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Chemoenzymatic Convergent Synthesis of 2′-*O*,4′-*C*-Methyleneribonucleosides

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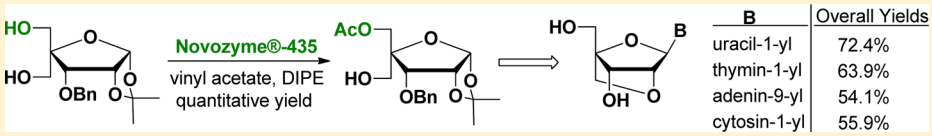
Supporting Information



B	Overall Yields
uracil-1-yl	72.4%
thymin-1-yl	63.9%
adenin-9-yl	54.1%
cytosin-1-yl	55.9%

ABSTRACT: Novozyme-435-catalyzed efficient regioselective acetylation of one of the two diastereotopic hydroxymethyl functions in 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose has been achieved. The enzymatic methodology has been successfully utilized for convergent synthesis of bicyclic nucleosides (LNA monomers) T, U, A, and C. Further, it has been demonstrated that Novozyme-435 can be used for 10 cycles of the acylation reaction without losing selectivity and efficiency.

The FDA approval of antisense drugs vitravene[1] and kynamro[2] has catalyzed the ongoing research on development of various types of backbone,[3] base,[4] and/or sugar-modified[5] antisense oligonucleotides (AONs). Among the numerous modified nucleic acid analogues, locked nucleic acid (LNA **2**, Figure 1) that has an N-type (C3′-*endo*) sugar

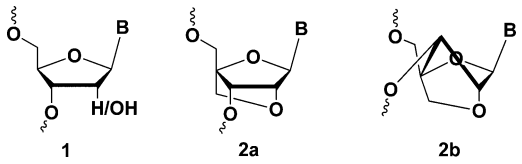


Figure 1. Structure of DNA/RNA **1**; structural representation of LNA monomer **2a**,**b**; B = nucleobase.

puckering due to the presence of a 2′-*O*,4′-*C* methylene linkage has shown promising applications in chemical biology.[6−9] Presently, more than five LNA-modified oligonucleotides are under active clinical trials, which demonstrates the commercial potential of these compounds.[10]

The linear approach employed for the synthesis of LNA monomers suffers from poor yields and could not be employed as a general method for the synthesis of all monomers.[11] In the convergent synthesis, a common glycosyl donor is synthesized for coupling with different nucleobases.[7,12,13] The earlier use of diol sugar precursor 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**3**) for the synthesis of a common glycosyl donor is limited because it requires selective protection of one of the two primary hydroxyl groups.[7] To overcome this limitation, di-*O*-mesylated derivative of the diol **3** has been employed, but this necessitates an additional two steps to remove the mesyl function toward the end of the synthesis (Supporting Information SI-Scheme 1).[12,13] It is at this juncture that the selectivity of lipases can be explored for discrimination between two primary hydroxyl groups in the precursor diol **3**.[14] We herein report efficient and novel chemoenzymatic synthesis of bicyclic nucleosides T, U, A, and C via lipase-mediated regioselective acetylation of diol sugar precursors **3**.

The diol **3** was synthesized from 1,2,5,6-di-*O*-isopropylidene-α-D-allofuranose (**5**) following a literature procedure,[15] which in turn was synthesized from its gluco-epimer **4** via DMSO/P_2O_5 oxidation and selective reduction using $NaBH_4$ (Scheme 1).[16] Lipases from different sources, such as *Candida antarctica* lipase-B (Novozyme-435), *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme TL IM), *Candida rugosa* lipase (CRL), and porcine pancreatic lipase (PPL) were screened in five sets of organic solvents, such as tetrahydrofuran (THF), acetonitrile (MeCN), toluene, diisopropyl ether (DIPE), and acetone using acetic anhydride/vinyl acetate as acetyl donor at 45 °C and at 200 rpm in an incubator shaker for the study of selective acetylation of one over the other primary hydroxyl function in diol **3** (Scheme 1). The solvent engineering, that is, the change of selectivity of lipases in different organic solvents, was first demonstrated by Klibanov et al. in the 1980s.[17,18] Since then, the use of lipases in organic solvents for acylation/deacylation reactions on suitable alcohols have been well studied to achieve optimum enantio-, regio-, and chemoselectivity.

Incubation of diol **3** with Novozyme-435 in the above-mentioned solvents in the presence of vinyl acetate as acetyl donor led to the formation of a single product with slightly higher R_f value than the diol, as indicated by TLC/HPLC

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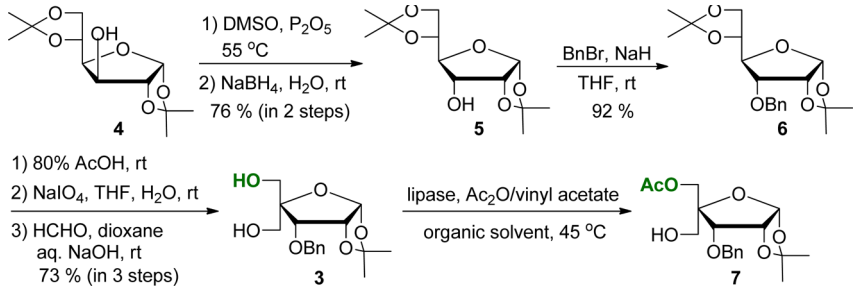
Scheme 1. Synthesis and Regioselective Novozyme-435-Catalyzed Acetylation Studies on Diol 3



examination. On comparison of the rate of acetylation reaction of diol catalyzed by Novozyme-435, it was observed that the reaction in DIPE was relatively faster. Further, regioselectivity was poor when acetic anhydride was used as acetyl donor in different organic solvents in the presence of the lipase. Lipozyme TL IM, CRL, and PPL did not accept diol **3** as a substrate. Based on the screening results, Novozyme-435 in DIPE in the presence of vinyl acetate was selected for regioselective acetylation of one of the two primary hydroxyl groups in sugar diol **3** (Figure 2).

