however, show any dimeric/polymeric product suggesting that the aggregation most probably occurred as a result of a stacking interaction[33] between the uracil bases. To confirm the stacking interaction, we monitored microstructural changes through SEM experiments at room temperature and low temperature as shown in

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Figure 7A–F. SEM images of **16** at room temperature showed self-assembled particles with no regular geometry, forming a long chain-like structure (Figure 7A). On cooling, the chain-like structure stacked one over the other with a contraction of the chain length due to molecular aggregation (Figure 7D). Macrocyclic trisuracil derivative **18**, however, exhibited an irregular geometry at room temperature with individual particles (Figure 7B), which transformed at low temperature to donut-shaped particles connected through weak linkages (Figure 7E). At room temperature, nucleoside analogue **20** consisted of a conglomeration of nanoparticles (Figure 7C), which at lower temperature furnishes a layered structure formed by the stacking of hexagon-shaped plates (Figure 7F). In the case of **16**, Figure 7D clearly shows the formation of a planar 3D aggregation, whereas Figure 7F shows that nucleoside **20** has probably undergone an aggregation in different directions (also exhibiting a 3D aggregation). It is interesting to see that nucleoside derivative **18** has replicated the cyclic nature of the molecule, resulting in the formation of donut-shaped particles after cooling followed by aggregation (Figure 7B,D).

According to the structural analysis, it is expected that the macrocyclic nucleosides have a higher tendency to form different noncovalent interactions such as H-bonding and nucleoside base stacking. In solution phase, the thermal mobility of these nucleosides decreases at low temperature. Due to the decrease in thermal mobility, the molecules come closer together leading to aggregation. Interestingly, this microstructural transformation was found to be reversible. On warming, the turbid aggregated solution reverted to a transparent solution, caused by a changeover of the microstructure to the original morphology as evidenced by SEM. We believe that these oligonucleosides have a tendency to form aggregated structures because of noncovalent interactions between the uracil bases that are reversible, such as intermolecular H-bonding and base stacking. The cyclic nature of the reversibility of the structures from room temperature to 4 °C and back to room temperature as evidenced by SEM image studies confirms unequivocally the absence of any kind of covalent interaction and supports strongly our idea that noncovalent interactions are the cause of molecular aggregation.

■ CONCLUSIONS

The work described herein involves the preparation of various sugar-based substrates from readily available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. Precursor assembly for RCM reaction by alkylation reaction appears simple, making the strategy an attractive one. This method has generated polyether-linked macrocycles of varied sizes, which have been transformed into their corresponding macrocyclic nucleoside analogues. X-ray analysis of one of the RCM products has conclusively established its structure, which also supports the structures of the other products. The strategy appears flexible enough to be utilized for synthesis of other ring systems through judicious manipulation of the substrates. Circular dichroism, absorbance, and fluorescence studies indicate that these nucleoside analogues can bind to small molecules and that they can also form aggregated structures as evidenced by SEM experiments. These properties of the nucleosides could be utilized in a nucleic acid targeted drug-delivery system.

■ EXPERIMENTAL SECTION

General. Melting points were taken in open capillaries and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on 300 and 600 MHz spectrometers, respectively, with tetramethylsilane and chloroform-d_1/methanol-d_4/dimethyl sulfoxide-d_6 as the internal standard. Data for ^{1}H NMR are reported as follows: chemical shift (multiplicity, number of hydrogens, coupling constant). Multiplicity is abbreviated as follows: s (singlet), d (doublet), dd (double doublet), brdd (broad double doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were recorded in ESI and EI mode. Specific rotations were measured at 589 nm. Precoated plates (0.25 mm, silica gel 60 F_{254}) were used for TLC. All of the solvents were distilled and purified as necessary. Thin-layer chromatography plates were visualized by exposure to UV light/iodine and/or by spraying with Liebermann reagent followed by heating on a hot plate.

(3a*R*,5*R*,6*S*,6a*R*)-6-(4-Methoxybenzyloxy)-2,2-dimethyl-5-vinyltetrahydrofuro[2,3-*d*][1,3]dioxole (1). To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (7.0 g, 26.9 mmol) in THF (60 mL) at 0 °C was added portionwise NaH (60% suspension in mineral oil, 1.62 g, 40.3 mmol), and the mixture was stirred for 15 min. A solution of *p*-methoxy benzyl chloride (4.75 mL, 35.0 mmol) in THF (5 mL) containing TBAI (1.0 g, 2.7 mmol) was added to the reaction flask, and the mixture was stirred at room temperature for 12 h. The reaction was then quenched with a saturated aqueous NH_4Cl solution (25 mL), and the solvent was evaporated in vacuo to a residue, which was extracted with EtOAc (3 × 30 mL). The combined organic extract was washed with water (2 × 15 mL), dried (Na_2SO_4), and the solvent was evaporated to a crude product, which was purified by column chromatography over silica gel (100–200 mesh) using a petroleum ether–EtOAc (9:1) mixture as the eluent to give 1,2:5,6-di-*O*-isopropylidene-3-*O*-*p*-methoxybenzyl-α-D-glucofuranose derivative (8.5 g, 83%) as a colorless gum. This sugar derivative (4.0 g, 10.53 mmol) was dissolved in 75% aqueous HOAc (40 mL) and stirred at room temperature for 12 h. The solvent was evaporated in vacuo, and the last trace of HOAc was removed by azeotropic distillation with toluene to yield a diol (3.5 g) as a gummy material. To a solution of the material in CH_2Cl_2 (40 mL) containing Et_3N (1.85 mL, 13.2 mmol) at 0 °C was added dropwise MsCl (1.03 mL, 13.2 mmol) with stirring. After stirring at 25 °C for 2 h, the reaction mixture was cooled to 0 °C followed by addition of few drops of water to destroy excess MsCl. The CH_2Cl_2 solvent was washed with a saturated aqueous $NaHCO_3$ solution (40 mL) and water and then dried (Na_2SO_4). Removal of the solvent afforded the corresponding 5,6-*O*-dimesylfuranose derivative (4.15 g) as pale-yellow oil. To a solution of this oil in dry DME (40 mL), NaI (12.1 g) was added, and the mixture was heated at 80 °C under N_2 for 8 h. The solvent was evaporated, and the residue was extracted with CH_2Cl_2 (3 × 20 mL). The combined extract was treated with an aqueous $Na_2S_2O_3$ solution (40 mL). The organic layer was washed with brine (10 mL), dried (Na_2SO_4), and evaporated to a crude product, which was purified by column chromatography over silica gel (100–200 mesh) using EtOAc–*n*-hexane (1:9) as the eluent to furnish alkene **1**[22] (1.65 g, 51%) as a colorless liquid. ^{1}H and ^{13}C NMR spectra were in good agreement with the reported values.

