ADP-ribose analogues: synthetic strategy towards inhibitors for viral macrodomains: SARS-CoV-2

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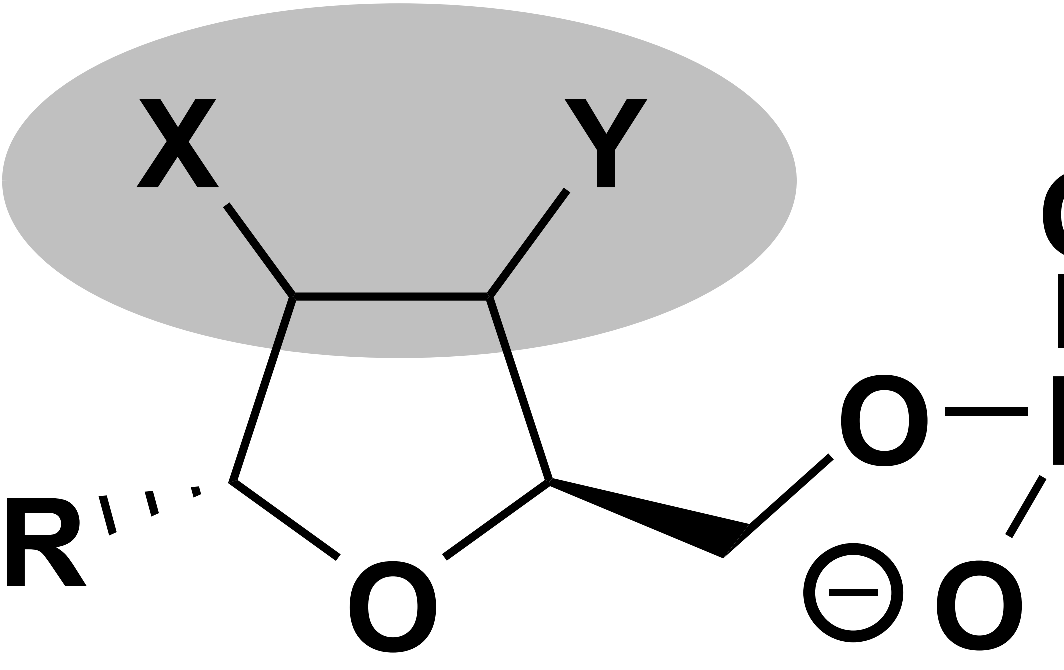
Chapter 3

Distal Ribose Part 2:
C2” and C3”



Introduction

In Chapter 2, modification of ADP-ribosides at the distal ribose anomeric position was shown to be a viable option when designing inhibitors for the SARS-CoV-2 macrodomain 1 (Mac1). In this Chapter, we aim to further explore modifications at the distal ribose, specifically at the C2” and C3” positions to see if their modification will generate better binders. To this end, the co-crystal structure of the SARS-CoV-2 Mac1 in complex with ADP-α-ribose was inspected for possible interaction candidates (Figure 1).[1,2] Notably, a phenylalanine residue (Phe132) was observed to be close to the distal ribose, making it a potential candidate for additional hydrophobic and π-π stacking interactions interactions.[3] While the aromatic ring of Phe132 is too close to the C3” position to allow for the introduction of a bulky aromatic moiety, the 2” position might be distant enough to allow for the accommodation of an apolar and aromatic benzyl group. Notably, the C3” hydroxyl participates in hydrogen bonding with Asn40 while the C2” position participates in hydrogen bonding with a water molecule (W6) which is hydrogen bonding to His45. Both of these interactions are favourable to binding of the inhibitor, but displacement of W6 by introduction of another group on the C2” hydroxyl should increase gain in entropy, potentially making it a net-favourable exchange. Moreover, the C3” position hydroxyl is deeper inside the Mac1 binding cleft than the C2”, which makes it more prone to unfavourable steric clashes when modified.

Thus, ADP-ribose analogues **1** and **2** were designed, based on their potent parent compounds synthesized in Chapter 2. To investigate the importance of H-bonding and polar interactions in binding the inhibitors, the *O*-methyl oxime derivatives **3** and **4** were chosen as candidates as well (Figure 2). The oximes, having a sp^2 hybridized carbon in the ribose ring,[4] are relatively small and may engage in different H-bond and polar interactions than the parent alcohol functions. The previously synthesized fluorinated analogue **5** is taken along in the binding studies as this close analogue may shed light on the importance of the H-bond formed by the C3” hydroxyl and Asn40.