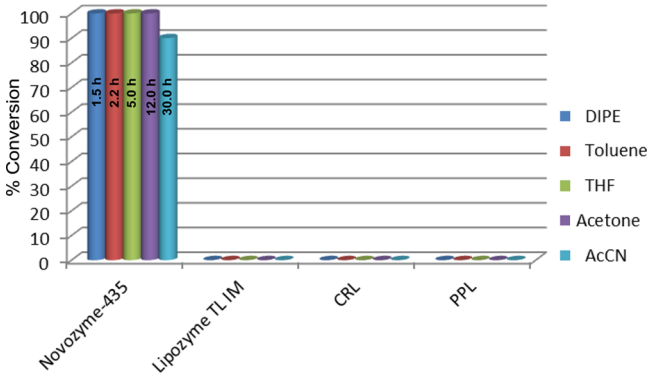


Figure 2. Screening of different lipases in organic solvents of varying polarity at 45 °C for selective acetylation of diol **3**. The acetylation reaction mediated by vinyl acetate in organic solvents did not yield any product when performed in the absence of Novozyme-435.

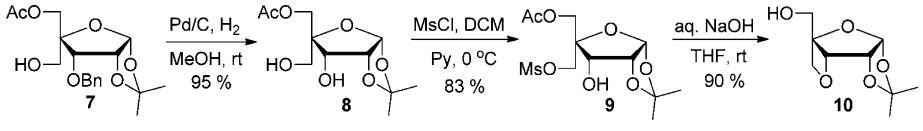
In a model reaction, a solution of compound **3** and vinyl acetate in DIPE was incubated with Novozyme-435 (10% w/w of **3**) at 45 °C and 200 rpm in an incubator shaker, and the progress of the reaction was monitored on TLC/HPLC. On completion, the reaction was quenched by filtering off the enzyme, and solvent was removed under reduced pressure to afford the crude product, which on stirring with hexane led to the isolation of pure monoacetylated compound 5-*O*-acetyl-3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**7**) in quantitative yield. Using optimized conditions, Novozyme-435 was utilized for 10 cycles for selective acetylation of **3** and was found to be efficient and equally regioselective for each cycle. Further, the lipase-mediated acetylation reaction was successfully scaled up under the same condition for selective acetylation of diol **3** up to 5 g scale. The acetylation reaction carried out on compound **3** in the absence of Novozyme-435 did not yield any product.

The structure of the monoacetylated compound **7** was confirmed by its chemical transformation to 1,2-*O*-isopropylidene-3-*O*,4-*C*-methylene-α-D-ribofuranose (**10**) (Scheme 2). Thus, the debenzylation of **7** afforded the dihydroxy compound **8**, which on selective mesylation of the primary hydroxyl group followed by the treatment of the resulting mesylated derivative **9** with aqueous NaOH solution resulted into concomitant intramolecular cyclization to yield the bicyclic compound **10**. The structure of bicyclic compound **10** was unambiguously confirmed by its spectral (^{1}H and ^{13}C NMR spectra and HRMS) data analysis and comparison of chemical shift value of oxetanomethylene protons with the chemical shift value of the corresponding protons in 3-*O*,4-*C*-methyleneribothymidine.[19]

The synthesis of LNA monomers T, U, A, and C **15a–d** was successfully achieved from monoacetylated compound **7** (Scheme 3). Thus, the common glycosyl donor **12** was synthesized by tosylation of monoacetylated compound **7** followed by acetolysis of the tosyl derivative **11** in 95% overall yield. The coupling reaction of compound **12** with nucleobases, uracil, thymine, 6-*N*-benzoyladenine, and cytosine under Vorbrüggen's conditions[20] yielded the corresponding nucleosides **13a–d** in 71–89% yields. Subsequently, deacetylation with concomitant intramolecular 2′-*O*,4′-*C*-cyclization in nucleosides **13a–d** under alkaline condition afforded 3′-*O*-benzyl-2′-*O*,4′-*C*-methyleneribonucleosides **14a–d** in 89–95% yields. The debenzylation of compounds **14a–d** with palladium hydroxide afforded the LNA monomers, that is, 2′-*O*,4′-*C*-methyleneuridine (**15a**), 2′-*O*,4′-*C*-methylenethymidine (**15b**), 2′-*O*,4′-*C*-methyleneadenosine (**15c**), and 2′-*O*,4′-*C*-methylenecytidine (**15d**) in 81–91% yields (Scheme 3).

The overall yields of LNA monomer synthesis starting from diol **3** were compared for the developed chemoenzymatic convergent synthesis to that of the reported classical chemical methodology (SI-Scheme 1).[12,13] The results revealed that the developed biocatalytic methodology is more efficient in all cases with remarkable improvement in yields particularly for the synthesis of LNA-A (SI-Table 1). Since the lipase Novozyme-435 exclusively acetylates the C-5 hydroxyl group of the sugar precursor, 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**3**), in quantitative yield and it could

Scheme 2. Chemical Transformation Studies on Novozyme-435-Catalyzed Acetylated Product 7