1,2:1′,2′-Di-*O*-isopropylidene-5-deoxy-5-(5-deoxy-α-D-xylofuranos-5-yl)-α-D-xylofuranose (2). To alkene **1** (2.1 g, 6.86 mmol) dissolved in dry benzene (30 mL) was added first-generation Grubbs catalyst (282 mg, 0.342 mmol, 5 mol %), and the mixture was heated at reflux for 12 h under N_2 atmosphere. The solvent was washed with water (2 × 20 mL), dried (Na_2SO_4), and evaporated in vacuo to furnish a crude residue (820 mg) containing product **2**. To a solution of the above residue in dry MeOH (20 mL) was added 10% Pd/C (100 mg); the mixture was degassed and then hydrogenated with H_2 under atmospheric pressure at room temperature for 12 h. The catalyst was filtered off, and the solvent was evaporated to a residue, which was purified by column chromatography over silica gel (100–200 mesh) using petroleum ether–EtOAc (5:1) as the eluent to afford **2** (748 mg, 63%) as a colorless liquid. $[\alpha]_D^{25}$ −16.3 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.31 (s, 6H), 1.50 (s, 6H), 1.58–1.66 (m, 2H), 1.90–1.99 (m, 2H), 2.72 (brs, 2H), 4.15–4.19 (m, 4H), 4.52 (d, 2H, *J*

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= 3.6 Hz), 5.90 (d, 2H, J = 3.6 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 25.7 ($2CH_2$), 26.6 ($2CH_3$), 27.3 ($2CH_3$), 76.3 (2CH), 81.8 (2CH), 87.1 (2CH), 106.1 (2CH), 112.6 (2C); HRMS (ESI−QToF, positive ion) m/z calcd for $C_{16}H_{26}NaO_8$, 369.1525; found, 369.1500.

1,2:1′,2′-Di-*O*-isopropylidene-3-*O*-allyl-5-deoxy-5-(3-*O*-allyl-5-deoxy-α-D-xylofuranos-5-yl)-α-D-xylofuranose (3). To a solution of **2** (500 mg, 1.45 mmol) in dry DMF (6 mL) at 0 °C was added NaH (60% suspension in mineral oil, 0.174 g, 4.35 mmol), and the mixture was stirred for 15 min. Allyl bromide (0.379 mL, 4.35 mmol) in dry DMF (5 mL) containing TBAI (53 mg, 0.145 mmol) was added to the reaction flask, and the mixture was stirred at room temperature for 3 h. The reaction was quenched with a saturated aqueous NH_4Cl solution (25 mL). The solvent was evaporated in vacuo, and the residue was extracted with EtOAc (3 × 30 mL). The combined organic extract was washed with water (2 × 15 mL), dried (Na_2SO_4), and evaporated to a residue, which was purified by column chromatography over silica gel (100−200 mesh). Elution was carried out with a petroleum ether−EtOAc (9:1) mixture to furnish **3** (510 mg, 83%) as a colorless gum. $[\alpha]_D^{25}$ −42.7 (c 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.32 (s, 3H), 1.34 (s, 3H), 1.49 (s, 3H), 1.52 (s, 3H), 1.76−1.92 (m, 4H), 3.74 (apparent t, 2H, J = 3.6 Hz), 3.94−4.16 (m, 6H), 4.55 (d, 2H, J = 3.6 Hz), 5.20 (brtd, 2H), 5.29 (td, 2H, J = 1.5, 17.4 Hz), 5.80−5.94 (m, 2H), 5.84 (d, 1H, J = 3.9 Hz), 5.89 (d, 1H, J = 3.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.9 ($2CH_2$), 26.1 ($2CH_3$), 26.6 ($2CH_3$), 70.8 ($2CH_2$), 79.7 (2CH), 81.6 (2CH), 82.2 (2CH), 104.6 (2CH), 111.2 (2C), 117.7 ($2CH_2$), 134.0 (2CH); HRMS (ESI−QToF, positive ion) m/z calcd for $C_{22}H_{34}NaO_8$, 449.2152; found, 449.2147.

(2*R*,3*R*,3a*S*,9a*S*,10*R*,11*R*,12a*R*,14a*R*)-2,3:10,11-Di-*O*-isopropylidene-tetradecahydrodifuro[3,2-*g*:2′,3′-*k*][1,6]-dioxacyclododecine (4). To a solution of **3** (400 mg, 0.94 mmol) in refluxing benzene (60 mL) was added dropwise a solution of first-generation Grubbs catalyst (39 mg, 0.047 mmol) in benzene (10 mL) under N_2 atmosphere; the reflux was continued for 18 h. The solution was cooled and then concentrated to afford a syrupy residue, which was purified by column chromatography over silica gel (100−200 mesh) using petroleum ether−EtOAc (5:1) as the eluent to produce a cis/trans mixture of the desired cyclized alkene (250 mg, 67%) as sticky liquid. 10% Pd/C (70 mg) was added to a solution of the alkene mixture in dry EtOAc (20 mL); the mixture was degassed and hydrogenated with H_2 under atmospheric pressure at room temperature for 12 h. The catalyst was filtered off, and the filtrate was evaporated to form a residue, which was purified by column chromatography over silica gel (100−200 mesh) using petroleum ether−EtOAc (6:1) as the eluent to afford **4** (210 mg, 56%) as a colorless sticky liquid. $[\alpha]_D^{25}$ −79.5 (c 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.32 (s, 6H), 1.51 (s, 6H), 1.58−1.61 (m, 2H), 1.66−1.70 (m, 2H), 1.72−1.75 (m, 2H), 1.77−1.80 (m, 2H), 3.64 (apparent t, 2H, J = 8.7 Hz), 3.70 (brdd, 2H, J = 6.0, 10.2 Hz), 3.74 (d, 2H, J = 2.4 Hz), 4.15 (brd, 2H, J = 10.8 Hz), 4.52 (d, 2H, J = 3.6 Hz), 5.88 (d, 2H, J = 3.6 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 23.2 ($2CH_2$), 26.1 ($2CH_3$), 26.62 ($2CH_2$), 26.64 ($2CH_3$), 69.4 ($2CH_2$), 80.0 (2CH), 80.6 (2CH), 82.3(2CH), 104.7 (2CH), 111.3 (2C); HRMS (ESI−QToF, positive ion) m/z calcd for $C_{20}H_{32}NaO_8$, 423.1995; found, 423.1980.

