# Synthesis and biological evaluation of nucleoside dicarboxylates as potential mimics of nucleoside diphosphates

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A series of nucleotide analogues wherein the diphosphate moiety has been replaced by a dicarboxylate were synthesized and tested for inhibitory activity against nucleoside diphosphate (NDP) kinase as well as several pathogenic bacterial strains.

## Introduction

The design of enzyme inhibitors often involves replacing the diphosphate or monophosphate by a more stable group. For example, the drug alendronate, a potent inhibitor of farnesyl diphosphate synthase,[1] contains a phosphonate, and there are several examples of phosphonate-containing analogues of UDP-*N*-acetylglucosamine.[2] Furthermore, malonates have also been used as a monophosphate mimic in analogues of mannose-6-phosphate[3] and in phosphotyrosyl mimetics,[4] among others. There is evidence suggesting that 1,2-dicarboxylates (*i.e.* maleates or succinates) can act as mimics of diphosphates and monophosphates. For example, several dicarboxylate-bearing natural products are potent inhibitors of enzymes that utilize diphosphorylated compounds as their natural substrates. Actinoplanic acids[5] A (**1**) and B (**2**) and chaetomellic acid A[6] (**3**) (Fig. 1) are tight binding inhibitors of mammalian Ras protein farnesyl transferase (PFTase), an enzyme responsible for the farnesylation of the oncogenic protein Ras. The actinoplanic acids contain one or two succinate units, whereas chaetomellic acid A bears a maleate moiety. It has been suggested that the dicarboxylate units in each of these compounds mimic the diphosphate of farnesyl diphosphate, the natural substrate of PFTase. Tautomycin[7] (**4**), a natural product containing a maleic anhydride moiety, is a very potent inhibitor of serine/threonine protein phosphatase (PP) 1 ($IC_{50}$ = 22 nM). It was proposed that tautomycin mimics the phosphorylated form of DARPP-32, an endogenous protein that acts to regulate PP1.[8] Baba and co-workers have also reported the synthesis of several derivatives of norcantharidin (**5**) and have shown them to be moderate inhibitors ($IC_{50}$ = 50–170 μM) of serine/threonine PP 2B (calcineurin).[9] Complementary to these experimental results are modeling studies that conclude that a maleate unit can achieve a spacing of the negatively charged oxygen atoms that is within 0.1 Å of that in the corresponding diphosphate.[10] These results strongly imply that the dicarboxylate is acting as a mimic of a mono- or diphosphate.

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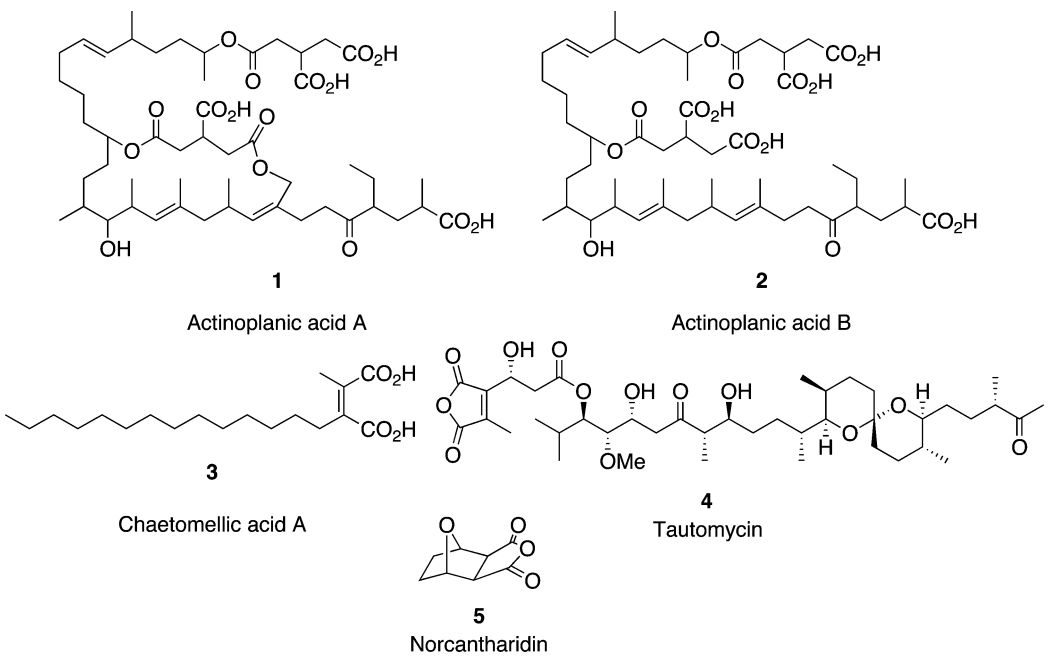



**Fig. 1** Selected compounds containing a maleyl or succinyl group.

We have reported an efficient synthesis of chaetomellic acid A and several of its analogues, and have studied their inhibition of yeast PFTase and yeast protein geranylgeranyl-transferase.[11] These compounds were shown to be moderate to fairly good inhibitors of these enzymes ($IC_{50}$ = 2.4–277 μM). Chaetomellic acid A and several of its analogues also inhibit the activity of rubber transferase ($K_i$ = 8.8–140 μM),[12] a chain elongating enzyme that uses farnesyl diphosphate as the initiating diphosphate. Several analogues of isopentenyl diphosphate (IPP) containing a dicarboxylate moiety in place of the diphosphate were shown to be moderate inhibitors of undecaprenyl diphosphate (UPP) synthase (lowest $IC_{50}$ = 135 μM) and PFTase (lowest $IC_{50}$ = 384 μM).[13] Finally, we have reported the synthesis and biological evaluation of mono- and disaccharide analogues of moenomycin and lipid II against penicillin-binding protein 1b.[14] All but one of the compounds were found to inhibit the transglycosylase activity of this enzyme, although with very modest potency (11–61% inhibition at 100 μM).

In further studies on dicarboxylates, it seemed interesting to extend this concept to inhibition of proteins utilizing nucleoside diphosphates. We thus decided to synthesize a series of uridine diphosphate analogues containing a dicarboxylate whose distance from the ribose unit is varied through the use of methylene spacers (Fig. 2). The key steps in this strategy would involve olefin cross metathesis (CM) between an olefinic uridine derivative and the appropriate alkene, as well as a *Z*-selective Horner–Emmons–Wadsworth (HEW) reaction between methyl diethylphosphonoacetate and an α-ketoester.[15] The *Z*-selectivity of the HEW reaction between phosphonoacetates and α-ketoesters has been rationalized to arise from the stability afforded to the incipient double bond in the transition state from the decomposition of the oxaphosphetane intermediate, due to its conjugation with the extra carbonyl group present in the α-ketoester.[16] This renders the formation of the intermediate adduct irreversible, precluding equilibration to the more stable fumarate.

Although nucleoside diphosphates are substrates for numerous enzymes, nucleoside diphosphate (NDP) kinase from *Dictyostelium discoideum*[17] seemed especially attractive for preliminary examination of the activity of these analogues. This enzyme catalyzes the phosphorylation of nucleoside diphosphates to their corresponding triphosphates. The enzyme is readily available, easy to handle, and several co-crystal structures with nucleoside diphosphate substrates in the active site clearly show a parallel arrangement of the oxygen atoms of the diphosphate (Fig. 3).[18] Molecular modeling suggests that this would be required to allow dicarboxylate oxygen atoms of analogues containing a maleate unit to mimic those of the diphosphate.

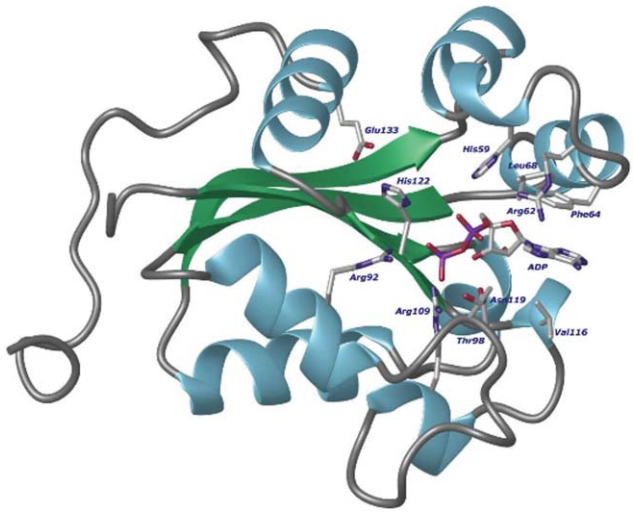



**Fig. 3** Co-crystal structure of *Dictyostelium discoideum* NDP kinase monomer with adenosine diphosphate in the active site.[14] Note the parallel arrangement of the oxygen atoms (red) of the diphosphate group. Nitrogen: purple; phosphorus: mauve; carbon: grey.