Results and discussion

3.1 Synthesis of ADP-ribose analogues modified at the C2" or C3” hydroxyl

The synthesis of the set of inhibitors started with the generation of analogues **1** and **2** (Scheme 1), which were based on the α-*O*-methyl and 1-azido ADP-ribose parent compounds, introduced in Chapter 2 (Ch2, **1** and **14**, respectively). While 1-azido ADPr is a more a potent inhibitor for SARS-CoV-2 Mac1 than α-*O*-methyl ADPr (IC_{50} = 0.49 μM vs. 7.0 μM, respectively), the ease of synthesis of the 1"-*O*-methyl compound as compared to its 1”-azido counterpart, makes it a tractable target. A common synthetic strategy was developed for both target compounds **1** and **2**. Benzylated 1"-*O*-methyl

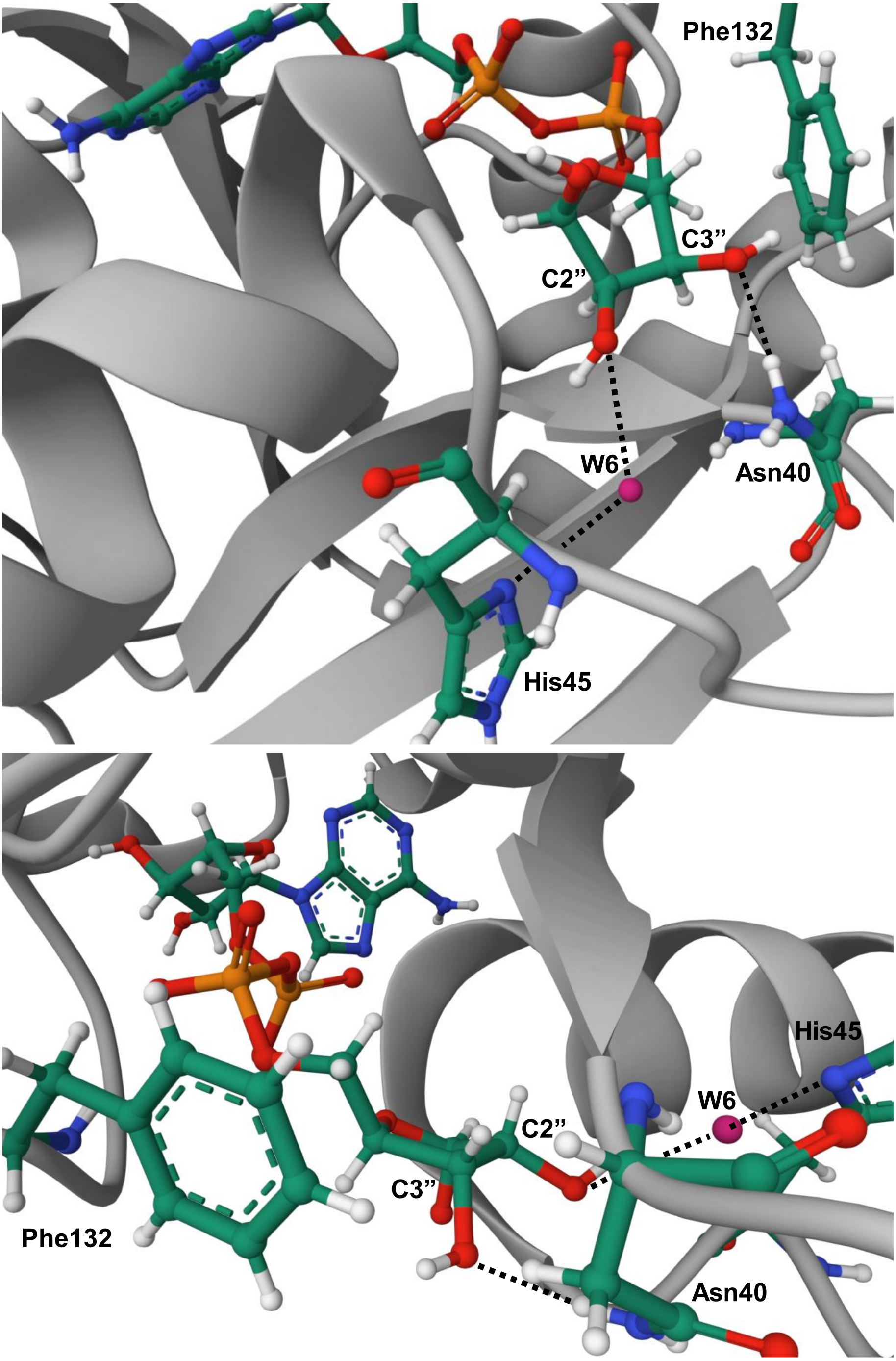


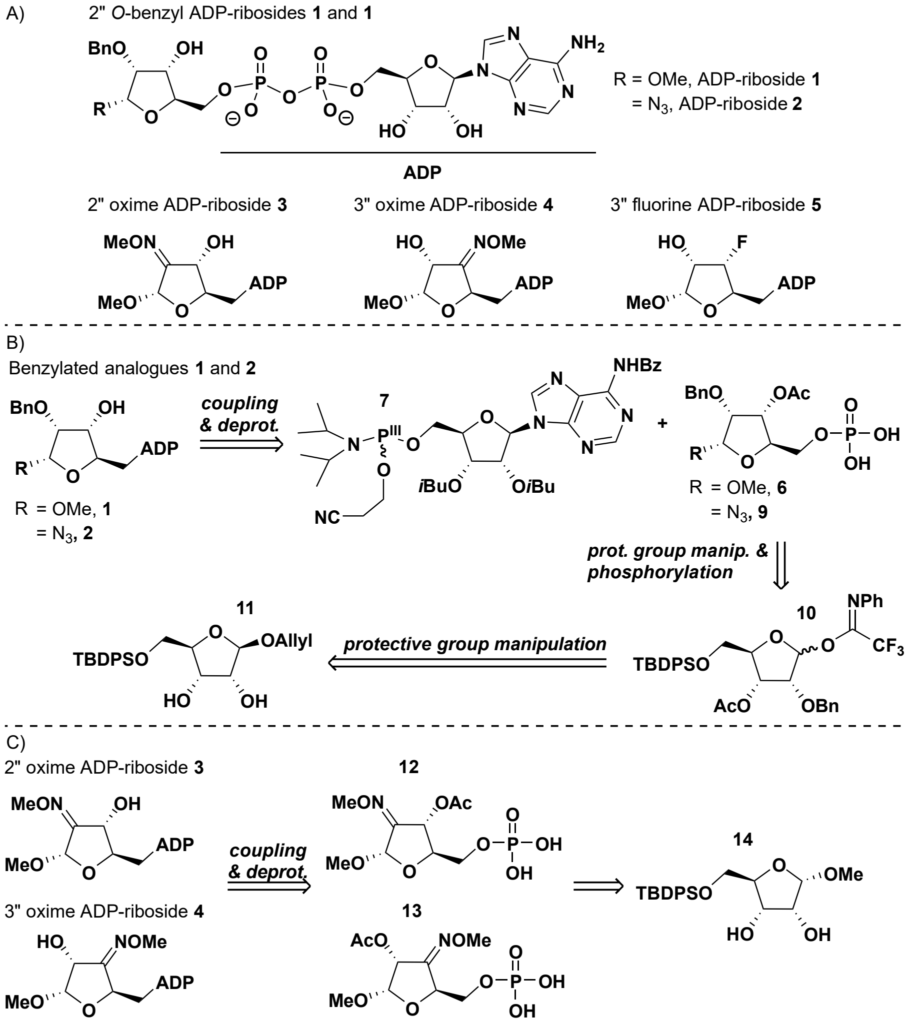
Figure 1 Co-crystal structure of SARS-CoV-2 nsp3 Mac1 with ADP-ribose. PDB: 7KQP.[1]

analogue **1** was envisioned to be obtained from phosphate **6** and phosphoramidite **7** (Ch2, **10**)[5] through the previously discussed P(III)-P(V) coupling methodology[6–8] followed by global basic deprotection.[9] In turn, phosphate **6** could be produced from the previously described tri-acetyl α-*O*-methyl ribose **8** (Scheme 1B) (Ch2, **5α**) through protective group manipulation and phosphorylation. Similarly, benzylated α-azide **2** was envisioned to be produced from monophosphate **9** through P(III)-P(V) coupling to phosphoramidite **7** followed by a basic global deprotection. Monophosphate **9** was to be obtained from imidate donor **10** through glycosylation followed by protective group manipulation and phosphorylation. Finally, donor **10** could be obtained from *O*-allyl riboside **11** through protective group manipulations followed by deallylation and imidate formation.

Next the *O*-methyl oxime-bearing ADP-ribose analogues **3** and **4** were designed (Scheme 1C). Both compounds were to be produced from their acetyl protected monophosphate counterparts **12** and **13**, which in turn could be afforded from common intermediate **14**. Here a global basic deprotection step was considered as the oxime moiety may be hydrolysed under acidic conditions. Thus, both monophosphates **18** and **19** were to be coupled to the previously described *iso*-butyryl/benzoyl protected adenosine phosphoramidite **7**[5] through the established P(III)-P(V) methodology.[6–8]