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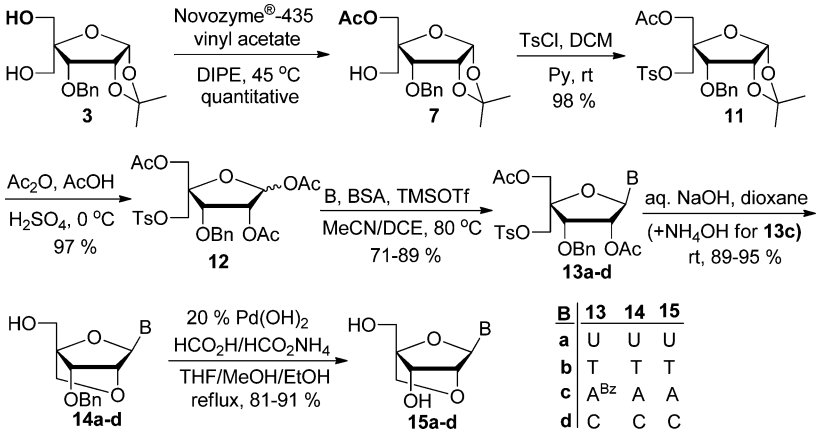
Scheme 3. Chemoenzymatic Convergent Synthesis of LNA Monomers 15a−d



be recovered and reused for the multiple cycles of selective acetylation reaction, the present methodology is greener than the reported methodologies.[12,13]

The structure of all the synthesized compounds **3**, **5**−**12**, **13a**−**d**, **14a**−**d**, and **15a**−**d** was unambiguously established on the basis of their spectral (IR, ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, ^{1}H−^{13}C HMQC, and HRMS) data analysis. The structures of known compounds **3**,[15] **5**,[16] **6**,[15] **14a**−**c**,[12,13] and **15a**−**d**[12,13] were further confirmed by the comparison of their physical and spectral data with those reported in the literature.

Herein, an efficient and environment friendly biocatalytic methodology has been developed for the first time for the convergent synthesis of LNA monomers T, U, A, and C with relatively shorter route and with significant improvement in overall yields. It has also been demonstrated that the selective enzymatic acetylation reaction on two diastereotopic hydroxymethyl functions of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose can be carried out in multiple-gram scale. The developed biocatalytic process will be useful for the commercial synthesis of LNA monomers required in various applications.

EXPERIMENTAL SECTION

Materials. The *Candida antarctica* lipase-B (CAL-B or Novozyme-435) immobilized on polyacrylate, *Candida rugosa* lipase (CRL), and porcine pancreatic lipase (PPL) were purchased from Sigma-Aldrich Co. (USA). *Theremomyces lanuginosus* lipase (Lipozyme TL IM) immobilized on silica was obtained as a gift from Novozymes Inc., Copenhagen, Denmark. All the enzymes were dried over P_2O_5 under vacuum for 24 h prior to use. For all the lipase-mediated reactions, AR-grade organic solvents were used, which were purchased from SD Fine-Chem Ltd., Mumbai, India.

5-*O*-Acetyl-3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (7). To a solution of diol **3** (2.0 g, 6.45 mmol) in DIPE (40 mL) was added vinyl acetate (0.64 mL, 7.10 mmol) followed by the addition of Novozyme-435 (10% w/w of **3**). The reaction mixture was stirred at 45 °C in an incubator shaker at 200 rpm, and the progress of the reaction was monitored periodically by TLC. After 1.5 h, the reaction was quenched by filtering off the Novozyme-435; the solvent was removed under reduced pressure, and the residue thus obtained was washed with hexane (2 × 30 mL) and dried in vacuo to afford the monoacetylated compound **7** as a white solid (2.24 g; quantitative yield): R_f = 0.3 (40% ethyl acetate in hexane); mp 55−56 °C; $[\alpha]_D^{32}$ = +93.3 (*c* 0.1, MeOH); IR (thin film) ν_{max} 3529, 3032, 2985, 2943, 1742, 1455, 1383, 1313, 1240, 1167, 1135, 1104, 1023, 873, 742, 700 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.26 and 1.48 (6H, 2s, 3H each), 1.94 (3H, s), 3.60−3.64 (1H, m), 3.82−3.87 (1H, m), 3.95 (1H, d, *J* = 11.6 Hz), 4.03 (1H, d, *J* = 5.2 Hz), 4.12 (1H, d, *J* = 11.6 Hz), 4.45 (1H, d, *J* = 8.0 Hz), 4.48 (1H, d, *J* = 12.0 Hz), 4.65 (1H, d, *J* = 12.0 Hz), 4.77 (1H, dd, *J* = 3.6 and 5.2 Hz), 5.70 (1H, d, *J* = 4.4 Hz), 7.28−7.35 (5H, m); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 20.6, 26.2, 26.5, 61.2, 64.5, 71.4, 78.2, 78.3, 85.4, 103.6, 112.5, 127.4, 127.6, 128.2, 137.9, 170.1; HR-ESI-TOF-MS *m*/*z* 375.1403 ([M + Na]$^+$), calcd for [$C_{18}H_{24}O_7$ + Na]$^+$ 375.1414.

5-*O*-Acetyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (8). To a stirred solution of compound **7** (0.50 g, 1.42 mmol) in dry methanol (10 mL) was added Pd−C (0.25 g). The reaction mixture was stirred for 4 h under H_2 atmosphere at 40 °C. The catalyst was filtered off and washed with methanol. The excess solvent was removed under reduced pressure, and the crude product thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford compound **8** as a white solid (0.35 g, 95%): R_f = 0.3 (5% methanol in chloroform); mp 54−56 °C; $[\alpha]_D^{32}$ = +30.46 (*c* 0.05, MeOH); IR (thin film) ν_{max} 3467, 2987, 2945, 1739, 1458, 1385, 1246, 1166, 1045, 1019, 874 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39 and 1.61 (6H, 2s, 3H each), 2.10 (3H, s), 2.50 (1H, t, *J* = 6.8 Hz), 3.09 (1H, d, *J* = 8.4 Hz), 3.82−3.84 (2H, m), 4.14 (1H, d, *J* = 11.6 Hz), 4.21−4.25 (2H, m), 4.71 (1H, dd, *J* = 3.6 and 6.4 Hz), 5.86 (1H, d, *J* = 4.4 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 20.8, 26.3, 26.5, 62.3, 65.8, 72.9, 79.4, 87.1, 104.7, 113.6, 170.7; HR-ESI-TOF-MS *m*/*z* 285.0940 ([M + Na]$^+$), calcd for [$C_{11}H_{18}O_7$ + Na]$^+$ 285.0945.