## Results and discussion

The synthesis of all uridine diphosphate analogues began with 1-(5,6-dideoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hex-5-enofuranosyl)-3-(butoxycarbonyl)uracil **6**, which can easily be accessed *via* oxidation of 2′,3′-*O*-isopropylideneuridine,[19] Wittig olefination of the resulting aldehyde,[20] and Boc-protection of the uracil

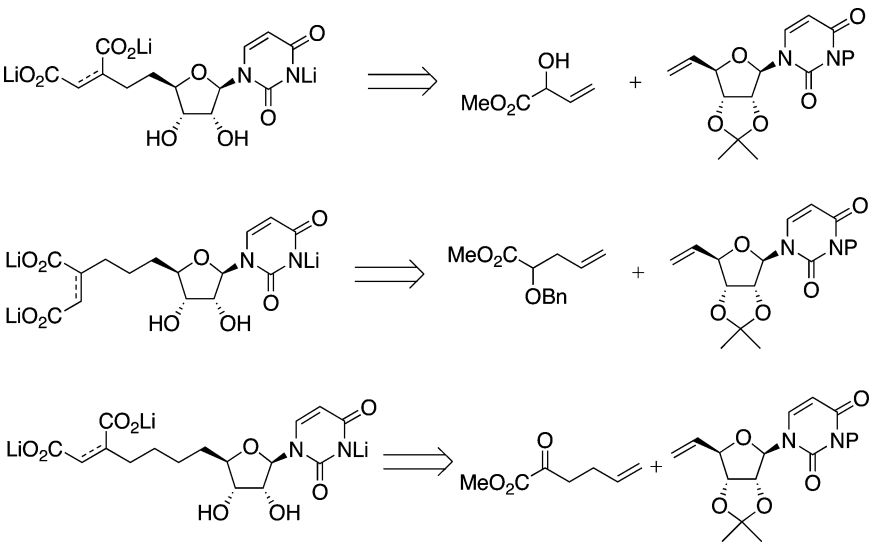
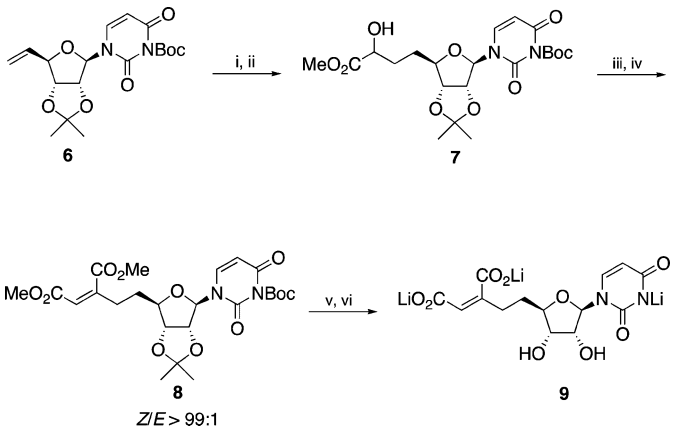



**Fig. 2** Retrosynthetic analysis of target compounds.

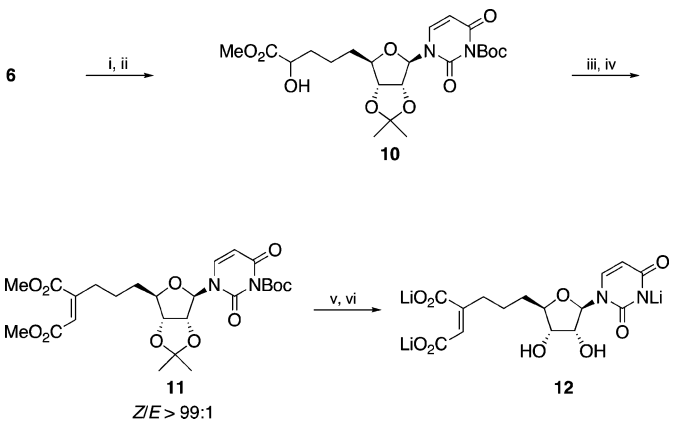
 

amide functionality.[21] The synthesis of the maleate analogue containing a 2-carbon spacer is shown in Scheme 1. Olefin CM with methyl 2-hydroxybut-3-enoate[22] was achieved using Grubbs' 2nd generation catalyst. Catalytic hydrogenation of the double bond gave alcohol **7**, which was oxidized with IBX to give the α-ketoester. Upon treatment with methyl diethylphosphonoacetate and sodium hydride, this underwent a HEW reaction to give maleate derivative **8** ($Z : E > 99 : 1$ by $^1$H NMR). Deprotection (Boc and isopropylidene) with TFA followed by ester hydrolysis with lithium hydroxide gave the lithium salt **9**.




**Scheme 1** *Reagents and conditions*: (i) methyl 2-hydroxybut-3-enoate, Grubbs' 2nd generation catalyst, $CH_2Cl_2$, reflux, 85% (ii) $H_2$ (1 atm), 5% Pd/C, EtOAc, 81% (iii) IBX, $CH_3CN$, reflux, 53% (iv) NaH, methyl diethylphosphonoacetate, DMF, −40 °C, 82% (v) TFA–$H_2O$ (7 : 3), 75% (vi) LiOH, $CD_3OD$–$D_2O$ (4 : 3), 100%.

The maleate analogue containing a 3-carbon spacer was accessed in a similar manner. Boc-protected derivative **6** underwent olefin CM with methyl 2-benzyloxypent-4-enoate (Scheme 2), the latter being readily available from benzyl protection[23] of known methyl 2-hydroxypent-4-enoate.[24] In contrast to the 2-carbon series, attempted olefin CM with the free alcohol in this case gave rise to a complex mixture of products and none of the expected CM product. Catalytic hydrogenation resulted in reduction of the double bond and removal of the benzyl group to give alcohol **10**, which was then oxidized with IBX to give the requisite α-ketoester. As in the previous case, this underwent the HEW reaction with methyl diethylphosphonoacetate in the presence of sodium hydride to give maleate derivative **11** with $Z : E > 99 : 1$ as shown by $^1$H NMR. Deprotection with TFA and subsequent lithium hydroxide-mediated hydrolysis afforded the lithium salt **12**.




**Scheme 2** *Reagents and conditions*: (i) methyl 2-benzyloxypent-4-enoate, Grubbs' 2nd generation catalyst, $CH_2Cl_2$, reflux, 61% (ii) $H_2$ (1 atm), 10% Pd/C, EtOAc, 77% (iii) IBX, $CH_3CN$, reflux, 64% (iv) NaH, methyl diethylphosphonoacetate, DMF, −40 °C (v) TFA–$H_2O$ (7 : 3), 74% (vi) LiOH, $CD_3OD$–$D_2O$ (4 : 3), 100%.

In an analogous fashion, the maleate analogue containing a 4-carbon spacer was obtained *via* olefin CM of Boc-protected derivative **6** with methyl 2-oxohex-5-enoate[24] (Scheme 3). Catalytic hydrogenation of the double bond was followed by the same sequence as described above, *i.e.* the HEW reaction ($Z : E >$ 99 : 1 by $^1$H NMR) to give **14**, then deprotection under acidic conditions, and basic hydrolysis to give the lithium salt **15**.

The succinate derivatives were obtained by catalytic hydrogenation of the respective maleates **8**, **11**, and **14**, as shown in Scheme 4. Deprotection and hydrolysis as previously described gave access to analogues **19**, **20**, and **21**.

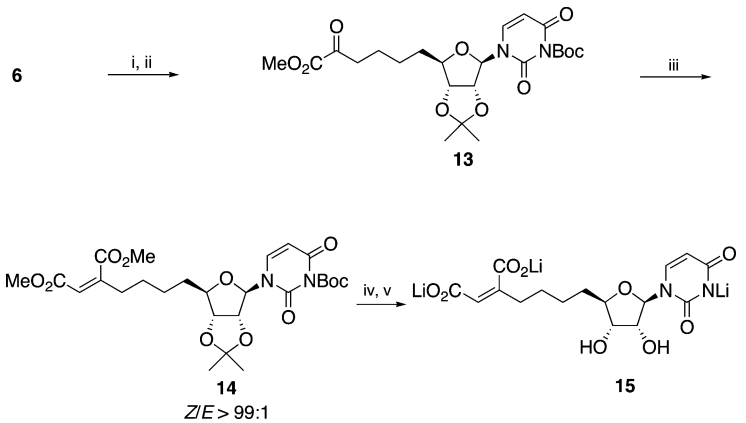
The six lithium dicarboxylate salts were tested for inhibitory activity against NDP kinase isolated from the slime mold *Dictyostelium discoideum*.† None of the compounds showed inhibition of the enzyme at a concentration of 1 mM. This may be a result of the constraints imposed by the methylene linkers, which can interact with enzyme residues in the active site and prevent the dicarboxylate oxygen atoms from occupying the positions normally assumed by the diphosphate oxygens of the parent compound.

The dicarboxylate salts as well as their diester precursors (without Boc or isopropylidene protecting groups) were also tested for their bacteristatic or bactericidal activity against several strains, including *E. coli* DH5α, *Pseudomonas aeruginosa* ATCC 14207, *Salmonella typhimurium* ATCC 23564, and *Staphylococcus aureus* ATCC 6538. Although the dicarboxylates may not penetrate negatively-charged bacterial cell membranes, the corresponding esters might be expected to diffuse through such a barrier more readily and could then potentially be hydrolyzed by intracellular esterases. Unfortunately, none of the 12 compounds tested inhibited growth of any of these strains.

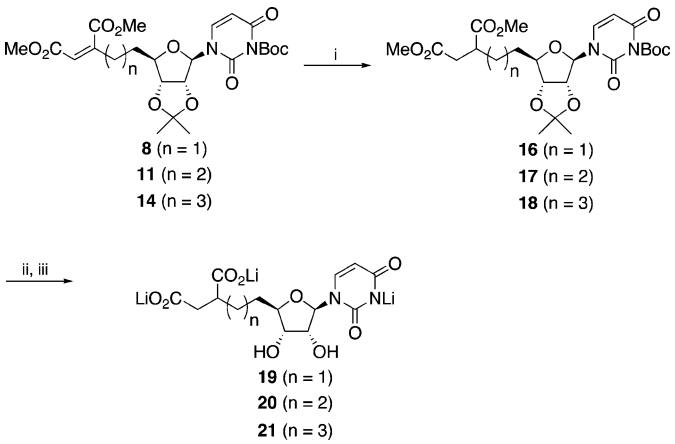
## Conclusions

A series of 6 analogues of uridine diphosphate containing either a maleate or a succinate unit were synthesized. The compounds did not exhibit any activity towards NDP kinase nor did they or their corresponding diesters inhibit growth of 4 different strains of pathogenic bacteria. Future endeavours may be directed towards inhibition studies of other enzymes that utilize substrates containing the nucleoside diphosphate unit. One example is the nucleoside monophosphate kinase class of enzymes, which are responsible for converting nucleoside monophosphates to their corresponding diphosphates. Another potential class of targets are the enzymes involved in bacterial peptidoglycan biosynthesis, for example GlmU, the enzyme responsible for uridylation of *N*-acetylglucosamine-1-phosphate to give UDP-*N*-acetyl-glucosamine.