1,2:1′,2′-Di-*O*-isopropylidene-3-*O*-allyl-5-*O*-(3-*O*-allyl-5-deoxy-α-D-xylofuranos-5-yl)-α-D-xylofuranose (7). A mixture of alcohol **5**[23] (2.77 g, 12.09 mmol), mesylate **6**[15c] (4.47 g, 14.51 mmol), and Bu_4NI (446 mg, 1.21 mmol) in 15% aqueous NaOH (70 mL) was heated at 60 °C for 60 h. The mixture was cooled and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extract was washed with brine (30 mL) and dried (Na_2SO_4). Evaporation of the solvent afforded a syrupy material, which was purified by column chromatography over silica gel (100−200 mesh) using petroleum ether−EtOAc (4:1) as the eluent to give **7** (4.43 g, 83%) as a colorless sticky oil. $[\alpha]_D^{25}$ −56.0 (c 3.3, $CHCl_3$), ref 15c $[\alpha]_D^{25}$ −54.0 (c 1.07, $CHCl_3$); ESIMS m/z 465 $(M + Na)^+$; ^{1}H and ^{13}C NMR spectra were in good agreement with the reported values.

(2*R*,3*R*,3a*S*,9a*S*,10*R*,11*R*,12a*R*,15a*R*)-2,3:10,11-Di-*O*-isopropylidene-tetradecahydrodifuro[3,2-*b*:2′,3′-*g*][1,5,9] Trioxacyclotridecane (8). RCM of **7** (720 mg, 1.63 mmol) was carried out in refluxing benzene (70 mL) using first-generation Grubbs catalyst (67.5 mg, 0.082 mmol) dissolved in benzene (10 mL) under N_2 atmosphere following the method as described for the preparation of **4** from **3**. Workup and purification by column chromatography over silica gel (100−200 mesh) using petroleum ether−EtOAc (39:11) as the eluent produced a cis/trans mixture of the desired alkene (418 mg, 62%) as sticky liquid. Hydrogenation of the olefin moiety was carried out in EtOAc (20 mL) using cis/trans mixture of the desired alkene (400 mg, 0.96 mmol), H_2 gas, and 10% Pd/C (80 mg). The usual workup and purification by column chromatography over silica gel (100−200 mesh) using petroleum ether−EtOAc (41:9) as the eluent afforded **8** (355 mg, 89%) as a colorless solid. Mp 182−183 °C; $[\alpha]_D^{25}$ −48.4 (c 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.34 (s, 6H), 1.50 (s, 6H), 1.54−1.64 (m, 2H), 1.80−1.84 (m, 2H), 3.60−3.74 (m, 6H), 3.82 (dd, 2H, J = 7.8, 9.3 Hz), 3.91 (d, 2H, J = 3.9 Hz), 4.34 (dt, 2H, J = 3.3, 3.9, 7.2 Hz), 4.55 (d, 2H, J = 3.9 Hz), 5.90 (d, 2H, J = 3.9 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.3 ($2CH_2$), 26.5 ($2CH_3$), 27.0 ($2CH_3$), 67.3 ($2CH_2$), 69.9 ($2CH_2$), 78.6 (2CH), 82.2 (2CH), 83.5 (2CH), 104.8 (2CH), 111.9 (2C); HRMS (EI, magnetic sector) m/z calcd for $C_{20}H_{32}O_9$, 416.2046; found, 416.2050.

1,2:1′,2′:1″,2″-Tri-*O*-isopropylidene-3-*O*-allyl-5-*O*-[3-*O*-(5-deoxy-3-*O*-allyl-α-D-xylo-furanos-5-yl)-5-deoxy-α-D-xylofuranos-5-yl)]-α-D-xylofuranose (10). Alkylation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (10.4 g, 40 mmol) with **6** (11.2 g, 40.4 mmol) was carried out following the procedure as described for the preparation of **7**, using Bu_4NI (1.47 g, 4.0 mmol) and 15% aqueous NaOH (100 mL) and heating at 70 °C for 72 h. The usual workup afforded an alkylated product. Selective removal of an acetonide group was carried out by 50% aqueous HOAc (70 mL) at room temperature for 15 h, and subsequent solvent evaporation furnished a gummy material. The material was dissolved in MeOH (30 mL) at 0 °C, and an aqueous solution (30 mL) of $NaIO_4$ (8.64 g) was added dropwise to the solution as it was stirred. The mixture was stirred at room temperature for 3 h and then filtered. The solvent was evaporated to a crude aldehyde (ν_{max}, 1730 cm^{-1}), which was dissolved in MeOH (50 mL). $NaBH_4$ (1.0 g) was added, and the mixture was stirred at room temperature for 3 h. The solvent was evaporated to a residue, which was purified by column chromatography over silica gel (230−400 mesh) using petroleum ether−EtOAc (5:1) as the eluent to give **9** (2.1 g, 52%) as a colorless thick oil. $[\alpha]_D^{25}$ −53.2 (c 1.35, $CHCl_3$), ref 23 $[\alpha]_D^{25}$ −54.8 (c 0.59, $CHCl_3$); ^{1}H and ^{13}C NMR spectra were in good agreement with the reported values.

The alkylation of **9** with **6** was similarly carried out using **9** (1.75 g, 4.35 mmol), Bu_4NI (162 mg, 0.44 mmol), and 15% aqueous NaOH (25 mL). The usual workup afforded a syrupy liquid, which was purified by column chromatography over silica gel (230−400 mesh) using petroleum ether−EtOAc (4:1) as the eluent to give **10** (1.82 g, 68%) as a colorless sticky liquid. $[\alpha]_D^{25}$ −72.7 (c 0.19, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (s, 3H), 1.31 (s, 6H), 1.49 (s, 9H), 3.63−3.87 (m, 7H), 3.91 (t, 2H, J = 3.3 Hz), 3.97 (dd, 1H, J = 1.2, 5.7 Hz), 4.02 (d, 1H, J = 5.7 Hz), 4.09−4.18 (m, 2H), 4.28−4.33 (m, 1H), 4.36−4.39 (m, 2H), 4.54−4.60 (m, 3H), 5.18−5.23 (m, 2H), 5.26−5.33 (m, 2H), 5.80−5.92 (m, 2H), 5.87 (d, 1H, J = 4.2 Hz), 5.90 (d, 1H, J = 3.9 Hz), 5.92 (d, 1H, J = 3.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.26−26.82 (6 × CH_3), 67.0 (CH_2), 69.0 (CH_2), 69.2 (CH_2), 70.9 (CH_2), 71.1 (CH_2), 78.6 (CH), 79.2 (CH), 79.3 (CH), 81.5 (CH), 81.6 (CH), 82.0 (CH), 82.1 (CH), 82.5 (CH), 83.0 (CH), 105.1 (3CH), 111.6 (2C), 111.7 (C), 117.4 (CH_2), 117.5 (CH_2), 133.9 (CH), 134.1 (CH); HRMS (ESI−QToF, positive ion) m/z calcd for $C_{30}H_{46}NaO_{13}$, 637.2836; found, 637.2838.