5-*O*-Acetyl-1,2-*O*-isopropylidene-4-*C*-methanesulfonyloxymethyl-α-D-ribofuranose (9). A solution of compound **8** (0.30 g, 1.14 mmol) and methanesulfonyl chloride (0.10 mL, 1.26 mmol) in anhydrous dichloromethane/pyridine (10 mL, 4:1) was stirred at 0 °C for 4 h. On completion, the reaction mixture was poured over 10% ice-cold hydrochloric acid solution and extracted with chloroform (3 × 100 mL). The combined organic extract was washed with saturated aqueous sodium bicarbonate solution (2 × 100 mL), dried over anhydrous sodium sulfate, and the excess solvent was removed under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using ethyl acetate in hexane as eluent to afford the mesylated compound **9** as a white solid (0.32 g, 83%): R_f = 0.5 (5% methanol in chloroform); mp 80−82 °C; $[\alpha]_D^{32}$ = +15.36 (*c* 0.05, MeOH); IR (thin film) ν_{max} 3490, 2989, 2941, 1742, 1353, 1239, 1174, 1134, 1093, 1024, 991, 967, 874, 828 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.37 and 1.64 (6H, 2s, 3H each), 2.08 (3H, s), 2.69 (1H, brs), 3.09 (3H, s), 4.12 (1H, d, *J* = 11.6 Hz), 4.20−4.24 (2H, m), 4.47 (1H, d, *J* = 11.2 Hz), 4.58 (1H, d, *J* = 11.6 Hz), 4.71 (1H, dd, *J* = 3.6 and 6.0 Hz), 5.85 (1H, d, *J* = 3.6 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 20.8, 26.1, 26.2, 38.0, 64.5, 68.6, 73.1, 79.2, 84.7, 104.7, 114.0, 170.4; HR-ESI-TOF-MS *m*/*z* 363.0711 ([M + Na]$^+$), calcd for [$C_{12}H_{20}O_9S$ + Na]$^+$ 363.0720.

1,2-*O*-Isopropylidene-3-*O*,4-*C*-methylene-α-D-ribofuranose (10). To a stirred solution of compound **9** (0.25 g, 0.73 mmol) in water/THF (2 mL, 1:1) was added 2 M NaOH (1.5 mL), and reaction mixture was stirred at rt for 1 h. The reaction mixture was extracted with ethyl acetate (3 × 100 mL), washed with saturated aqueous

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sodium bicarbonate solution (2 × 100 mL) and brine solution (2 × 100 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using ethyl acetate in hexane as gradient solvent system to give **10** as a colorless viscous oil (0.13 g, 90%): R_f = 0.5 (5% methanol in chloroform); $[\alpha]_D^{32}$ = −39.65 (*c* 0.05, MeOH); IR (thin film) ν_{max} 3372, 2922, 2119, 1740, 1372, 1222, 1177, 1017, 864 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 and 1.67 (6H, 2s, 3H each), 1.83 (1H, brs), 3.85 (2H, dd, *J* = 12.4 and 16.8 Hz), 4.56 (1H, d, *J* = 7.6 Hz), 4.60 (1H, t, *J* = 4.4 Hz), 4.68 (1H, d, *J* = 7.2 Hz), 4.89 (1H, d, *J* = 6.0 Hz), 6.10 (1H, d, *J* = 4.4 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 26.3, 27.2, 63.2, 78.1, 79.9, 84.7, 90.0, 109.1, 113.6; HR-ESI-TOF-MS *m*/*z* 225.0728 ([M + Na]$^+$), calcd for [$C_9H_{14}O_5$ + Na]$^+$ 225.0733.

5-*O*-Acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-4-*C*-*p*-toluenesulfonyloxymethyl-α-D-ribofuranose (11). A solution of compound **7** (2.0 g, 5.68 mmol) and *p*-toluenesulfonyl chloride (1.29 g, 6.82 mmol) in anhydrous dichloromethane/pyridine (40 mL, 4:1) was stirred at rt for 6 h. On completion, the reaction mixture was poured over 10% ice-cold hydrochloric acid solution and extracted with chloroform (3 × 100 mL). The combined organic extract was washed with saturated aqueous sodium bicarbonate solution (2 × 100 mL), dried over anhydrous sodium sulfate, and the excess solvent was removed under reduced pressure. The residue thus obtained was coevaporated with toluene (2 × 50 mL) and dried in vacuo to afford the tosylated compound **11** as a white solid (2.82 g; 98%): R_f = 0.3 (40% ethyl acetate in hexane); mp 80−81 °C; $[\alpha]_D^{31}$ = +43.4 (*c* 0.1, MeOH); IR (thin film) ν_{max} 3065, 3031, 2988, 2942, 2876, 1747, 1598, 1496, 1455, 1360, 1310, 1292, 1234, 1190, 1177, 1099, 1023, 979, 913, 874, 839, 816, 753, 700, 666 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28 and 1.36 (6H, 2s, 3H each), 1.91 (3H, s), 2.42 (3H, s), 3.96−4.00 (2H, m), 4.18 (1H, d, *J* = 11.6 Hz), 4.35 (1H, d, *J* = 11.2 Hz), 4.48−4.55 (2H, m), 4.58 (1H, dd, *J* = 3.6 and 4.8 Hz), 4.70 (1H, d, *J* = 12.0 Hz), 5.69 (1H, d, *J* = 3.6 Hz), 7.28−7.35 (7H, m), 7.80 (2H, d, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 20.6, 21.6, 25.8, 26.1, 64.1, 69.1, 72.4, 78.1, 78.8, 83.1, 104.1, 113.7, 127.83, 128.1, 128.2, 128.5, 129.7, 132.8, 136.8, 144.6, 170.1; HR-ESI-TOF-MS *m*/*z* 529.1501 ([M + Na]$^+$), calcd for [$C_{25}H_{30}O_9S$ + Na]$^+$ 529.1503.