† Professor Ioan Lascu (University of Bordeaux-2, Bordeaux, France) generously provided us with NDP kinase from *Dictyostelium discoideum*.




**Scheme 3** *Reagents and conditions*: (i) methyl 2-oxohex-5-enoate, Grubbs' 2nd generation catalyst, $CH_2Cl_2$, reflux, 40% (ii) $H_2$ (1 atm), 5% Pd/C, EtOAc, 80% (iii) NaH, methyl diethylphosphonoacetate, DMF, −40 °C, 53% (iv) TFA–$H_2O$ (7 : 3), 75% (v) LiOH, $CD_3OD$–$D_2O$ (4 : 3), 100%.




**Scheme 4** *Reagents and conditions*: (i) $H_2$ (1 atm), 10% Pd/C, EtOAc (ii) TFA–$H_2O$ (7 : 3) (iii) LiOH, $CD_3OD$–$D_2O$ (4 : 3). For % yields, refer to Experimental section.

## Experimental

### General

All reactions except those involving aqueous conditions were done in flame-dried glassware under an atmosphere of pre-purified argon using solvents freshly distilled under argon. $CH_2Cl_2$ and $CH_3CN$ were distilled over CaH, EtOAc was distilled over $K_2CO_3$, and THF was distilled over sodium and benzophenone. DMF was HPLC grade. IBX was prepared according to Frigerio *et al.*[25] All other reagents were used as obtained, unless otherwise indicated. Thin layer chromatography was performed using glass-backed plates coated with silica gel 60 $F_{254}$ (EMD Chemicals Inc.) and visualized using UV fluorescence or phosphomolybdic acid (PMA). Flash column chromatography was performed using 230–400 mesh silica gel (Silicycle). HPLC separations were performed on a Varian ProStar instrument and monitored at 220 and 280 nm, using a Vydac $C_8$ column. $^1$H NMR data are reported in ppm relative to the residual solvent as internal standard: $CHCl_3$, $\delta$ 7.24; $CH_3OH$, $\delta$ 3.30; $H_2O$, $\delta$ 4.79. The coupling constants have been rounded to the nearest 0.1 Hz. $^{13}$C NMR spectra are reported in ppm relative to: $CDCl_3$, $\delta$ 77.0; $CD_3OD$, $\delta$ 49.0; $D_2O$ referenced to 1% acetone, $\delta$ 31.1. Chemical shifts are reported to within 0.1 ppm except where close peaks necessitate an additional significant figure.

### Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyl-uracil-1-yl)-7-hydroxy-β-D-*ribo*-oct-5-eno-1,4-furanuronate

To a solution of **6** (569 mg, 1.50 mmol) and methyl 2-hydroxy-but-3-enoate (529 mg, 4.56 mmol) in $CH_2Cl_2$ (7.50 mL) was added Grubbs' 2nd generation catalyst (183 mg, 0.21 mmol). The dark brown mixture was stirred at reflux for 24 h, then extra catalyst (81 mg, 0.10 mmol) was added and the reaction allowed to continue for another 6 h. After cooling to rt, DMSO (2.80 mL, 50 equiv. relative to catalyst) was added and stirring continued at rt for 12 h. The $CH_2Cl_2$ was removed *in vacuo*, and the crude product was purified by flash chromatography on silica (pentane–EtOAc, 3 : 1 to 1 : 1) to give a clear gum, isolated as a 1 : 1 mixture of diastereomers (596 mg, 85%). $[\alpha]_D^{20}$ +37.6 (*c* 0.004, $CHCl_3$); $\nu_{max}$(cast)/cm$^{-1}$ 3600–3300, 2987, 2956, 1785, 1744, 1722, 1680, 1633 and 1148; $\delta_H$(500 MHz; $CDCl_3$) 1.35 (3 H, s, $CH_3$), 1.57 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 2.94 (1 H, br s, OH), 3.82 (3 H, s, $OCH_3$), 4.59–4.61 (1 H, m, H4′), 4.70–4.76 (2 H, m, H3′, $CH_\alpha$), 5.03 (1 H, dd, *J* 6.0, 1.8, H2′), 5.64 (1 H, d, *J* 1.8, H1′), 5.75 (1 H, d, *J* 8.0, H5), 5.90–5.95 (1 H, m, CH=C*H*CHOH), 6.05–6.11 (1 H, m, C*H*=CHCHOH), 7.21 (1 H, d, *J* 8.0, H6); $\delta_C$(125 MHz; $CDCl_3$) 25.2, 25.3, 27.02, 27.05, 27.4, 52.85, 52.90, 70.3, 70.4, 70.5, 83.88, 83.91, 84.56, 84.58, 87.1, 87.27, 87.33, 94.2, 94.3, 102.0, 114.56, 114.58, 128.5, 128.6, 128.9, 129.0, 130.5, 130.6, 141.4, 147.42, 147.45, 148.2, 160.36, 160.38 172.97, 173.02; *m*/*z* (ES+) calcd for $C_{21}H_{28}N_2O_{10}Na$ 491.1636, found 491.1635 [$MNa^+$].

### Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyl-uracil-1-yl)-7-hydroxy-β-D-*ribo*-octa-1,4-furanuronate (7)

To a solution of methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-7-hydroxy-β-D-*ribo*-oct-5-eno-1,4-furanuronate (357 mg, 0.77 mmol) in EtOAc (6.00 mL) was added 5% Pd/C (359 mg), and the mixture was stirred under 1 atm $H_2$ for 24 h. The mixture was filtered through Celite and washed with hot EtOAc. Removal of the solvent *in vacuo* followed by flash chromatography on silica (pentane–EtOAc, 1 : 1) gave a gum, isolated as a 1 : 1 mixture of diastereomers (291 mg, 81%). $[\alpha]_D^{20}$ +19.50 (*c* 0.0012, $CHCl_3$); $\nu_{max}$(cast)/cm$^{-1}$ 3600–3250, 3097, 2986,

2955, 1785, 1723, 1681, 1633 and 1094; $\delta_H$(600 MHz; $CDCl_3$) 1.34 (3 H, s, $CH_3$), 1.56 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 1.94–2.05 (2 H, m, $CH_2$), 2.76–2.98 (2 H, m, $CH_2$), 3.80 (3 H, s, $OCH_3$), 4.05–4.07 (1 H, m, H4′), 4.24 (1 H, br s, $CH_a$), 4.58 (1 H, dd, *J* 6.6, 4.8, H3′), 4.94 (1 H, dd, *J* 4.8, 2.3, H2′), 5.64 (1 H, d, *J* 2.3, H1′), 5.76 (1 H, d, *J* 8.1, H5), 7.22 (1 H, d, *J* 8.1, H6); $\delta_C$(125 MHz; $CDCl_3$) 25.3, 27.1, 27.4, 28.5, 28.7, 29.7, 29.9, 52.4, 52.5, 69.8, 69.9, 83.4, 83.5, 84.3, 86.3, 86.5, 86.9, 93.3, 93.4, 102.0, 102.1, 114.75, 114.76, 141.1, 147.5, 148.0, 148.1, 160.4, 175.1; *m/z* (ES+) calcd for $C_{21}H_{30}N_2O_{10}Na$ 493.1793, found 493.1793 [$MNa^+$].

### Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-7-oxo-β-D-*ribo*-octa-1,4-furanuronate

IBX (397 mg, 1.42 mmol) was added to a solution of **7** (267 mg, 0.57 mmol) in $CH_3CN$ (4.10 mL), and the white suspension was heated at reflux for 2 h, then cooled to 0 °C and filtered through a sintered glass funnel. After solvent removal *in vacuo*, the product was purified by flash chromatography on silica gel (petroleum ether–EtOAc, 2 : 1) to afford a white foam (142 mg, 53%). $[\alpha]_D^{20}$ +26.41 (*c* 0.0012, $CHCl_3$); $\nu_{max}$(cast)/$cm^{-1}$ 3096, 2986, 2939, 1785, 1725, 1682, 1633 and 1086; $\delta_H$ (600 MHz; $CDCl_3$) 1.33 (3 H, s, $CH_3$), 1.53 (3 H, s, $CH_3$), 1.60 (9 H, s, Boc), 2.08 (2 H, app q, *J* 7.2, $CH_2$), 2.94 (1 H, dt, *J* 18.7, 7.1, C$H_a$H$_b$), 3.01 (1 H, dt, *J* 18.7, 7.1, $CH_aCH_b$), 3.85 (3 H, s, $OCH_3$), 4.03 (1 H, dd, *J* 7.2, 5.3, H4′), 4.61 (1 H, dd, *J* 6.6, 5.3, H3′), 4.97 (1 H, dd, *J* 6.6, 2.1, H2′), 5.56 (1 H, d, *J* 2.1, H1′), 5.75 (1 H, d, *J* 7.8, H5), 7.17 (1 H, d, *J* 7.8, H6); $\delta_C$(125 MHz; $CDCl_3$) 25.4, 26.5, 27.2, 27.4, 35.6, 53.0, 83.4, 84.3, 85.8, 87.1, 94.1, 102.3, 115.0, 141.3, 147.4, 148.0, 160.2, 161.1, 193.0; *m/z* (ES+) calcd for $C_{21}H_{28}N_2O_{10}Na$ 491.1636, found 491.1636 [$MNa^+$].