(2*R*,3*R*,3a*S*,5a*R*,7*R*,8*R*,8a*S*,14a*S*,15*R*,16*R*,17a*R*,20a*R*)-2,3:7,8:15,16-Tri-*O*-isopro-pylidene-octadecahydro-2*H*-trifuro-[3,2-*b*:3′,2′-*f*:2″,3″-*k*][1,5,9,13]tetraoxacyclo-heptadecane (11). RCM was carried out using **10** (850 mg, 1.38 mmol), second-generation Grubbs catalyst (59 mg, 0.069 mmol), and benzene (50 mL) following the procedure as described for the preparation of **4** from **3**. The usual workup and purification by column chromatography over silica gel (100−200 mesh) using petroleum ether−EtOAc (4:1) as the eluent produced a cis/trans mixture of the expected cyclic alkene (510 mg) as sticky liquid. Hydrogenation of the alkene mixture

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was carried out as described earlier using 10% Pd/C (90 mg) in dry EtOAc (20 mL). Workup and purification by column chromatography over silica gel (100−200 mesh) using petroleum ether−EtOAc (5:1) as the eluent afforded **11** (480 mg, 59%) as a colorless sticky liquid. $[\alpha]_D^{25}$ −39.1 (*c* 0.08, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) δ 1.31 (s, 3H), 1.32 (s, 3H), 1.33 (s, 3H), 1.48 (s, 3H), 1.49 (s, 3H), 1.51 (s, 3H), 1.61 (brs, 2H), 1.66−1.68 (m, 2H), 3.49−3.51 (m, 2H), 3.69−3.77 (m, 4H), 3.86 (d, 1H, *J* = 3.6 Hz), 3.80−3.85 (m, 3H), 3.91 (d, 1H, *J* = 3.6 Hz), 3.99 (d, 1H, *J* = 3.0 Hz), 4.34−4.39 (m, 3H), 4.52−4.59 (m, 1H), 4.54 (d, 1H, *J* = 3.6 Hz), 4.56 (d, 1H, *J* = 3.6 Hz), 4.59 (d, 1H, *J* = 3.6 Hz), 5.86 (d, 1H, *J* = 3.6 Hz), 5.88 (t, 2H, *J* = 3.0, 3.6 Hz); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 25.9 (CH_2), 26.3 (CH_3), 26.4 ($2CH_3$), 26.8 (CH_3), 26.85 (CH_3), 26.9 (CH_3), 27.0 (CH_2), 67.4 (CH_2), 67.7 (CH_2), 69.2 (CH_2), 69.7 (CH_2), 70.1 (CH_2), 78.2 (CH), 78.2 (CH), 78.4 (CH), 81.6 (CH), 81.8 (CH), 82.2 (CH), 82.4 (CH), 83.0 (CH), 83.4 (CH), 104.8 (CH), 104.8 (CH), 105.0 (CH), 111.7 (C), 111.8 (C), 111.9 (C); HRMS (ESI−QToF, positive ion) *m*/*z* calcd for $C_{28}H_{44}NaO_{13}$, 611.2680; found, 611.2690.

1,2:1′,2′:1″,2″:1‴,2‴-Tetra-*O*-isopropylidene-3-*O*-allyl-5-*O*-[5-*O*-[3-*O*-(5-deoxy-3-*O*-allyl-α-D-xylofuranos-5-yl)-5-deoxy-α-D-xylofuranos-5-yl]-3-deoxy-α-D-xylo-furanos-3-yl]-α-D-xylofuranose (12). Mesylation of **9** was carried out according to the procedure described for the preparation of **6**[15c] using **9** (2.65 g, 6.59 mmol), CH_3SO_2Cl (0.71 mL, 9.23 mmol), Et_3N (1.29 mL, 9.23 mmol), and CH_2Cl_2 (60 mL). The usual workup afforded the respective mesyl derivative of **9** (3.08 g) as pale-yellow oil, which was used in the next step without further purification. Alkylation of **9** by the mesyl derivative was carried out by following the method as described for the preparation of **10** using **9** (2.1 g, 5.22 mmol), the mesyl derivative of **9** (3.0 g, 6.25 mmol), Bu_4NI (195 mg, 0.53 mmol), and 15% aqueous NaOH (40 mL). The usual workup and purification by column chromatography over silica gel (230−400 mesh) using petroleum ether−EtOAc (5:1) as the eluent afforded **12** (2.25 g, 55%) as a colorless sticky liquid. $[\alpha]_D^{25}$ −56.5 (*c* 0.48, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) δ 1.29 (s, 6H), 1.32 (s, 6H), 1.49 (s, 6H), 1.50 (s, 6H), 3.65−3.70 (m, 4H), 3.77−3.85 (m, 4H), 3.86 (d, 2H, *J* = 3.6 Hz), 3.92 (dd, 2H, *J* = 3.6, 13.8 Hz), 3.96 (dd, 2H, *J* = 6.6, 13.8 Hz), 4.14 (dd, 2H, *J* = 3.6, 12.0 Hz), 4.29−4.32 (m, 2H), 4.37−4.39 (m, 2H), 4.54 (d, 2H, *J* = 3.6 Hz), 4.56 (dd, 2H, *J* = 3.6, 7.2 Hz), 5.21 (d, 2H, *J* = 10.8 Hz), 5.30 (d, 2H, *J* = 17.4 Hz), 5.85−5.90 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 26.2 ($2CH_3$), 26.3 ($2CH_3$), 26.8 ($2CH_3$), 26.8 ($2CH_3$), 66.9 ($2CH_2$), 69.2 ($2CH_2$), 70.9 ($2CH_2$), 78.5 (2CH), 79.3 (2CH), 81.5 (2CH), 81.9 (2CH), 82.1 (2CH), 83.0 (2CH), 105.1 (2CH), 105.1 (2CH), 111.6 (2C), 111.7 (2C), 117.4 ($2CH_2$), 133.9 (2CH); HRMS (ESI−QToF, positive ion) *m*/*z* calcd for $C_{38}H_{58}NaO_{17}$, 809.3572; found, 809.3553.