1,2,5-Tri-*O*-acetyl-3-*O*-benzyl-4-*C*-*p*-toluenesulfonyloxymethyl-α,β-D-ribofuranose (12). Acetic anhydride (1.9 mL, 19.8 mmol) and concentrated sulfuric acid (0.01 mL, 0.19 mmol) were added to a stirred solution of compound **11** (1.0 g, 1.98 mmol) in acetic acid (11.3 mL, 198 mmol) at 0 °C, and the resulting reaction mixture was stirred for 6 h at rt. The reaction was quenched by the addition of cold water (100 mL) and stirred for 30 min at rt and was extracted with ethyl acetate (3 × 200 mL). The organic layer was washed with saturated aqueous sodium bicarbonate solution (3 × 100 mL) and brine solution (2 × 100 mL) and then dried over anhydrous sodium sulfate. The excess solvent was removed under reduced pressure, and the residue thus obtained was purified by silica gel column chromatography using ethyl acetate in petroleum ether as gradient solvent system to afford an anomeric mixture (α:β = ∼1:16, based on comparison of integration of anomeric proton) of **12** (1.05 g, 97%) as a colorless viscous oil: R_f = 0.3 (40% ethyl acetate in hexane); $[\alpha]_D^{30}$ = −17.15 (*c* 0.1, MeOH); IR (thin film) ν_{max} 2926, 1750, 1367, 1219, 1190, 1177, 1096, 1043, 969, 839, 739, 701, 667 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.32 (d, *J* = 4.4 Hz, C-1H$_\alpha$), 5.96 (s, C-1H$_\beta$); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 20.5, 20.6, 21.0, 21.6, 64.5, 68.4, 73.7, 73.8, 79.1, 83.1, 97.3, 127.8, 128.2, 128.3, 128.5, 129.6, 132.6, 136.6, 144.8, 168.6, 169.3, 170.0; HR-ESI-TOF-MS *m*/*z* 573.1389 ([M + Na]$^+$), calcd for [$C_{26}H_{30}O_{11}S$ + Na]$^+$ 573.1401.

General Procedure for the Synthesis of 2′,5′-Di-*O*-acetyl-3′-*O*-benzyl-4′-*C*-*p*-toluenesulfonyloxymethylribonucleosides 13a−d. To the stirred solution of tri-*O*-acetylated sugar derivative **12** (0.50 g, 0.91 mmol) and uracil/thymine/cytosine (1.36 mmol) in anhydrous acetonitrile (MeCN) (20 mL) was added dropwise *N*,*O*-bis(trimethylsilyl)acetamide (0.91 mL, 3.64 mmol). Dichloroethane was used as solvent for coupling of 6-*N*-benzoyladenine (1.36 mmol) instead of acetonitrile. The reaction mixture was stirred at reflux for 1 h and then cooled to 0 °C. In the cooled reaction mixture was added dropwise trimethylsilyltrifluoromethanesulfonate (0.28 mL, 1.55 mmol) under stirring, and the solution was heated at 70−80 °C for 6−10 h. The reaction was quenched with a cold saturated aqueous solution of sodium bicarbonate (100 mL), and extraction was performed with dichloromethane (3 × 100 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate solution (2 × 100 mL) and brine solution (2 × 100 mL) and was dried over anhydrous sodium sulfate. The excess solvent was removed under reduced pressure, and the residue thus obtained was purified by silica gel column chromatography using methanol in chloroform as eluent to afford nucleosides **13a−d** in 71−89% yields.

2′,5′-Di-*O*-acetyl-3′-*O*-benzyl-4′-*C*-*p*-toluenesulfonyloxymethyluridine (13a). It was obtained as a white solid (0.49 g, 89%): R_f = 0.3 (5% methanol in chloroform); mp 70−72 °C; $[\alpha]_D^{32}$ = +4.09 (*c* 0.1, MeOH); IR (thin film) ν_{max} 3199, 3033, 1748, 1697, 1458, 1366, 1228, 1190, 1177, 1109, 1049, 981, 814, 755, 700, 667 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.96 and 2.06 (6H, 2s, 3H each), 2.42 (3H, s), 4.13−4.17 (3H, m), 4.30 (1H, d, *J* = 10.8 Hz), 4.42−4.45 (2H, m), 4.53 (1H, d, *J* = 11.6 Hz), 5.42−5.44 (1H, m), 5.61 (1H, d, *J* = 3.6 Hz), 5.71 (1H, dd, *J* = 1.2 and 8.0 Hz), 7.21−7.33 (8H, m), 7.75 (2H, d, *J* = 8.0 Hz), 9.39 (1H, brs); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 20.6, 20.6, 21.6, 63.7, 67.8, 73.8, 74.5, 84.4, 91.1, 102.8, 128.0, 128.1, 128.3, 128.5, 129.8, 132.5, 136.6, 141.3, 145.1, 149.8, 163.1, 169.8, 169.9; HR-ESI-TOF-MS *m*/*z* 625.1468 ([M+ Na]$^+$), calcd for [$C_{28}H_{30}N_2O_{11}S$ + Na]$^+$ 625.1463.