### Methyl-2-benzyloxypent-4-enoate

To a solution of methyl 2-hydroxypent-4-enoate (3.51 g, 26.98 mmol) in THF (45.00 mL) at 0 °C was added NaH (60% suspension in mineral oil, 1.11 g, 27.78 mmol), followed by *n*-$Bu_4NI$ (0.99 g, 2.69 mmol) and benzyl bromide (3.50 mL, 29.45 mmol). The mixture was warmed to rt and stirred for 5 h. A saturated $NH_4Cl$ solution (30 mL) was added, then the aqueous layer extracted with $Et_2O$ (3 × 30 mL). The combined organic layers were dried ($MgSO_4$), and the solvent removed *in vacuo.* Purification by flash chromatography on silica gel (petroleum ether–$Et_2O$, 95 : 5), followed by solvent removal *in vacuo* at 0 °C (product is volatile) afforded a colourless liquid (4.39 g, 74%). $\nu_{max}$(neat)/$cm^{-1}$ 3066, 3032, 2952, 1752, 1642, 1497, 1455 and 1115; $\delta_H$(400 MHz; $CDCl_3$) 2.53–2.56 (2 H, m, allylic $CH_2$), 3.75 (3 H, s, $OCH_3$), 4.03 (1 H, t, *J* 6.2, $CH_a$), 4.46 (1 H, d, *J* 11.8, benzylic C$H_a$H$_b$), 4.72 (1 H, d, *J* 11.8, benzylic $CH_aH_b$), 5.08–5.15 (2 H, m, CH=C$H_2$), 5.83 (1 H, dddd, *J* 17.2, 14.0, 10.2, 6.9, C*H*=$CH_2$), 7.28–7.37 (5 H, m, Ar); $\delta_C$(125 MHz; $CDCl_3$) 37.3, 51.8, 72.3, 77.8, 117.9, 127.8, 127.9, 128.4, 133.0, 137.4, 172.6; *m/z* (ES+) calcd for $C_{13}H_{16}O_3Na$ 243.0992, found 243.0992 [$MNa^+$].

### Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyl-uracil-1-yl)-8-benzyloxy-β-D-*ribo*-non-5-eno-1,4-furanuronate

To a solution of **6** (702 mg, 1.85 mmol) and methyl-2-benzyloxypent-4-enoate (1.02 g, 4.63 mmol) in $CH_2Cl_2$ (9.00 mL) was added Grubbs' 2nd generation catalyst (160 mg, 0.19 mmol), and the dark brown mixture was stirred at reflux for 24.5 h. After cooling to rt, DMSO (730 μL, 50 equiv. relative to catalyst) was added, and the mixture was stirred at rt for an extra 12 h. The $CH_2Cl_2$ was removed *in vacuo*, and the crude product was purified by flash chromatography on silica gel (petroleum ether–EtOAc, 3 : 1 to 2 : 1) to give an off-white foam, isolated as a 1 : 1 mixture of diastereomers (730 mg, 69%). $[\alpha]_D^{20}$ +62.49 (*c* 0.0008, $CHCl_3$); $\nu_{max}$(cast)/$cm^{-1}$ 3091, 2986, 2937, 1785, 1749, 1722, 1682, 1631, 1497 and 1085; $\delta_H$(500 MHz; $CDCl_3$) 1.35 (3 H, s, $CH_3$), 1.57 (3 H, s, $CH_3$), 1.60 (9 H, s, Boc), 2.52–2.55 (2 H, m, allylic $CH_2$), 3.74 (3 H, s, $OCH_3$), 4.00–4.02 (1 H, m, $CH_a$), 4.43 (1 H, d, *J* 11.7, benzylic C$H_a$H$_b$), 4.51–4.55 (1 H, m, H4′), 4.62–4.66 (1 H, m, H3′), 4.71 (0.5 H, d, *J* 11.7, benzylic $CH_aH_b$), 4.72 (0.5 H, d, *J* 11.7, benzylic $CH_aH_b$), 4.95 (0.5 H, app t, *J* 6.5, H2′), 4.96 (0.5 H, app t, *J* 6.5, H2′), 5.60–5.66 (2 H, m, CH=CH), 5.68 (0.5 H, d, *J* 8.1, H5), 5.70 (0.5 H, d, *J* 8.1, H5), 5.79–5.87 (1 H, m, H1′), 7.21 (0.5 H, d, *J* 8.1, H6), 7.22 (0.5 H, d, *J* 8.1, H6), 7.28–7.36 (5 H, m, Ar); $\delta_C$(125 MHz; $CDCl_3$) 25.4, 27.1, 27.5, 35.7, 35.8, 51.9, 72.4, 77.3, 83.9, 84.0, 84.87, 84.92, 87.0, 88.0, 88.1, 94.30, 94.33, 102.0, 102.1, 114.49, 114.52, 127.96, 127.99, 128.0, 128.4, 129.8, 130.0, 130.38, 130.44, 137.2, 137.3, 140.8, 140.9, 147.5, 148.2, 160.3, 172.3; *m/z* (ES+) calcd for $C_{29}H_{36}N_2O_{10}Na$ 595.2262, found 595.2263 [$MNa^+$].

### Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyl-uracil-1-yl)-8-hydroxy-β-D-*ribo*-nona-1,4-furanuronate (10)

10% Pd/C (73 mg) was added to a solution of methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-8-benzyloxy-β-D-*ribo*-non-5-eno-1,4-furanuronate (723 mg, 1.26 mmol) in EtOAc (13.00 mL), and the mixture was stirred under 1 atm $H_2$ for 7 h. The Pd/C was removed by filtration through Celite and washed with hot EtOAc. The solvent was removed *in vacuo*, and the resulting viscous gum was purified by flash chromatography on silica gel (petroleum ether–EtOAc, 1 : 1) to yield a white foam, isolated as a 1 : 1 mixture of diastereomers (567 mg, 93%). $\nu_{max}$(cast)/$cm^{-1}$ 3600–3300, 3094, 2985, 2939, 1785, 1722, 1681, 1632 and 1084; $\delta_H$(500 MHz; $CDCl_3$) 1.34 (3 H, s, $CH_3$), 1.48–1.89 (6 H, m, 3 × $CH_2$), 1.55 (3 H, s, $CH_3$), 1.60 (9 H, s, Boc), 3.79 (3 H, s, $OCH_3$), 4.01–4.06 (1 H, m, H4′), 4.18–4.21 (1 H, m, $CH_a$), 4.55 (1 H, m, H3′), 4.92 (0.5 H, d, *J* 6.8, H2′), 4.93 (0.5 H, d, *J* 6.8, H2′), 5.63 (1 H, br s, H1′), 5.751 (0.5 H, d, *J* 8.1, H5), 5.755 (0.5 H, d, *J* 8.1, H5), 7.21 (0.5 H, d, *J* 8.1, H6), 7.22 (0.5 H, d, *J* 8.1, H6); $\delta_C$(125 MHz; $CDCl_3$) 21.0, 21.1, 25.4, 27.2, 27.5, 32.8, 32.9, 33.90, 33.93, 52.6, 70.08, 70.12, 83.6, 84.4, 84.5, 86.66, 86.69, 87.0, 93.6, 93.7, 102.2, 114.78, 114.82, 140.70, 140.74, 147.5, 148.1, 160.3, 175.5; *m/z* (ES+) calcd for $C_{22}H_{32}N_2O_{10}Na$ 507.1949, found 507.1949 [$MNa^+$].

### Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyl-uracil-1-yl)-8-oxo-β-D-*ribo*-nona-1,4-furanuronate

To a solution of **10** (560 mg, 1.16 mmol) in $CH_3CN$ (8.00 mL) was added IBX (1.02 g, 3.63 mmol), and the white suspension was stirred at reflux for 1.5 h. It was then cooled to 0 °C and filtered through a sintered glass funnel. The solvent was removed *in vacuo*, and the crude product was purified by flash chromatography on silica (pet ether–EtOAc, 2 : 1 to 1 : 1) to give a white foam (413 mg, 74%). $[\alpha]_D^{20}$ +10.44 (*c* 0.0009, $CHCl_3$); $\nu_{max}$(cast)/$cm^{-1}$ 3096, 2985,

2938, 1784, 1724, 1683, 1633 and 1083; $\delta_H$(500 MHz; $CDCl_3$) 1.34 (3 H, s, $CH_3$), 1.55 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 1.73–1.81 (4 H, m, 2 × $CH_2$), 2.91 (2 H, t, *J* 6.8, $CH_{2\alpha}$), 3.86 (3 H, s, $OCH_3$), 4.00–4.04 (1 H, m, H4′), 4.56 (1 H, dd, *J* 6.6, 5.0, H3′), 4.93 (1 H, dd, *J* 6.6, 2.2, H2′), 5.62 (1 H, d, *J* 2.2, H1′), 5.77 (1 H, d, *J* 8.3, H5), 7.21 (1 H, d, *J* 8.3, H6); $\delta_C$(125 MHz; $CDCl_3$) 19.2, 25.4, 27.2, 27.5, 32.3, 38.7, 53.0, 83.5, 84.4, 86.5, 87.1, 93.7, 102.3, 114.9, 140.8, 147.4, 148.0, 160.3, 161.4, 193.5; *m/z* (ES+) calcd for $C_{22}H_{30}N_2O_{10}Na$ 505.1793, found 505.1793 [$MNa^+$].

### Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyl-uracil-1-yl)-9-oxo-β-D-*ribo*-dec-5-eno-1,4-furanuronate

To a solution of **6** (383 mg, 1.01 mmol) and methyl 2-oxohex-5-enoate (405 mg, 2.59 mmol) in $CH_2Cl_2$ (5.00 mL) was added Grubbs' 2nd generation catalyst (125 mg, 0.15 mmol), and the dark brown mixture was stirred at reflux for 16 h. Extra catalyst (43 mg, 0.051 mmol) was added, and stirring continued at reflux for another 8 h. The reaction mixture was then cooled to rt, and DMSO (770 μL, 50 equiv. relative to catalyst) was added. After stirring at rt for 12 h, the $CH_2Cl_2$ was removed *in vacuo* and the crude product was purified by flash chromatography on silica gel (pentane–EtOAc, 3 : 1 to 2 : 1) to give a white foam (206 mg, 40%). $[\alpha]_D^{20}$ +32.99 (*c* 0.0016, $CHCl_3$); $\nu_{max}$(cast)/$cm^{-1}$ 3097, 2987, 2937, 1785, 1724, 1682, 1632 and 1081; $\delta_H$(600 MHz; $CDCl_3$) 1.35 (3 H, s, $CH_3$), 1.57 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 2.42 (2 H, app qd, *J* 6.9, 1.4, allylic $CH_2$), 2.97 (2 H, t, *J* 6.9, $CH_{2\alpha}$), 3.88 (3 H, s, $OCH_3$), 4.51 (1 H, dd, *J* 7.9, 4.2, H4′), 4.69 (1 H, dd, *J* 6.5, 4.2, H3′), 5.02 (1 H, dd, *J* 6.5, 2.2, H2′), 5.60 (1 H, d, *J* 2.2, H1′), 5.65 (1 H, ddt, *J* 15.5, 7.9, 1.4, CH=C*H*), 5.76 (1 H, d, *J* 8.4, H5), 5.83 (1 H, dtd, *J* 15.5, 6.9, 0.9, C*H*=CH), 7.23 (1 H, d, *J* 8.4, H6); $\delta_C$(125 MHz; $CDCl_3$) 25.3, 25.4, 27.1, 27.4, 38.3, 53.0, 83.9, 84.8, 86.9, 88.3, 94.5, 102.0, 114.5, 128.1, 133.5, 141.2, 147.4, 148.1, 160.3, 161.1, 193.0; *m/z* (ES+) calcd for $C_{23}H_{30}N_2O_{10}Na$ 517.1793, found 517.1794 [$MNa^+$].

### Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyl-uracil-1-yl)-9-oxo-β-D-*ribo*-deca-1,4-furanuronate (13)

1,5,6-Trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-9-oxo-β-D-*ribo*-dec-5-eno-1,4-furanuronate (199 mg, 0.39 mmol) was dissolved in EtOAc (3.10 mL) and 5% Pd/C (25 mg) was added. The mixture was stirred under 1 atm $H_2$ for 7 h, then filtered through Celite and washed with hot EtOAc. After solvent removal *in vacuo*, the crude product was purified by flash chromatography on silica gel to afford a white foam (155 mg, 80%). $[\alpha]_D^{20}$ +18.82 (*c* 0.0017, $CHCl_3$); $\nu_{max}$(cast)/$cm^{-1}$ 3097, 2986, 2939, 1785, 1725, 1683, 1633 and 1082; $\delta_H$(600 MHz; $CDCl_3$) 1.34 (3 H, s, $CH_3$), 1.40–1.52 (2 H, m, $CH_2$), 1.56 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 1.65–1.74 (4 H, m, 2 × $CH_2$), 2.87 (2 H, t, *J* 7.2, $CH_{2\alpha}$), 3.87 (3 H, s, $OCH_3$), 4.01 (1 H, m, H4′), 4.55 (1 H, dd, *J* 6.8, 4.8, H3′), 4.93 (1 H, dd, *J* 6.8, 2.2, H2′), 5.62 (1 H, d, *J* 2.2, H1′), 5.77 (1 H, d, *J* 8.4, H5), 7.21 (1 H, d, *J* 8.4, H6); $\delta_C$(125 MHz; $CDCl_3$) 22.6, 24.9, 25.4, 27.2, 27.4, 32.9, 39.0, 52.9, 83.5, 84.4, 86.5, 87.0, 93.4, 102.1, 114.8, 140.8, 147.5, 148.1, 160.3, 161.4, 193.9; *m/z* (ES+) calcd for $C_{23}H_{32}N_2O_{10}Na$ 519.1949, found 519.1948 [$MNa^+$].

### General procedure for Horner–Emmons–Wadsworth reaction on α-ketoesters

To a suspension of NaH (60% in mineral oil, 1.5 equiv.) in DMF at 0 °C was added dropwise methyl diethylphosphonoacetate (0.8 equiv) in DMF, and the mixture was stirred for 30 min. It was then cooled to −40 °C and the α-ketoester (1.0 equiv.) in DMF was added dropwise. The orange mixture was stirred at −40 °C for 5 h, then $H_2O$ was added. After warming to rt, the aqueous layer was extracted 3 times with EtOAc. The combined organic layers were dried ($MgSO_4$) and the solvent was removed *in vacuo.* Purification was done by flash chromatography on silica gel (petroleum ether–EtOAc).

**Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-7-methoxycarbonyl-β-D-*ribo*-non-7-eno-1,4-furanuronate (8).** Isolated as a colourless glass (41 mg, 49%). $[\alpha]_D^{20}$ +18.00 (*c* 0.0009, $CHCl_3$); $\nu_{max}$(cast)/$cm^{-1}$ 2925, 2854, 1786, 1724, 1682 and 1150; $\delta_H$(500 MHz; $CDCl_3$) 1.34 (3 H, s, $CH_3$), 1.55 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 1.91 (2 H, app q, *J* 7.7, $CH_2$), 2.41–2.55 (2 H, m, $CH_2$), 3.73 (3 H, s, $OCH_3$), 3.83 (3 H, s, $OCH_3$), 3.98–4.04 (1 H, m, H4′), 4.61 (1 H, dd, *J* 6.5, 5.0, H3′), 4.98 (1 H, dd, *J* 6.5, 2.5, H2′), 5.55 (1 H, d, *J* 2.5, H1′), 5.76 (1 H, d, *J* 8.0, H5), 5.88 (1 H, s, C=CH), 7.17 (1 H, d, *J* 8.0, H6); $\delta_C$(125 MHz; $CDCl_3$) 25.4, 27.2, 27.5, 30.4, 30.6, 51.9, 52.4, 83.5, 84.3, 85.9, 87.1, 94.4, 102.3, 114.9, 120.5, 141.3, 147.4, 148.0, 148.6, 160.2, 165.3, 168.8; *m/z* (ES) calcd for $C_{24}H_{32}N_2O_{11}Na$ 547.1898, found 547.1897 [$MNa^+$].

**Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-8-methoxycarbonyl-β-D-*ribo*-dec-8-eno-1,4-furanuronate (11).** Isolated as a gum (279 mg, 82%). $[\alpha]_D^{20}$ +7.75 (*c* 0.0008, $CHCl_3$); $\nu_{max}$(cast)/$cm^{-1}$ 3092, 2985, 2951, 2927, 2853, 1784, 1723, 1682, 1633 and 1085; $\delta_H$(500 MHz; $CDCl_3$) 1.34 (3 H, s, $CH_3$), 1.55 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 1.62–1.77 (4 H, m, 2 × $CH_2$), 2.41 (2 H, t, *J* 7.5, allylic $CH_2$), 3.72 (3 H, s, $OCH_3$), 3.83 (3 H, s, $OCH_3$), 3.98–4.02 (1 H, m, H4′), 4.55 (1 H, dd, *J* 6.6, 5.0, H3′), 4.92 (1 H, dd, *J* 6.6, 2.2, H2′), 5.62 (1 H, d, *J* 2.2, H1′), 5.76 (1 H, d, *J* 8.0, H5), 5.83 (1 H, s, C=CH), 7.21 (1 H, d, *J* 8.0, H6); $\delta_C$(125 MHz; $CDCl_3$) 23.1, 25.4, 27.2, 27.5, 32.3, 33.9, 51.9, 52.4, 83.5, 84.4, 86.4, 87.0, 93.6, 102.3, 114.9, 119.9, 140.8, 147.4, 148.1, 149.7, 160.3, 165.2, 169.0; *m/z* (ES+) calcd for $C_{25}H_{34}N_2O_{11}Na$ 561.2055, found 561.2054 [$MNa^+$].

**Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-9-methoxycarbonyl-β-D-*ribo*-undec-9-eno-1,4-furanuronate (14).** Isolated as a colourless glass (35 mg, 53%). $[\alpha]_D^{20}$ +20.00 (*c* 0.0022, $CHCl_3$); $\nu_{max}$(cast)/$cm^{-1}$ 3099, 2987, 2950, 2867, 1785, 1724, 1683, 1633 and 1086; $\delta_H$(600 MHz; $CDCl_3$) 1.33 (3 H, s, $CH_3$), 1.38–1.53 (3 H, m, $CH_2$ + C$H_a$H$_b$), 1.55 (3 H, s, $CH_3$), 1.60 (9 H, s, Boc), 1.65–1.72 (3 H, m, $CH_2$ + CH$_a$H$_b$), 2.37 (2 H, td, *J* 7.5, 1.5, allylic $CH_2$), 3.72 (3 H, s, $OCH_3$), 3.82 (3 H, s, $OCH_3$), 3.98–4.01 (1 H, m, H4′), 4.54 (1 H, dd, *J* 6.6, 4.8, H3′), 4.92 (1 H, dd, *J* 6.6, 2.4, H2′), 5.61 (1 H, d, *J* 2.4, H1′), 5.75 (1 H, d, *J* 8.4, H5), 5.81 (1 H, t, *J* 1.5, C=CH), 7.21 (1 H, d, *J* 8.4, H6); $\delta_C$(125 MHz; $CDCl_3$) 24.9, 25.4, 26.7, 27.2, 27.5, 32.9, 34.1, 51.8, 52.3, 83.6, 84.4, 86.6. 87.0, 93.6, 102.2, 114.8, 119.5, 140.8, 147.5, 148.1, 150.2, 160.3, 165.3, 169.2; *m/z* (ES+) calcd for $C_{26}H_{36}N_2O_{11}Na$ 575.2211, found 575.2211 [$MNa^+$].