(2*R*,3*R*,3a*S*,5a*R*,7*S*,8*R*,8a*S*,14a*S*,15*R*,16*R*,17a*R*,19a*S*,20*R*,21*R*,22a*R*,25a*R*)-2,3:7,8:15,16:20,21-Tetra-*O*-isopropylidene-tetracosahydrotetrafuro[3,2-*b*:3′,2′-*f*:2″,3‴-*k*:2‴,3⁗-*o*][1,5,9,13,17]pentaoxacyclohenicosane (13). RCM of **12** (1.20 g, 1.53 mmol) in refluxing benzene (80 mL) was carried out using second-generation Grubbs catalyst (65.4 mg, 0.077 mmol) in benzene (10 mL) under N_2 following the procedure described for the preparation of **4**. Workup and purification by column chromatography over silica gel (230−400 mesh) using petroleum ether−EtOAc (3:1) as the eluent furnished a cis/trans mixture of a cyclic alkene (640 mg, 55%) as sticky liquid, which was finally hydrogenated following the method described earlier using 10% Pd/C (85 mg) and EtOAc (30 mL). The product was purified by column chromatography over silica gel (230−400 mesh) using petroleum ether−EtOAc (4:1) as the eluent to afford **13** (478 mg, 75%) as a colorless sticky liquid. $[\alpha]_D^{25}$ −39.1 (*c* 0.22, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.30 (s, 6H), 1.33 (s, 6H), 1.48 (s, 6H), 1.51 (s, 6H), 1.61 (brs, 4H), 3.41 (brd, 2H, *J* = 9.3 Hz), 3.63−3.82 (m, 10H), 3.64 (brd, 2H, *J* = 9.6 Hz), 3.95 (d, 2H, *J* = 3.0 Hz), 4.32−4.39 (m, 4H), 4.53 (d, 2H, *J* = 3.6 Hz), 4.59 (d, 2H, *J* = 3.9 Hz), 5.87 (d, 2H, *J* = 3.6 Hz), 5.89 (d, 2H, *J* = 3.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.1 ($2CH_2$), 26.2 ($2CH_3$), 26.3 ($2CH_3$), 26.7 ($2CH_3$), 26.9 ($2CH_3$), 67.7 ($2CH_2$), 68.5 ($2CH_2$), 69.5 ($2CH_2$), 78.1 (2CH), 78.6 (2CH), 82.1 (2CH), 82.4 (2CH), 82.7 (2CH), 82.8 (2CH), 105.0 (2CH), 105.1 (2CH), 111.6 (2C), 111.8 (2C); HRMS (ESI−QToF, positive ion) *m*/*z* calcd for $C_{36}H_{56}NaO_{17}$, 783.3415; found, 783.3404.

(2*R*,3*R*,3a*S*,9a*S*,10*R*,11*R*,12a*R*,14a*R*)-2,11-Bis(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetradecahydrodifuro[3,2-*g*:2′,3′-*k*][1,6]dioxacyclododecine-3,10-diyldiacetate (14). Ac_2O (0.3 mL, 3.2 mmol) was added to **4** (200 mg, 0.5 mmol) in HOAc (15 mL), and the mixture was cooled to 0 °C. TfOH (0.002 mL, 0.02 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction was quenched with a cold saturated $NaHCO_3$ solution (10 mL), and the mixture was extracted with CH_2Cl_2 (3 × 25 mL). The combined extract was washed with water (2 × 10 mL), dried (Na_2SO_4), and evaporated to a crude mixture of anomeric products, which was dried via coevaporation with anhydrous CH_3CN (2 × 15 mL) followed by storage in a vacuum desiccator over P_2O_5 for 12h. To a solution of the anomeric products in CH_3CN (20 mL) were added uracil (246 mg, 2.2 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (BSA, 0.73 mL, 3.0 mmol), and the mixture was heated at reflux for 45 min. The reaction mixture was cooled to 0 °C. TMSOTf (0.27 mL, 1.48 mmol) was added dropwise, and then the mixture was heated at 50 °C for 17 h. The solvent was evaporated in vacuo to a residue, and a saturated $NaHCO_3$ solution (10 mL) was added to it. The mixture was extracted with EtOAc (3 × 25 mL). The solvent was washed with water (2 × 10 mL), dried (Na_2SO_4), evaporated, and finally the crude product was purified by column chromatography over silica gel (230−400 mesh) using petroleum ether−EtOAc (1:4) as the eluent to afford **14** (196 mg, 66%) as sticky liquid. $[\alpha]_D^{25}$ −13.4 (*c* 0.08, CH_3OH); ^1H NMR (CD_3OD, 300 MHz) δ 1.59−1.87 (m, 8H), 2.13 (s, 6H), 3.76 (brs, 4H), 3.95 (d, 2H, *J* = 2.7 Hz), 4.21 (brs, 2H), 5.09 (s, 2H), 5.70 (d, 2H, *J* = 8.1 Hz), 5.95 (s, 2H), 7.74 (d, 2H, *J* = 8.1 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 20.9 ($2CH_3$), 24.6 ($2CH_2$), 28.0 ($2CH_2$), 70.8 ($2CH_2$), 80.4 (2CH), 81.6 (2CH), 84.6 (2CH), 90.6 (2CH), 102.4 (2CH), 142.8 (2CH), 152.3 (2C), 166.4 (2C), 171.5 (2C); HRMS (ESI−QToF, positive ion) *m*/*z* calcd for $C_{26}H_{32}N_4NaO_{12}$, 615.1914; found, 615.1917.

1,1′-((2*R*,3*R*,3a*R*,9a*R*,10*R*,11*R*,12a*R*,14a*R*)-3,10-Dihydroxytetradecahydrodifuro[3,2-*g*:2′,3′-*k*][1,6]-dioxacyclododecine-2,11-diyl)dipyrimidine-2,4(1*H*,3*H*)-dione (15). To a solution of **14** (150 mg, 0.253 mmol) in dry MeOH (15 mL) at 0 °C under N_2 was added K_2CO_3 (105 mg, 0.76 mmol), and the mixture was stirred at room temperature for 3 h. The solvent was evaporated in vacuo, the K_2CO_3 was neutralized with a NH_4Cl solution, and the mixture was extracted using EtOAc (2 × 25 mL). The solvent was evaporated, and the crude product was purified by column chromatography over silica gel (230−400 mesh) using EtOAc−MeOH (49:1) as the eluent to afford **15** (110 mg, 86%) as sticky liquid. $[\alpha]_D^{25}$ −19.7 (*c* 0.16, CH_3OH); ^1H NMR (CD_3OD, 600 MHz) δ 1.51−1.54 (m, 2H), 1.79 (d, 2H, *J* = 6.0 Hz), 1.88 (d, 4H, *J* = 4.2 Hz), 3.62−3.64 (m, 4H), 3.76 (d, 2H, *J* = 3.0 Hz), 4.19 (s, 2H), 4.34 (brt, 2H, *J* = 2.4 Hz), 5.65 (d, 2H, *J* = 7.8 Hz), 5.78 (s, 2H), 7.75 (d, 2H, *J* = 8.4 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 24.9 ($2CH_2$), 28.1 ($2CH_2$), 70.6 ($2CH_2$), 79.7 (2CH), 82.7 (2CH), 84.9 (2CH), 93.7 (2CH), 101.4 (2CH), 143.3 (2CH), 152.4 (2C), 166.6 (2C); HRMS (ESI−QToF, positive ion) *m*/*z* calcd for $C_{22}H_{28}N_4NaO_{10}$, 531.1703; found, 531.1752.