2′,5′-Di-*O*-acetyl-3′-*O*-benzyl-4′-*C*-*p*-toluenesulfonyloxymethylthymidine (13b). It was obtained as a white solid (0.49 g, 88%): R_f = 0.3 (5% methanol in chloroform); mp 78−80 °C; $[\alpha]_D^{31}$ = −2.28 (*c* 0.1, MeOH); IR (thin film) ν_{max} 3034, 2928, 1750, 1701, 1364, 1227, 1190, 1177, 1112, 1048, 981, 839, 754, 669 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.89 (3H, s), 1.96 and 2.05 (6H, 2s, 3H each), 2.42 (3H, s), 4.13−4.17 (3H, m), 4.28 (1H, d, *J* = 11.2 Hz), 4.41−4.45 (2H, m), 4.53 (1H, d, *J* = 10.8 Hz), 5.42 (1H, dd, *J* = 4.4 and 6.0 Hz), 5.61 (1H, d, *J* = 3.6 Hz), 7.00 (1H, s), 7.21−7.33 (7H, m), 7.75 (2H, d, *J* = 8.0 Hz), 8.65 (1H, brs); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 14.9, 23.1, 23.1, 24.1, 66.3, 70.3, 76.2, 77.0, 86.7, 93.2, 113.8, 130.5, 130.6, 130.8, 131.0, 132.3, 135.0, 139.2, 139.6, 147.5, 152.4, 166.2, 172.3, 172.5; HR-ESI-TOF-MS *m*/*z* 639.1616 ([M + Na]$^+$), calcd for [$C_{29}H_{32}N_2O_{11}S$ + Na]$^+$ 639.1619.

2′,5′-Di-*O*-acetyl-3′-*O*-benzyl-4′-*C*-*p*-toluenesulfonyloxymethyl-6-*N*-benzoyladenosine (13c). It was obtained as a white solid (0.47 g, 71%): R_f = 0.4 (5% methanol in chloroform); mp 208−210 °C; $[\alpha]_D^{32}$ = +0.95 (*c* 0.1, MeOH); IR (thin film) ν_{max} 3256, 3065, 3031, 2930, 1748, 1702, 1610, 1584, 1508, 1484, 1455, 1365, 1227, 1190, 1177, 1110, 1097, 1050, 981, 838, 816, 755, 704, 667 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.77 and 2.08 (6H, 2s, 3H each), 2.42 (3H, s), 4.12−4.22 (3H, m), 4.41 (1H, d, *J* = 11.2 Hz), 4.52 (1H, d, *J* = 11.6 Hz), 4.60 (1H, d, *J* = 10.8 Hz), 4.81 (1H, d, *J* = 2.8 Hz), 5.98−6.01 (2H, m), 7.27−7.35 (8H, m), 7.50 (2H, t, *J* = 7.2 Hz), 7.59 (1H, dd, *J* = 7.2 and 14.8 Hz), 7.77 (2H, d, *J* = 8.0 Hz), 8.03−8.08 (3H, m), 8.72 (1H, s); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 23.1, 23.2, 24.2, 65.8, 70.4, 76.3, 77.0, 87.2, 90.1, 125.7, 130.6, 130.7, 130.9, 131.1, 131.2, 131.5, 132.4, 135.2, 135.6, 135.7, 139.1, 145.0, 147.7, 152.1, 153.9, 154.3, 167.2, 172.2, 172.4; HR-ESI-TOF-MS *m*/*z* 730.2149 ([M + H]$^+$), calcd for [$C_{36}H_{35}N_5O_{10}S$ + H]$^+$ 730.2177.

2′,5′-Di-*O*-acetyl-3′-*O*-benzyl-4′-*C*-*p*-toluenesulfonyloxymethylcytidine (13d). It was obtained as a sticky solid (0.43 g, 78%): R_f = 0.3 (10% methanol in chloroform); $[\alpha]_D^{32}$ = +15.44 (*c* 0.05, MeOH); IR (thin film) ν_{max} 3355, 3206, 3033, 1744, 1663, 1497, 1457, 1366, 1227, 1190, 1177, 1109, 1049, 1031, 981, 839, 815, 756, 668 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.91 and 2.03 (6H, 2s, 3H each), 2.40 (3H, s), 4.07−4.10 (2H, m), 4.16 (1H, d, *J* = 12.4 Hz), 4.32 (1H, d, *J* = 11.2 Hz), 4.37−4.40 (2H, m), 4.51 (1H, d, *J* = 11.6 Hz), 5.45 (1H, s), 5.64 (1H, d, *J* = 2.4 Hz), 6.25 (1H, d, *J* = 7.6 Hz), 7.18−7.20 (2H, m), 7.27−7.29 (5H, m), 7.52 (1H, d, *J* = 6.8 Hz), 7.72 (2H, d, *J* = 8.0 Hz), 8.46 (1H, brs), 9.32 (1H, brs); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 20.6, 20.6, 21.6, 63.3, 68.1, 73.9, 74.2, 84.7, 91.4, 96.1, 127.9, 128.1, 128.27, 128.5, 129.9, 132.3, 136.7, 143.6, 145.2, 151.2, 162.4, 169.8, 170.0; HR-ESI-TOF-MS *m*/*z* 602.1793 ([M + H]$^+$), calcd for [$C_{28}H_{31}N_3O_{10}S$ + H]$^+$ 602.1803.

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General Procedure for the Synthesis of 3′-*O*-Benzylated LNA Monomers 14a–d. To a stirred solution of compounds **13a–d** (0.67 mmol) in water/dioxane (2 mL, 1:1) was added 2 M NaOH (1.5 mL) (+25% NH_4OH, 1 mL for **13c** only), and reaction mixture was stirred at rt for 2–8 h. The reaction mixture was neutralized with acetic acid, and excess solvent was evaporated under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to give **14a–d** in 89–95% yields, respectively.