**General procedure for hydrogenation of dimethyl maleates to dimethyl succinates**

10% Pd/C was added to a solution of the maleate starting material in EtOAc, and hydrogenation was carried out at 1 atm $H_2$ for 12–16 h, at which point the mixture was filtered through Celite and washed with hot EtOAc. After solvent removal *in vacuo*, purification was done by flash chromatography on silica gel (petroleum ether–EtOAc) to isolate the product as a mixture of diastereomers.

**Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-7-methoxycarbonyl-β-D-*ribo*-nona-1,4-furanuronate (16).** Isolated as a colourless gum (19 mg, 95%). $[\alpha]_D^{20}$ +25.45 (*c* 0.0011, $CHCl_3$); $\nu_{max}$(cast)/cm$^{-1}$ 2924, 2852, 1786, 1724, 1683, 1633 and 1150; $\delta_H$(500 MHz; $CDCl_3$) 1.33 (3 H, s, $CH_3$), 1.55 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 1.62–1.74 (4 H, m, 2 × $CH_2$), 2.45 (1 H, ddd, *J* 16.5, 5.5, 2.5, $MeO_2CCH_aH_b$), 2.73 (1 H, ddd, *J* 16.5, 9.0, 1.0, $CH_3O_2CCH_aH_b$), 2.84–2.94 (1 H, m, $CH_3O_2CCH$), 3.67 (3 H, s, $OCH_3$), 3.70 (3 H, s, $OCH_3$), 3.96–4.04 (1 H, m, H4′), 4.56 (1 H, dd, *J* 6.5, 4.5, H3′), 4.94 (1 H, dd, *J* 6.5, 2.0, H2′), 5.59 (1 H, d, *J* 2.0, H1′), 5.76 (1 H, d, *J* 8.3, H5), 7.20 (1 H, d, *J* 8.3, H6); $\delta_C$(125 MHz; $CDCl_3$) 25.4, 27.2, 27.5, 27.8, 27.9, 30.6, 30.8, 35.7, 35.9, 40.7, 40.9, 51.8, 52.0, 83.5, 83.6, 86.6, 86.7, 87.1, 94.0, 94.1, 102.2, 114.8, 114.9, 140.9, 141.0, 147.4, 148.0, 160.3, 172.1, 172.2, 174.8; *m*/*z* (ES+) calcd for $C_{24}H_{34}N_2O_{11}Na$ 549.2055, found 549.2056 [$MNa^+$].

**Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-8-methoxycarbonyl-β-D-*ribo*-deca-1,4-furanuronate (17).** Isolated as a colourless glass (21 mg, 71%). $[\alpha]_D^{20}$ +20.63 (*c* 0.0009, $CHCl_3$); $\nu_{max}$(cast)/cm$^{-1}$ 2986, 2938, 1785, 1724, 1683, 1635 and 1085; $\delta_H$(500 MHz; $CDCl_3$) 1.34 (3 H, s, $CH_3$), 1.36–1.52 (3 H, m, $CH_2$ + $CH_aH_b$), 1.55 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 1.64–1.73 (3 H, m, $CH_2$ + $CH_aH_b$), 2.45 (1 H, dd, *J* 16.7, 5.5, $MeO_2CCH_aH_b$), 2.73 (1 H, dd, *J* 16.7, 9.0, $CH_3O_2CCH_aH_b$), 2.83–2.89 (1 H, m, $CH_3O_2CCH$), 3.68 (3 H, s, $OCH_3$), 3.70 (3 H, s, $OCH_3$), 3.98–4.01 (1 H, m, H4′), 4.53 (1 H, dd, *J* 6.5, 5.0, H3′), 4.91 (1 H, dd, *J* 6.5, 2.3, H2′), 5.62 (1 H, d, *J* 2.3, H1′), 5.76 (1 H, d, *J* 8.3, H5), 7.20 (1 H, d, *J* 8.3, H6); $\delta_C$(125 MHz; $CDCl_3$) 23.12, 23.14, 25.4, 27.2, 27.5, 31.6, 33.0, 35.8, 41.0, 51.8, 51.9, 83.5, 84.4, 86.5, 87.0, 93.5, 102.2, 114.9, 140.66, 140.70, 147.5, 148.1, 160.3, 172.2, 175.1; *m*/*z* (ES+) calcd for $C_{25}H_{36}N_2O_{11}Na$ 563.2211, found 563.2209 [$MNa^+$].

**Methyl 1,5,6-trideoxy-2,3-*O*-isopropylidene-1-(3-butoxycarbonyluracil-1-yl)-9-methoxycarbonyl-β-D-*ribo*-undeca-1,4-furanuronate (18).** Isolated as a colourless glass (10 mg, 21%). $[\alpha]_D^{20}$ +10.09 (*c* 0.0019, $CHCl_3$); $\nu_{max}$(cast)/cm$^{-1}$ 2987, 2938, 1785, 1724, 1683, 1632 and 1085; $\delta_H$(500 MHz; $CDCl_3$) 1.34 (3 H, s, $CH_3$), 1.35–1.54 (4 H, m, 2 × $CH_2$), 1.55 (3 H, s, $CH_3$), 1.61 (9 H, s, Boc), 1.63–1.70 (2 H, m, $CH_2$), 2.43 (1 H, dd, *J* 16.6, 5.3, $MeO_2CCH_aH_b$), 2.72 (1 H, dd, *J* 16.6, 9.0, $CH_3O_2CCH_aH_b$), 2.82–2.88 (1 H, m, $CH_3O_2CCH$), 3.68 (3 H, s, $OCH_3$), 3.70 (3 H, s, $OCH_3$), 3.98–4.02 (1 H, m, H4′), 4.53 (1 H, app t, *J* 6.3, H3′), 4.91 (1 H, dd, *J* 6.3, 2.2, H2′), 5.63 (1 H, d, *J* 2.2, H1′), 5.76 (1 H, d, *J* 8.0, H5), 7.20 (1 H, d, *J* 8.0, H6); $\delta_C$(MHz; $CDCl_3$) 25.3, 26.7, 27.2, 27.5, 31.7, 33.0, 35.82, 35.83, 41.01, 41.03, 51.77, 51.84, 83.5, 84.5, 86.6, 86.7, 87.0, 93.38, 93.42, 102.2, 114.83, 114.85, 140.6, 147.5, 148.1, 160.3, 172.3, 175.2; *m*/*z* (ES+) calcd for $C_{26}H_{38}N_2O_{11}Na$ 577.2368, found 577.2368 [$MNa^+$].

**General conditions for Boc and isopropylidene deprotection**

The starting material was stirred in 70% TFA in $H_2O$ at rt for 3 h, then the solvent was removed *in vacuo*. The crude dimethyl maleates were purified by RP HPLC on $C_8$ ($CH_3CN$ gradient in $H_2O$ with 0.1% TFA) then lyophilized from $H_2O$. The crude dimethyl succinates were purified by flash chromatography on silica gel (EtOAc–MeOH) and isolated as a mixture of diastereomers.

**Methyl 1,5,6-trideoxy-1-(uracil-1-yl)-7-methoxycarbonyl-β-D-*ribo*-non-7-eno-1,4-furanuronate.** Isolated as a white powder (8 mg, 75%). $[\alpha]_D^{20}$ +24.20 (*c* 0.001, $CHCl_3$); $\nu_{max}$(cast)/cm$^{-1}$ 3650–3100, 2955, 2925, 2854, 1713 and 1128; $\delta_H$(500 MHz; $CD_3OD$) 1.80–1.88 (1 H, m, $CH_aH_b$), 1.90–1.97 (1 H, m, $CH_aH_b$), 2.45–2.51 (1 H, m, $CH_cH_d$), 2.54–2.60 (1 H, m, $CH_cH_d$), 3.70 (3 H, s, $OCH_3$), 3.78 (3 H, s, $OCH_3$), 3.85–3.91 (2 H, m, H3′, H4′), 4.18 (1 H, dd, *J* 5.3, 4.1, H2′), 5.71 (1 H, d, *J* 8.0, H5), 5.75 (1 H, d, *J* 4.1, H1′), 5.99 (1 H, t, *J* 1.5, C=CH), 7.56 (1 H, d, *J* 8.0, H6); $\delta_C$(125 MHz; $CDCl_3$) 31.5, 32.0, 52.3, 52.9, 74.7, 74.9, 83.7, 92.2, 103.0, 121.6, 142.9, 150.6, 152.2, 166.1, 167.0, 170.6; *m*/*z* (ES+) calcd for $C_{16}H_{20}N_2O_9Na$ 407.1061, found 407.1060 [$MNa^+$].

**Methyl 1,5,6-trideoxy-1-(uracil-1-yl)-8-methoxycarbonyl-β-D-*ribo*-dec-8-eno-1,4-furanuronate.** Isolated as a white powder (7 mg, 74%). $[\alpha]_D^{20}$ +37.79 (*c* 0.001, $CHCl_3$); $\nu_{max}$(cast)/cm$^{-1}$ 3600–3150, 2956, 2924, 2853 and 1712; $\delta_H$(500 MHz; $CD_3OD$) 1.58–1.80 (4 H, m, 2 × $CH_2$), 2.41–2.46 (2 H, m, allylic $CH_2$), 3.69 (3 H, s, $OCH_3$), 3.77 (3 H, s, $OCH_3$), 3.84–3.87 (2 H, m, H3′, H4′), 4.13–4.15 (1 H, m, H2′), 5.71 (1 H, d, *J* 8.0, H5), 5.77 (1 H, d, *J* 4.0, H1′), 5.93 (1 H, s, C=CH), 7.56 (1 H, d, *J* 8.0, H6); $\delta_C$(125 MHz; $CDCl_3$) 24.6, 33.4, 34.9, 52.3, 52.8, 74.8, 75.1, 84.5, 91.9, 102.9, 121.0, 142.6, 151.6, 152.2, 166.1, 166.9, 170.8; *m*/*z* (ES+) calcd for $C_{17}H_{22}N_2O_9Na$ 421.1218, found 421.1222 [$MNa^+$].