(2*R*,3*R*,3a*S*,9a*S*,10*R*,11*R*,12a*R*,15a*R*)-2,11-Bis(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetradecahydrodifuro[3,2-*b*:2′,3′-*g*][1,5,9]trioxacyclotridecine-3,10-diyldiacetate (16). Deprotection and peracetylation of the acetonide rings of **8** (400 mg, 0.96 mmol) were carried out using HOAc (20 mL), Ac_2O (0.54 mL, 5.76 mmol), and TfOH (0.003 mL, 0.032 mmol) to furnish a mixture of acetates, which was subjected to nucleosidation according to the method described for the preparation of **14** using uracil (473 mg, 4.22 mmol), BSA (1.4 mL, 5.74 mmol), TMSOTf (0.52 mL, 2.86 mmol), and CH_3CN (20 mL). The usual workup and purification by column chromatography over silica gel (100−200 mesh) using petroleum ether−EtOAc (1:4) as the eluent afforded **16** (350 mg, 60%) as sticky liquid. $[\alpha]_D^{25}$ −3.4 (*c* 0.15, CH_3OH); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.69−1.77 (m, 4H), 2.14 (s, 6H), 3.60−3.63 (m, 2H), 3.71−3.77 (m, 4H), 3.98−4.03 (m, 4H), 4.28−4.31 (m, 2H), 5.19 (t, 2H, *J* = 2.4 Hz), 5.71 (dd, 2H, *J* = 1.5, 8.1 Hz), 6.08 (d, 2H, *J* = 3.0 Hz),7.78 (d, 2H, *J* =

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8.4 Hz), 9.12 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.7 ($2CH_3$), 26.3 ($2CH_2$), 67.8 ($2CH_2$), 70.0 ($2CH_2$), 79.1 (2CH), 79.7 (2CH), 80.1 (2CH), 87.0 (2CH), 102.4 (2CH), 140.3 (2CH), 150.4 (2C), 163.4 (2C), 169.8 (2C); HRMS (ESI−QToF, positive ion) m/z calcd for $C_{26}H_{33}N_4O_{13}$, 609.2044; found, 609.2044.

1,1′-((2*R*,3*R*,3a*R*,9a*R*,10*R*,11*R*,12a*R*,15a*R*)-3,10-Dihydroxytetradecahydrodifuro[3,2-*b*:2′,3′-*g*][1,5,9]-trioxacyclotridecine-2,11-diyl)dipyrimidine-2,4(1*H*,3*H*)-dione (17). Deacetylation of **16** (220 mg, 0.362 mmol) in dry MeOH (20 mL) at 0 °C was carried out following the procedure described for the preparation of **15** from **14** using K_2CO_3 (152 mg, 1.1 mmol). Workup and purification by column chromatography over silica gel (230−400 mesh) using EtOAc−MeOH (19:1) as the eluent afforded **17** (170 mg, 90%) as sticky liquid. $[\alpha]_D^{25}$ −3.6 (c 0.1, CH_3OH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.59 (brs, 2H), 1.76 (brs, 2H), 3.68 (s, 4H), 3.74 (dd, 2H, J = 2.4, 9.6 Hz), 3.90 (q, 2H, J = 2.4 Hz), 4.01 (dd, 2H, J = 7.2, 9.6 Hz), 4.23 (t, 2H, J = 2.4 Hz), 4.39−4.41 (m, 2H), 5.69 (d, 2H, J = 8.4 Hz), 5.82 (d, 2H, J = 2.4 Hz), 7.94 (d, 2H, J = 8.4 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 27.8 ($2CH_2$), 69.1 ($2CH_2$), 71.5 ($2CH_2$), 79.9 (2CH), 82.1 (2CH), 84.0 (2CH), 91.9 (2CH), 102.0 (2CH), 143.0 (2CH), two quaternary carbons were not discernible; HRMS (ESI−QToF, positive ion) m/z calcd for $C_{22}H_{29}N_4O_{11}$, 525.1833; found, 525.1845.