3′-*O*-Benzyl-2′-*O*,4′-*C*-methyleneuridine (14a).[13] It was obtained as a white solid (220 mg, 95%): R_f = 0.4 (10% MeOH in $CHCl_3$); mp 216–218 °C (lit.[11b] mp 217–219 °C); $[\alpha]_D^{23}$ = +108.7 (*c* 0.3, MeOH) {Lit[11b] $[\alpha]_D^{23}$ = +108.4 (*c* 0.3, MeOH)}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.55 (1H, brs), 3.69 (1H, d, *J* = 8.0 Hz), 3.78 (2H, s), 3.86 (1H, d, *J* = 8.0 Hz), 3.92 (1H, s), 4.45 (1H, s), 4.60 (2H, s), 5.48 (1H, d, *J* = 2.4 Hz), 5.61 (1H, d, *J* = 8.0 Hz), 7.28–7.35 (5H, m), 7.73 (1H, dd, *J* = 2.0 and 8.0 Hz), 11.36 (1H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 55.9, 71.1, 71.5, 75.8, 76.4, 86.5, 88.3, 100.9, 127.4, 127.6, 128.2, 137.9, 139.0, 150.0, 163.3; HR-ESI-TOF-MS *m*/*z* 369.1064 ([M + Na]$^+$), calcd for $[C_{17}H_{18}N_2O_6 + Na]^+$ 369.1057.

3′-*O*-Benzyl-2′-*O*,4′-*C*-methylenethymidine (14b).[12] It was obtained as a white solid (224 mg, 93%): R_f = 0.4 (10% MeOH in $CHCl_3$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.78 (3H, s), 3.69 (1H, d, *J* = 7.6 Hz), 3.80 (2H, d, *J* = 5.2 Hz), 3.86 (1H, d, *J* = 7.2 Hz), 3.96 (1H, s), 4.44 (1H, s), 4.60 (2H, s), 5.34 (1H, t, *J* = 4.8 Hz), 5.48 (1H, s), 7.27–7.33 (5H, m), 7.60 (1H, s), 11.37 (1H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 12.3, 55.9, 71.0, 71.5, 75.6, 76.4, 86.3, 88.2, 108.3, 127.3, 127.4, 128.1, 134.6, 137.8, 149.8, 163.8; HR-ESI-TOF-MS *m*/*z* 361.1396 ([M + H]$^+$), calcd for $[C_{18}H_{20}N_2O_6 + H]^+$ 361.1394.

3′-*O*-Benzyl-2′-*O*,4′-*C*-methyleneadenosine (14c).[12] It was obtained as a white solid (220 mg, 89%): R_f = 0.4 (25% MeOH in $CHCl_3$); mp 217–219 °C (lit.[12] mp 218–219.5 °C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.64 (1H, brs), 3.82–3.84 (3H, m), 3.96 (1H, d, *J* = 8.0 Hz), 4.36 (1H, s), 4.63 (2H, s), 4.74 (1H, s), 5.97 (1H, s), 7.27–7.31 (5H, m), 7.35 (2H, brs), 8.14 (1H, s), 8.19 (1H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 56.7, 71.1, 72.0, 76.9, 77.1, 85.2, 87.9, 119.0, 127.5, 127.6, 128.2, 137.9,, 137.9, 148.6, 152.7, 156.0; HR-ESI-TOF-MS *m*/*z* 370.1520 ([M + H]$^+$), calcd for $[C_{18}H_{19}N_5O_4 + H]^+$ 370.1510.

3′-*O*-Benzyl-2′-*O*,4′-*C*-methylenecytidine (14d). It was obtained as a white solid (217 mg, 94%): R_f = 0.4 (25% methanol in chloroform); mp 76–78 °C; $[\alpha]_D^{32}$ = +112.89 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3357, 2945, 1645, 1482, 1396, 1282, 1152, 1087, 1048, 938, 789, 699 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.70 (1H, d, *J* = 7.2 Hz), 3.79 (2H, s), 3.84–3.86 (2H, m), 4.41 (1H, s), 4.56 (2H, dd, *J* = 11.6 and 16.8 Hz), 5.26 (1H, brs), 5.48 (1H, s), 5.73 (1H, d, *J* = 7.2 Hz), 7.18 (1H, brs), 7.21 (1H, brs), 7.27–7.34 (5H, m), 7.72 (1H, d, *J* = 7.6 Hz); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 56.1, 70.9, 71.4, 75.5, 76.4, 86.9, 87.9, 93.3, 127.3, 127.4, 128.1, 137.7, 139.6, 154.6, 165.7; HR-ESI-TOF-MS *m*/*z* 346.1390 ([M + H]$^+$), calcd for $[C_{17}H_{19}N_3O_5 + H]^+$ 346.1397.