**Methyl 1,5,6-trideoxy-1-(uracil-1-yl)-9-methoxycarbonyl-β-D-*ribo*-undec-9-eno-1,4-furanuronate.** Isolated as a white powder (2 mg, 75%). $[\alpha]_D^{20}$ +34.99 (*c* 0.001, $CHCl_3$); $\nu_{max}$(cast)/cm$^{-1}$ 3700–3000, 2951, 2858 and 1694; $\delta_H$(600 MHz; $CD_3OD$) 1.43–1.50 (1 H, m, $CH_aH_b$), 1.51–1.58 (3 H, m, $CH_2$ + $CH_aH_b$), 1.64–1.70 (1 H, m, $CH_cH_d$), 1.72–1.78 (1 H, m, $CH_cH_d$), 2.39 (2 H, td, *J* 7.3, 1.5, allylic $CH_2$), 3.69 (3 H, s, $OCH_3$), 3.77 (3 H, s, $OCH_3$), 3.84–3.87 (2 H, m, H3′, H4′), 4.14 (1 H, dd, *J* 5.4, 4.2 H2′), 5.71 (1 H, d, *J* 8.1, H5), 5.77 (1 H, d, *J* 4.2, H1′), 5.92 (1 H, t, *J* 1.5, C=CH), 7.56 (1 H, d, *J* 8.1, H6); $\delta_C$(125 MHz; $CDCl_3$) 26.1, 28.0, 34.0, 35.0, 52.3, 52.8, 74.8, 75.1, 84.8, 91.6, 103.0 120.7, 142.6, 151.9, 152.3, 166.1, 167.0, 170.9; *m*/*z* (ES+) calcd for $C_{18}H_{24}N_2O_9Na$ 435.1374, found 435.1372 [$MNa^+$].

**Methyl 1,5,6-trideoxy-1-(uracil-1-yl)-7-methoxycarbonyl-β-D-*ribo*-nona-1,4-furanuronate.** Isolated as a colourless glass (13 mg, 100%). $[\alpha]_D^{20}$ +20.30 (*c* 0.0013, $CH_3OH$); $\nu_{max}$(cast)/cm$^{-1}$ 3600–3100, 3026, 2954, 1693 and 1107; $\delta_H$(500 MHz; $CD_3OD$) 1.63–1.78 (4 H, m, 2 × $CH_2$), 2.54 (1 H, dd, *J* 16.8, 5.3, $CH_3O_2CCH_aH_b$), 2.69 (1 H, dd, *J* 16.8, 9.3, $CH_3O_2CCH_aH_b$), 2.85–2.91 (1 H, m, $CH_3O_2CCH$), 3.64 (3 H, s, $OCH_3$), 3.68 (3 H, s, $OCH_3$), 3.80–3.86 (2 H, m, H3′, H4′), 4.14–4.17 (1 H, m,

H2′), 5.71 (1 H, d, *J* 8.0, H5), 5.75 (1 H, d, *J* 4.5, H1′), 7.56 (1 H, d, *J* 8.0, H6); $\delta_C$(125 MHz; $CD_3OD$) 29.2, 29.3, 31.8, 36.6, 36.7, 42.2, 42.3, 52.2, 52.4, 74.7, 74.8, 74.9, 75.0, 84.5, 92.0, 102.9, 103.0, 142.8, 152.2, 166.1, 174.0, 176.8; *m/z* (ES+) calcd for $C_{16}H_{22}N_2O_9Na$ 409.1218, found 409.1216 [$MNa^+$].

**Methyl 1,5,6-trideoxy-1-(uracil-1-yl)-8-methoxycarbonyl-β-D-*ribo*-deca-1,4-furanuronate.** Isolated as a glass (23 mg, 96%). $\nu_{max}$(cast)/$cm^{-1}$ 3600–3000, 3061, 2952, 2863, 1693 and 1053; $\delta_H$(500 MHz; $CD_3OD$) 1.39–1.78 (6 H, m, 3 × $CH_2$), 2.50 (1 H, dd, *J* 16.5, 5.0, $CH_3O_2CCH_aH_b$), 2.67 (1 H, dd, *J* 16.5, 9.2, $CH_3O_2CCH_aH_b$), 2.81–2.87 (1 H, m, $CH_3O_2CCH$), 3.64 (3 H, s, $OCH_3$), 3.66 (3 H, s, $OCH_3$), 3.83–3.86 (2 H, m, H3′, H4′), 4.14 (1 H, app t, *J* 4.0, H2′), 5.71 (0.5 H, d, *J* 8.0, H5), 5.72 (0.5 H, d, *J* 8.0, H5), 5.76 (1 H, d, *J* 4.0, H1′), 7.55 (0.5 H, d, *J* 8.0, H6), 7.56 (0.5 H, d, *J* 8.0, H6); $\delta_C$(125 MHz; $CD_3OD$) 24.38, 24.41, 32.8, 34.0, 34.1, 36.66, 36.68, 42.4, 52.2, 52.3, 74.7, 75.0, 75.1, 84.56, 84.61, 91.7, 103.0, 142.6, 152.3, 166.2, 174.1, 176.98, 177.01; *m/z* (ES+) calcd for $C_{17}H_{24}N_2O_9Na$ 423.1374, found 423.1376 [$MNa^+$].

**Methyl 1,5,6-trideoxy-1-(uracil-1-yl)-9-methoxycarbonyl-β-D-*ribo*-undeca-1,4-furanuronate.** Isolated as a colourless glass (15 mg, 89%). $[\alpha]_D^{20}$ +33.73 (*c* 0.0011, $CHCl_3$); $\nu_{max}$(cast)/$cm^{-1}$ 3550–3100, 3025, 2948, 2860, 1717 and 1049; $\delta_H$(300 MHz; $CD_3OD$) 1.28–1.71 (8 H, m, 4 × $CH_2$), 2.49 (1 H, dd, *J* 16.6, 5.3, $CH_3O_2CCH_aH_b$), 2.66 (1 H, dd, *J* 16.6, 9.3, $CH_3O_2CCH_aH_b$), 2.77–2.86 (1 H, m, $CH_3O_2CCH$), 3.64 (3 H, s, $OCH_3$), 3.66 (3 H, s, $OCH_3$), 3.82–3.86 (2 H, m, H3′, H4′), 4.12–4.15 (1 H, m, H2′), 5.72 (1 H, d, *J* 8.1, H5), 5.77 (1 H, d, *J* 4.2, H1′), 7.56 (1 H, dd, *J* 8.1, 0.9, H6); $\delta_C$(125 MHz; $CD_3OD$) 26.7, 27.79, 27.80, 32.8, 34.1, 36.66, 36.68, 42.39, 42.41, 52.2, 52.3, 74.8, 75.066, 75.074, 84.8, 91.6, 103.0, 142.6, 152.3, 166.1, 174.1, 177.1; *m/z* (ES+) calcd for $C_{18}H_{26}N_2O_9Na$ 437.1531, found 437.1530 [$MNa^+$].

### General procedure for hydrolysis of dimethyl diesters

The dimethyl diester was dissolved in a 4 : 3 $CD_3OD$–$D_2O$ mixture (700 μL) and placed in an NMR tube. A solution of LiOH in $D_2O$ (1 M, 4.0 equiv.) was then added, and the reaction progress was monitored by $^1H$ NMR. In the case of the dimethyl maleates, hydrolyses were conducted at 40 °C, and for the dimethyl succinates, hydrolyses were conducted at rt. Upon completion of the reaction, the solvent was removed and the remaining residue was dissolved in $H_2O$. Lyophilization gave the respective lithium salt.

**1,5,6-Trideoxy-1-(uracil-1-yl)-7-hydroxycarbonyl-β-D-*ribo*-non-7-eno-1,4-furanuronic acid, trilithium salt (9).** Isolated as a solid (5 mg, 100%). $[\alpha]_D^{20}$ +3.82 (*c* 0.0011, $H_2O$); $\nu_{max}$(μscope)/$cm^{-1}$ 3650–2700, 1684 and 1135; $\delta_H$(500 MHz; $D_2O$) 1.81–1.97 (2 H, m, $CH_2$), 2.30–2.37 (1 H, m, $CH_aH_b$), 2.41–2.47 (1 H, m, $CH_aH_b$), 4.02–4.07 (2 H, m, H3′, H4′), 4.28 (1 H, app t, *J* 5.0, H2′), 5.55 (1 H, s, C=CH), 5.82 (1 H, d, *J* 7.7, H5), 5.92 (1 H, d, *J* 5.0, H1′), 7.53 (1 H, d, *J* 7.7, H6); $\delta_C$(125 MHz; $D_2O$) 31.4, 31.9, 73.8, 74.6, 83.7, 90.3, 103.8, 121.5, 141.1, 150.8, 160.5, 169.0, 175.2, 177.9; *m/z* (ES−) calcd for $C_{14}H_{15}N_2O_9$ 355.0772, found 355.0775 [$MH^-$].

**1,5,6-Trideoxy-1-(uracil-1-yl)-8-hydroxycarbonyl-β-D-*ribo*-dec-8-eno-1,4-furanuronic acid, trilithium salt (12).** Isolated as a white solid (4 mg, 100%). $[\alpha]_D^{20}$ +16.15 (*c* 0.0013, $H_2O$); $\nu_{max}$(μscope)/$cm^{-1}$ 3700–3000, 2942, 1688, 1568 and 1072; $\delta_H$(600 MHz; $D_2O$) 1.54–1.68 (2 H, m, $CH_2$), 1.70–1.84 (2 H, m, $CH_2$), 2.29–2.31 (2 H, m, allylic $CH_2$), 4.03 (2 H, br s, H3′, H4′), 4.26–4.28 (1 H, m, H2′), 5.50 (1 H, s, C=CH), 5.81 (1 H, d, *J* 7.8, H5), 5.90 (1 H, d, *J* 4.8, H1′), 7.54 (1 H, d, *J* 7.8, H6); $\delta_C$(125 MHz; $D_2O$) 24.0, 33.0, 35.1, 73.8, 74.5, 84.3, 90.2, 103.8, 1221.2, 141.1, 151.8, 160.0, 167.0, 175.3, 177.2; *m/z* (ES−) calcd for $C_{15}H_{17}N_2O_9$ 369.0929, found 369.0928 [$MH^-$].