(2*R*,3*R*,3a*S*,5a*R*,7*R*,8*R*,8a*S*,14a*S*,15*R*,16*R*,17a*R*,20a*R*)-2,7,16-Tris(2,4-dioxo-3,4-dihydro-pyrimidin-1(2*H*)-yl)octadecahydro-2*H*-trifuro[3,2-*b*:3′,2′-*f*:2″,3″-*k*][1,5,9,13]tetraoxacyclo-heptadecine-3,8,15-triyl Triacetate (18). A mixture of CH_3CN−H_2O−concentrated H_2SO_4 (18:6:1, 10 mL) was added to **11** (200 mg, 0.34 mmol), and the mixture was stirred at 25 °C for 12 h. The solution was neutralized by adding solid $CaCO_3$ and then filtered. The residue was washed with CH_3CN (2 × 5 mL), and the combined filtrate was concentrated under reduced pressure to afford a crude alcohol (anomeric mixture). To a solution of the alcohol (140 mg) in pyridine (8 mL) at 0 °C were added Ac_2O (0.25 mL, 2.70 mmol) and DMAP (4 mg, 0.03 mmol), and the solution was stirred at 0 °C for 1 h and then at 25 °C for 13 h. The solvent was removed, crushed ice was added to the residue, and the crude product was extracted with $CHCl_3$ (3 × 20 mL). The solvent was successively washed with HCl (20 mL, 1 M), a saturated $NaHCO_3$ solution (2 × 15 mL), and brine (2 × 15 mL), then dried (Na_2SO_4) and evaporated to a residue, which was purified by column chromatography over silica gel (100−200 mesh) using petroleum ether− EtOAc (3:2) to afford a mixture of acetates (160 mg, 0.222 mmol) as a colorless oil. Nucleosidation on the acetate mixture was carried out using uracil (165 mg, 1.47 mmol), BSA (0.49 mL, 2.0 mmol), TMSOTf (0.18 mL, 1.0 mmol), and CH_3CN (20 mL) following the method used for the preparation of **14**. The usual workup and purification by column chromatography over silica gel (230−400 mesh) using EtOAc−MeOH (19:1) as the eluent furnished **18** (117 mg, 60%) as sticky liquid. $[\alpha]_D^{25}$ +23.7 (c 0.3, CH_3OH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.15−1.61 (m, 2H), 1.72−1.79 (m, 2H), 2.12 (s, 3H), 2.13 (s, 3H), 2.14 (s, 3H), 3.64−3.66 (m, 2H), 3.68−3.71 (m, 1H), 3.73−3.75 (m, 1H), 3.81 (dd, 1H, J = 3.0, 10.2 Hz), 3.85 (dd, 1H, J = 3.6, 10.2 Hz), 3.93 (d, 1H, J = 4.2 Hz), 3.97−4.03 (m, 3H), 4.11 (t-like, 1H, J = 3.6, 4.8 Hz), 4.19 (q, 1H, J = 6.0 Hz), 4.28 (t−like, 1H, J = 3.6, 4.8 Hz), 4.37 (q, 1H, J = 6.0 Hz), 4.40−4.45 (m, 2H), 5.11 (s, 1H), 5.25 (t, 1H, J = 3.0 Hz), 5.33 (t, 1H, J = 3.6 Hz), 5.68 (d, 1H, J = 8.4 Hz), 5.72 (d, 1H, J = 8.4 Hz), 5.75 (d, 1H, J = 8.4 Hz), 5.99 (d, 1H, J = 3.0 Hz), 6.00 (d, 1H, J = 3.6 Hz), 6.03 (d, 1H, J = 1.8 Hz), 7.73 (d, 1H, J = 8.4 Hz), 7.86 (d, 1H, J = 8.4 Hz), 7.91 (d, 1H, J = 8.4 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 20.8 (CH_3), 20.85 (CH_3), 20.87 (CH_3), 27.0 (CH_2), 27.5 (CH_2), 69.0 (CH_2), 69.8 (CH_2), 69.9 (CH_2), 70.5 (CH_2), 71.9 (CH_2), 79.8 (CH), 81.0 (CH), 81.05 (CH), 81.09 (CH), 81.2 (CH), 81.4 (CH), 81.5 (CH), 81.9 (CH), 82.1 (CH), 88.5 (CH), 88.6 (CH), 90.2 (CH), 102.7 (CH), 102.9 (CH), 103.0 (CH), 142.2 (CH), 142.4 (CH), 142.5 (CH), 152.18 (C), 152.25 (C), 152.33 (C), 166.06 (C), 166.14 (C), 171.6 (C), 171.9 (2C), one quaternary carbon not discernible; HRMS (ESI−QToF, positive ion) m/z calcd for $C_{37}H_{44}N_6NaO_{19}$, 899.2559; found, 899.2567.

1,1′,1″-((2*R*,3*R*,3a*R*,5a*R*,7*R*,8*R*,8a*R*,14a*R*,15*R*,16*R*,17a*R*,20a*R*)-3,8,15-Trihydroxy-octadecahydro-2H-trifuro[3,2-*b*:3′,2′-*f*:2″,3″-*k*][1,5,9,13]tetraoxacycloheptadecine-2,7,16-triyl)-tripyrimidine-2,4(1*H*,3*H*)-dione (19). Hydrolysis of triacetate **18** (110 mg, 0.126 mmol) in dry MeOH (15 mL) at 0 °C using K_2CO_3 (78 mg, 0.567 mmol) was carried out according to the procedure described for the preparation of **15** from **14**. The usual workup and column chromatography over silica gel (230−400 mesh) using EtOAc−MeOH (17:3) as the eluent furnished **19** (78 mg, 82%) as sticky liquid. $[\alpha]_D^{25}$ −0.3 (c 0.14, CH_3OH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.54−1.59 (m, 2H), 1.72−1.80 (m, 2H), 3.57−3.68 (m, 4H), 3.81 (dd, 1H, J = 3.6, 9.6 Hz), 3.83−3.87 (m, 2H), 3.86 (d, 1H, J = 3.0 Hz), 3.96−4.01 (m, 3H), 4.11 (t, 1H, J = 4.8 Hz), 4.15 (dd, 1H, J = 6.6, 10.8 Hz), 4.25 (s, 1H), 4.28−4.30 (m, 2H), 4.42−4.46 (m, 3H), 5.67 (d, 1H, J = 8.4 Hz), 5.70 (t, 2H, J = 7.8, 8.4 Hz), 5.81 (d, 1H, J = 4.2 Hz), 5.82 (d, 1H, J = 3.0 Hz), 5.86 (d, 1H, J = 3.6 Hz), 7.74 (d, 1H, J = 7.8 Hz), 7.80 (d, 1H, J = 7.8 Hz), 7.91 (d, 1H, J = 8.4 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 27.4 (CH_2), 27.5 (CH_2), 68.9 (CH_2), 69.0 (CH_2), 70.6 ($2CH_2$), 71.3 (CH_2), 78.9 (CH), 79.3 (CH), 80.1 (CH), 81.2 (CH), 81.9 (CH), 82.2 (CH), 83.5 (CH), 83.7 (CH), 84.6 (CH), 91.1 (CH), 92.0 (CH), 93.1 (CH), 102.0 (CH), 102.02 (CH), 102.6 (CH), 142.7 (CH), 142.9 (CH), 142.91 (CH), 152.5 (C), 152.6 (C), 152.8 (C), 166.5 (C), 166.6 (C), 166.8 (C); HRMS (ESI−QToF, positive ion) m/z calcd for $C_{31}H_{38}N_6NaO_{16}$, 773.2242; found, 773.2219.