2′-*O*,4′-*C*-Methyleneuridine (15a).[13] To a solution of nucleoside **14a** (0.10 g, 0.29 mmol) in anhydrous THF/MeOH (6 mL, 9:1, v/v) were added $Pd(OH)_2$-C (20 wt %, 0.02 g) and 88% formic acid (0.09 mL, 2.31 mmol). The reaction mixture was refluxed for 3 h, whereupon it was cooled to rt. The catalyst was carefully filtered off, washed with excess MeOH, and the combined filtrate was concentrated. The crude product thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford LNA-U **15a** as a white solid (0.07 g, 91%): R_f = 0.2 (10% MeOH in $CHCl_3$); mp 242–244 °C (lit.[11b] mp 239–243 °C); $[\alpha]_D^{23}$ = +91.6 (*c* 0.3, MeOH) {lit.[11b] $[\alpha]_D^{23}$ = +92.2 (*c* 0.3, MeOH)}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.61 (1H, d, *J* = 8.4 Hz), 3.72 (2H, d, *J* = 4.8 Hz), 3.80 (1H, d, *J* = 7.2 Hz), 3.84 (1H, d, *J* = 4.4 Hz), 4.12 (1H, s), 5.13 (1H, t, *J* = 5.2 Hz), 5.40 (1H, s), 5.60 (1H, dd, 2.4 and *J* = 8.0 Hz), 5.66 (1H, d, *J* = 4.4 Hz), 7.73 (1H, d, *J* = 8.0 Hz), 11.34 (1H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 55.9, 68.6, 71.0, 78.9, 86.3, 88.9, 100.8, 139.1, 150.2, 163.3; HR-ESI-TOF-MS *m*/*z* 279.0598 ([M + Na]$^+$), calcd for $[C_{10}H_{12}N_2O_6 + Na]^+$ 279.0588.

2′-*O*,4′-*C*-Methylenethymidine (15b). To a solution of nucleoside **14b** (0.10 g, 0.28 mmol) in anhydrous MeOH (6 mL) were added $Pd(OH)_2$-C (20 wt %, 0.05 g) and ammonium formate (0.05 g, 0.85 mmol). The reaction mixture was refluxed for 10 min, whereupon it was cooled to rt. The catalyst was carefully filtered off, washed with excess MeOH, and the combined filtrate was concentrated. The crude product thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford LNA-T **15b** as a white solid (0.06 g, 83%): R_f = 0.2 (10% MeOH in $CHCl_3$); mp 194–196 °C (lit.[12] mp 196–198 °C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.77 (3H, s), 3.62 (1H, d, *J* = 7.2 Hz), 3.76 (2H, d, *J* = 5.2 Hz), 3.82 (1H, d, *J* = 7.2 Hz), 3.90 (1H, d, *J* = 3.6 Hz), 4.11 (1H, s), 5.19 (1H, t, *J* = 6.0 Hz), 5.41 (1H, s), 5.65 (1H, d, *J* = 4.4 Hz), 7.63 (1H, d, *J* = 1.6 Hz), 10.79 (1H, brs); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 12.2, 55.8, 68.5, 70.9, 78.8, 86.1, 88.7, 108.2, 134.7, 149.8, 163.7; HR-ESI-TOF-MS *m*/*z* 293.0754 ([M + Na]$^+$), calcd for $[C_{11}H_{14}N_2O_6 + Na]^+$ 293.0744.

2′-*O*,4′-*C*-Methyleneadenosine (15c).[12] To a solution of nucleoside **14c** (0.10 g, 0.27 mmol) in anhydrous EtOH (6 mL) were added $Pd(OH)_2$-C (20 wt %, 0.03 g) and ammonium formate (0.09 g, 1.36 mmol). The reaction mixture was refluxed for 1 h, whereupon it was cooled to rt. The catalyst was carefully filtered off, washed with excess MeOH, and the combined filtrate was concentrated. The crude product thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford LNA-A **15c** as a white solid (0.07 g, 91%): R_f = 0.2 (25% MeOH in $CHCl_3$); mp 267–270 °C (lit.[12] mp 266–268 °C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.75–3.80 (3H, m), 3.92 (1H, d, *J* = 8.0 Hz), 4.25 (1H, d, *J* = 4.4 Hz), 4.40 (1H, s), 5.07 (1H, t, *J* = 6.0 Hz), 5.70 (1H, d, *J* = 4.4 Hz), 5.90 (1H, s), 7.33 (2H, brs), 8.14 (1H, s), 8.23 (1H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 56.7, 69.9, 71.4, 79.2, 85.3, 88.5, 119.0, 137.9, 148.4, 152.7, 156.0; HR-ESI-TOF-MS *m*/*z* 280.1048 ([M + H]$^+$), calcd for $[C_{11}H_{13}N_5O_4 + H]^+$ 280.1040.

2′-*O*,4′-*C*-Methylenecytidine (15d). A mixture of compound **14d** (0.25 g, 0.72 mmol), 20% $Pd(OH)_2$-C (0.12 g), and ammonium formate (0.14 g, 2.17 mmol) in methanol (4 mL) was refluxed for 10 min. The catalyst was carefully filtered off, washed with excess MeOH, and the combined filtrate was concentrated. The crude product thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford LNA-C **15d** as a white solid (0.15 g, 81%): R_f = 0.2 (25% MeOH in $CHCl_3$); mp 274–276 °C (lit.[12] mp 275–278 °C); R_f = 0.2 (25% MeOH in $CHCl_3$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.62 (1H, d, *J* = 7.2 Hz), 3.72 (2H, d, *J* = 5.2 Hz), 3.79–3.81 (2H, m), 4.07 (1H, s), 5.13 (1H, t, *J* = 5.2 Hz), 5.37 (1H, s), 5.64 (1H, d, *J* = 4.4 Hz), 5.74 (1H, d, *J* = 7.6 Hz), 7.12 (1H, brs), 7.23 (1H, brs), 7.73 (1H, d, *J* = 7.6 Hz); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 56.1, 68.4, 71.0, 79.0, 86.9, 88.6, 93.4, 139.9, 154.8, 165.8; HR-ESI-TOF-MS *m*/*z* 278.0757 ([M + Na]$^+$), calcd for $[C_{10}H_{13}N_3O_5 + Na]^+$ 278.0747.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

SI-Scheme 1, SI-Table 1, and ^{1}H and ^{13}C NMR spectra of compounds **7–12**, **13a–d**, **14a–d**, and **15a–d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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