**1,5,6-Trideoxy-1-(uracil-1-yl)-9-hydroxycarbonyl-β-D-*ribo*-undec-9-eno-1,4-furanuronic acid, trilithium salt (15).** Isolated as a white solid (2 mg, 100%). $[\alpha]_D^{20}$ −4.14 (*c* 0.0014, $H_2O$); $\nu_{max}$(μscope)/$cm^{-1}$ 3650–3000, 2940, 1688, 1565 and 1137; $\delta_H$(500 MHz; $D_2O$) 1.42–1.55 (4 H, m, 2 × $CH_2$), 1.71–1.79 (2 H, m, $CH_2$), 2.24–2.27 (2 H, m, allylic $CH_2$), 4.01–4.04 (2 H, m, H3′, H4′), 4.28 (1 H, app t, *J* 5.0, H2′), 5.49 (1 H, s, C=CH), 5.82 (1 H, d, *J* 7.8, H5), 5.91 (1 H, d, *J* 5.0, H1′), 7.54 (1 H, d, *J* 7.8, H6); $\delta_C$(125 MHz; $D_2O$) 25.4, 27.8, 33.2, 35.2, 73.8, 74.5, 84.5, 90.1, 103.8, 120.7, 141.1, 152.5, 161.1, 165.7, 175.4, 176.3; *m/z* (ES−) calcd for $C_{16}H_{19}N_2O_9$ 383.1085, found 383.1084 [$MH^-$].

**1,5,6-Trideoxy-1-(uracil-1-yl)-7-hydroxycarbonyl-β-D-*ribo*-nona-1,4-furanuronic acid, trilithium salt (19).** Isolated as a white solid (7 mg, 100%). $[\alpha]_D^{20}$ +7.00 (*c* 0.001, $H_2O$); $\nu_{max}$(μscope)/$cm^{-1}$ 3700–2800, 1675, 1570 and 1130; $\delta_H$(400 MHz; $D_2O$) 1.50–1.74 (4 H, m, 2 × $CH_2$), 2.19 (1 H, dd, *J* 14.6, 9.8, $CH_3O_2CCH_aH_b$), 2.47 (1 H, dd, *J* 14.6, 5.6, $CH_3O_2CCH_aH_b$), 2.55–2.62 (1 H, m, $CH_3O_2CCH$), 3.97–4.04 (2 H, m, H3′, H4′), 4.27 (1 H, app t, *J* 3.5, H2′), 5.84 (0.5 H, d, *J* 7.6, H5), 5.86 (0.5 H, d, *J* 8.0, H5), 5.88 (0.5 H, d, *J* 3.5, H1′), 5.89 (0.5 H, d, *J* 3.5, H1′), 7.57 (0.5 H, d, *J* 7.6, H6), 7.58 (0.5 H, d, *J* 8.0, H6); $\delta_C$(125 MHz; $D_2O$) 28.9, 29.0, 31.80, 31.84, 41.85, 41.90, 46.9, 73.7, 73.8, 74.5, 74.6, 84.6, 84.7, 89.9, 103.76, 103.80, 141.2, 159.2, 165.1, 176.1, 182.3, 184.86, 184.89; *m/z* (ES−) calcd for $C_{14}H_{17}N_2O_9$ 357.0940, found 357.0939 [$MH_2^-$].

**1,5,6-Trideoxy-1-(uracil-1-yl)-8-hydroxycarbonyl-β-D-*ribo*-deca-1,4-furanuronic acid, trilithium salt (20).** Isolated as a white solid (6 mg, 88%). $[\alpha]_D^{20}$ +6.31 (*c* 0.0013, $H_2O$); $\nu_{max}$(μscope)/$cm^{-1}$ 3600–3000, 2941, 1695, 1576, 1426 and 1088; $\delta_H$(600 MHz; $D_2O$) 1.32–1.80 (6 H, m, 3 × $CH_2$), 2.18 (0.56 H, dd, *J* 14.4, 10.2, $CH_3O_2CCH_aH_b$), 2.37 (0.44 H, dd, *J* 15.9, 5.7, $CH_3O_2CCCH_aH_b$), 2.47 (1 H, m, $CH_3O_2CCH_aH_b$), 2.58 (0.56 H, m, $CH_3O_2CCH$), 2.79–2.85 (0.44 H, m, $CH_3O_2CCH$), 3.99–4.05 (2 H, m, H3′, H4′), 4.29 (1 H, app q, *J* 4.8 H2′), 5.85 (1 H, br s, H5), 5.88 (0.44 H, d, *J* 4.2, H1′), 5.89 (0.56 H, d, *J* 4.8, H1′), 7.55 (0.44 H, dd, *J* 7.8, 4.8, H6), 7.58 (0.56 H, d, *J* 7.8, 2.4, H6); $\delta_C$(125 MHz; $D_2O$) 23.5, 24.0, 33.6, 40.5, 42.1, 43.3, 43.4, 46.9, 73.68, 73.73, 73.8, 74.45, 74.48, 84.2, 84.3, 84.7, 84.8, 89.99, 90.05, 90.5, 103.6, 141.68, 141.74, 149.2, 162.5, 182.5, 185.4; *m/z* (ES−) calcd for $C_{15}H_{19}N_2O_9$ 371.1096, found 371.1097 [$MH_2^-$].

**1,5,6-Trideoxy-1-(uracil-1-yl)-9-hydroxycarbonyl-β-D-*ribo*-undeca-1,4-furanuronic acid, trilithium salt (21).** Isolated as a white solid (5 mg, 76%). $[\alpha]_D^{20}$ +6.11 (*c* 0.0009, $H_2O$); $\nu_{max}$(μscope)/$cm^{-1}$ 3700–3000, 2935, 1681, 1579 and 1087; $\delta_H$(600 MHz; $D_2O$) 1.30–1.52 (6 H, m, 3 × $CH_2$), 1.67–1.77 (2 H, m, allylic $CH_2$), 2.17 (0.78 H, dd, *J* 14.4, 9.6, $CH_3O_2CCH_aH_b$), 2.37 (0.22 H, dd, *J* 15.6, 6.0, $CH_3O_2CCH_aH_b$), 2.44 (0.78 H, dd, *J* 14.4, 5.4, $CH_3O_2CCH_aH_b$), 2.47 (0.22 H, dd, *J* 15.6, 9.0, $CH_3O_2CCH_aH_b$), 2.56 (0.78 H, m, $CH_3O_2CCH$), 2.77–2.82

(0.22 H, m, $CH_3O_2CCH$), 4.00–4.04 (2 H, m, H3′, H4′), 4.28–4.30 (1 H, m, H2′), 5.84 (1 H, d, *J* 7.8, H5), 5.88 (0.22 H, d, *J* 4.8, H1′), 5.89 (0.78 H, d, *J* 5.4, H1′), 7.55 (0.22 H, d, *J* 7.8, H6), 7.56 (0.78 H, d, *J* 7.8, H6); $\delta_C$(125 MHz; $D_2O$) 25.5, 25.8, 27.7, 32.2, 32.6, 33.2, 33.4, 40.5, 42.1, 47.1, 73.8, 74.5, 84.4, 84.4, 84.7, 84.8, 90.1, 103.7, 141.5, 157.7, 163.0, 181.4, 182.6, 185.7; *m/z* (ES−) calcd for $C_{16}H_{21}N_2O_9$ 385.1253, found 385.1243 [$MH_2^-$].

### NDP kinase assay

The coupled assay using pyruvate kinase and lactate dehydrogenase described by Kezdi *et al.*[26] was used and adapted to microtiter plates. The final assay volume was 100 μL, with the final concentrations as follows: 50 mM Tris-HCl (pH 7.6), 75 mM KCl, 5 mM $MgCl_2$, 1 mM phosphoenolpyruvate, 1 mM NADH, 0.2 mM ATP, 0.6 mM 8-bromoinosine diphosphate, 1 mg $mL^{-1}$ bovine serum albumin, and 50 U $mL^{-1}$ pyruvate kinase–lactate dehydrogenase. The reaction was initiated by adding NDP kinase (0.08 nM) with a multi-channel pipette to wells in the absence or presence of nucleotide analogue, and the activity was monitored at 30 °C by the decrease in absorbance at 340 nm.

### Antibacterial activity assay

The spot-on-lawn overlay method was used. Luria broth was used to grow *E. coli* DH5α, *Pseudomonas aeruginosa* ATCC 14207, *Salmonella typhimurium* ATCC 23564, and tryptic soy broth was used to grow *Staphylococcus aureus* ATCC 6538. A culture tube containing liquid media was inoculated from a frozen stock solution of the bacterial strain, and all strains were incubated overnight at 37 °C. The dimethyl diesters were dissolved in EtOH–$H_2O$ (2 : 1) and the lithium dicarboxylate salts were dissolved in $H_2O$. A 10 μL portion of a 20 mg $mL^{-1}$ solution of the nucleotide analogue was pipetted on a solid agar plate and allowed to dry. A 100 μL portion of the test organism from the culture tube was added to a tube containing melted soft agar (40 °C), and after gentle vortexing, this was poured onto the agar plate. After allowing the agar to solidify, the plates were incubated overnight at 37 °C. Activity was detected by the appearance of a circular zone of growth inhibition around the area where the nucleotide analogue was spotted.

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