(2*R*,3*R*,3a*S*,5a*R*,7*R*,8*R*,8a*S*,14a*S*,15*R*,16*R*,17a*R*,19a*S*,20*R*,21*R*,22a*R*,25a*R*)-2,7,16,21-Tetrakis(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetracosahydrotetrafuro[3,2-*b*:3′,2′-*f*:2″,3″-*k*:2‴,3‴-*o*][1,5,9,13,17]pentaoxacyclohenicosine-3,8,15,20-tetrayltetraacetate (20). Deprotection of acetonide groups and subsequent acetylation was carried out with **13** (250 mg, 0.329 mmol) using CH_3CN−H_2O−concentrated H_2SO_4 (18:6:1, 15 mL), Ac_2O (0.35 mL, 3.72 mmol), and DMAP (4 mg, 0.03 mmol) according to the method described for the preparation of **18**. The usual workup and purification by column chromatography over silica gel (100−200 mesh) using petroleum ether−EtOAc (3:2) as the eluent afforded a mixture of anomeric acetates (240 mg, 0.256 mmol) as a colorless oil, which was dried under vacuum over P_2O_5. Installation of a uracil base was carried out on the above acetates using uracil (252 mg, 2.25 mmol), BSA (0.75 mL, 3.07 mmol), TMSOTf (0.28 mL, 1.54 mmol), and CH_3CN (20 mL) following the method described for the preparation of **14**. The usual workup and purification by column chromatography over silica gel (230−400 mesh) using EtOAc−MeOH (23:2) as the eluent gave **20** (190 mg, 65%) as sticky liquid. $[\alpha]_D^{25}$ +49.7 (c 0.16, CH_3OH); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.59−1.64 (m, 4H), 2.13 (s, 12H), 3.55−3.57 (m, 2H), 3.71−3.73 (m, 2H), 3.88 (dd, 2H, J = 4.8, 10.2 Hz), 3.98 (quint, 4H, J = 5.4 Hz), 4.04 (dd, 2H, J = 4.2, 11.4 Hz), 4.10 (dd, 2H, J = 2.4, 4.8 Hz), 4.21 (dd, 2H, J = 3.0, 4.2 Hz), 4.43 (q, 2H, J = 2.4 Hz), 4.49 (q, 2H, J = 4.8 Hz), 5.19 (t, 2H, J = 2.4 Hz), 5.30 (t, 2H, J = 3.0 Hz), 5.66 (d, 2H, J = 8.4 Hz), 5.74 (d, 2H, J = 7.8 Hz), 6.02 (d, 4H, J = 3.0 Hz), 7.79 (d, 2H, J = 8.4 Hz), 7.87 (d, 2H, J = 7.8 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 20.85 ($2CH_3$), 20.88 ($2CH_3$), 27.6 ($2CH_2$), 69.7 ($2CH_2$), 70.2 ($2CH_2$), 71.4 ($2CH_2$), 80.6 (2CH), 80.9 (2CH), 81.4 (2CH), 81.6 (2CH), 82.1 (2CH), 82.2 (2CH), 89.1 (2CH), 89.4 (2CH), 102.9 (2CH), 103.1 (2CH), 142.2 (2CH), 142.7 (2CH), 152.3 (2C), 152.34 (2C), 166.1 (2C), 171.7 (2C), 172.0 (2C), two quaternary carbons not discernible; HRMS (ESI−QToF, positive ion) m/z calcd for $C_{48}H_{56}N_8NaO_{25}$, 1167.3254; found, 1167.3269.

1,1′,1′,1″-((2*R*,3*R*,3a*R*,5a*R*,7*R*,8*R*,8a*R*,14a*R*,15*R*,16*R*,17a*R*,19-a*R*,20*R*,21*R*,22a*R*,25a*R*)-3,8,15,20-Tetrahydroxytetracosahy-drotetrafuro[3,2-*b*:3′,2′-*f*:2″,3″-*k*:2‴,3‴-*o*][1,5,9,13,17]-pentaoxacyclohenicosine-2,7,16,21-tetrayl)tetrakis(pyridine-2,4(1*H*,3*H*)-dione) (21). To a solution of **20** (20 mg, 0.017 mmol) in dry MeOH (8 mL) at 0 °C under N_2 was added metallic Na (5 mg), and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was neutralized with Amberlite IR120 H resin. The resin was filtered off and the solvent was evaporated to a residue, which was washed with petroleum ether−EtOAc (1:19, 3 × 5 mL) and dried to afford **21** (13 mg, 76%) as a foam sufficiently pure for characterization. $[\alpha]_D^{25}$ +9.7 (c 0.14, CH_3OH); ^{1}H NMR (DMSO-d_6,

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600 MHz) δ 1.53 (brs, 4H), 3.29 (d, 4H, J = 5.4 Hz), 3.74−3.96 (m, 12H), 4.14 (brs, 2H), 4.16 (brs, 2H), 4.30 (brs, 4H), 5.52 (d, 2H, J = 8.4 Hz), 5.63 (d, 2H, J = 7.8 Hz), 5.73 (s, 2H), 5.75 (s, 2H), 5.92 (s, 2H), 6.01 (s, 2H), 7.63 (d, 2H, J = 7.8 Hz), 7.68 (d, 2H, J = 7.8 Hz), 11.33 (brs, 4H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 26.2 ($2CH_2$), 67.6 ($2CH_2$), 68.4 ($2CH_2$), 69.2 ($2CH_2$), 76.7 (2CH), 77.6 (2CH), 79.1 (2CH), 79.4 (2CH), 82.3 (2CH), 82.9 (2CH), 89.3 (4CH), 101.4 (2CH), 101.6 (2CH), 140.7 (2CH), 141.1 (2CH), 150.6 (2C), 150.7 (2C), 163.1 (2C), 163.2 (2C); HRMS (ESI−QToF, positive ion) m/z calcd for $C_{40}H_{48}N_8NaO_{21}$, 999.2832; found, 999.2851.

Materials and Methods. Berberine (BER), palmatine (PAL), and ethidium bromide (EB) were obtained from a chemical supplier. Concentrations were determined by absorbance measurements using wavelengths and molar absorption coefficients (M^{-1} cm^{-1}) as reported in the literature.[34] Concentrations of macrocyclic oligonucleosides were determined by weighing accurately. Glass-distilled deionized water and analytical-grade reagents were used throughout all the experiments.

Biophysical Studies. All absorption spectral studies were carried out at 25 ± 0.5 °C on a spectrophotometer in 1 cm path length cuvettes. After each addition of an aliquot, the solution was allowed to re-equilibrate for at least 10 min before noting the absorbance at the desired wavelength maximum. Steady-state fluorescence emission spectra were recorded on a spectrofluorimeter in 1 cm path length cuvettes with an excitation and emission bandpass of 5 nm. The excitation wavelengths for BER, PAL, and EB were 345, 346, and 495 nm, respectively.

A spectropolarimeter equipped with a temperature controller was used for measuring CD spectra at 25 ± 0.5 °C as described previously.[35] The instrument parameters were set as scanning speed of 100 nm/min, bandwidth of 1.0 nm, and sensitivity of 100 millidegree. Each spectrum represents an average of five scans; the baseline was corrected and smoothed within permissible limits using the inbuilt software of the unit.

SEM imaging was carried out using digital microscopy imaging. SEM measurements were carried out on a glass slide using 10 μL of samples dried completely under a dryer. The samples were fastened on the sample holder using carbon tape. Gold coating was carried out with current 10 mA at 10^{-6}−10^{-8} mbar/Pa.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of new compounds **2**−**4**, **8**, and **10**−**21** and known compounds **1**, **7**, and **9**; ORTEP diagram of **8**; CIF file for the X-ray data of **8**; absorption and CD spectra of **14**, **15**, **17**, **19**, and **21**; absorption and fluorescence spectra of complexes between berberine, palmatine, or ethidium bromide and nucleosides **16**−**20**; low-temperature aggregation studies of **16**, **18**, and **20**; and structures of possible intermolecular H-bonding between two uracil moieties. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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