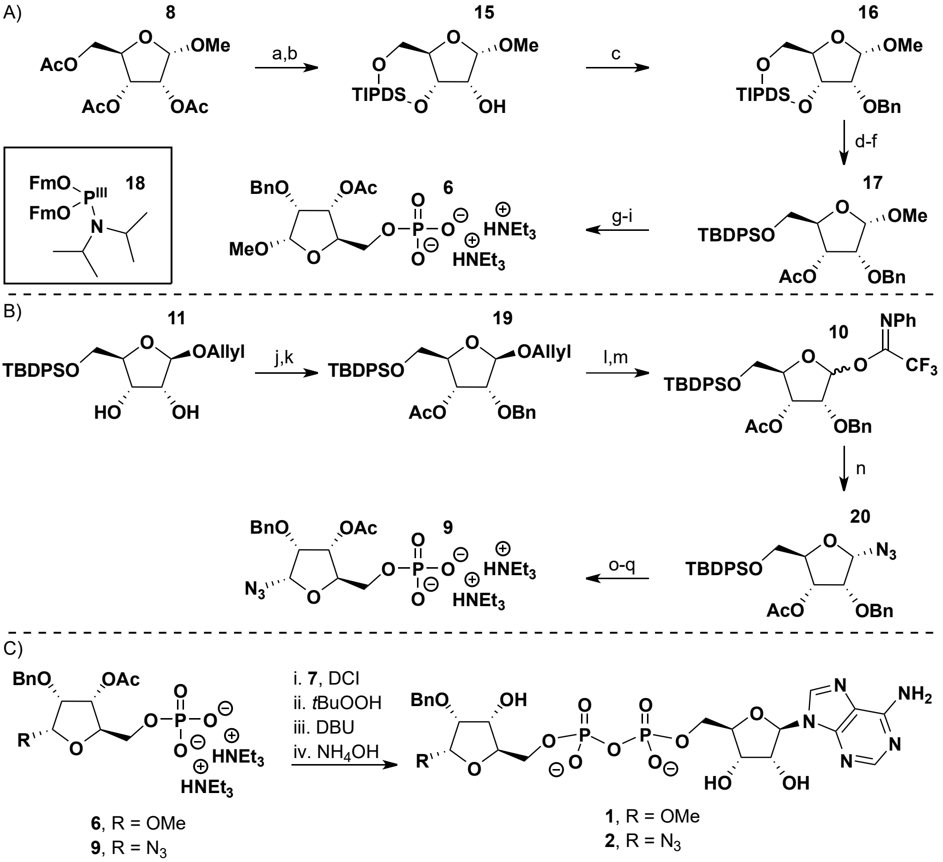
Synthesis of benzylated *O*-methyl monophosphate **6** commenced from anomerically pure tri-acetyl α-*O*-methyl ribose **8** (Scheme 2A), which was deacetylated using methanolic sodium methoxide, to afford the corresponding triol in good yield (82%). This compound was then dissolved in dry pyridine and selectively protected on the C3 and C5 hydroxyls by slow addition of tetra-*iso*-propyl-disiloxane dichloride ($TIPDSCl_2$) to afford **15** protected with a cyclic siloxane in 48% yield. Compound **15** was then subjected to Williamson etherification conditions[10,11] using benzyl bromide to afford benzyl ether **16** (64%), which was subsequently treated with HF in pyridine to remove the cyclic siloxane protective group, providing the diol in near quantitative yield. The primary hydroxyl in this diol was selectively protected with a bulky silyl ether (TBDPS) and the resulting alcohol was then subjected to acetylation conditions using acetic anhydride to afford ester **17**. Next, the silyl ether in ester **17** was selectively removed using HF in pyridine to liberate the primary hydroxyl (64%) which was phosphorylated using $(FmO)_2PN(iPr)_2$ **18** and the resulting phosphite was subsequently oxidized to afford the corresponding protected phosphate in 74% yield. Finally, this phosphate was deprotected using triethylamine which provided monophosphate **6** as the triethylammonium salt.

Synthesis of benzylated α-azide mono-phosphate **9** (Scheme 2B) commenced from riboside **11** (See Chapter 2), which was subjected to Williamson etherification



Scheme 1 A) Target inhibitors **1** – **5**. B) and C) Synthetic approach towards inhibitors **1** - **4**.

conditions using 1.1 equivalents of sodium hydride to furnish a mixture of mono and dibenzylated constructs that could be separated to afford the desired 2” benzyl ether (37%). This alcohol was subsequently acetylated using acetic anhydride to furnish ester **19** in 49% yield. Next, the anomeric *O*-allyl protective group was isomerized by treatment with a hydrogen activated iridium catalyst after which the resulting enol ether was cleaved using elemental iodine and sodium bicarbonate in the same pot, to afford the lactol in 69% yield. This compound was then transformed into donor **10** by treatment with *N*-



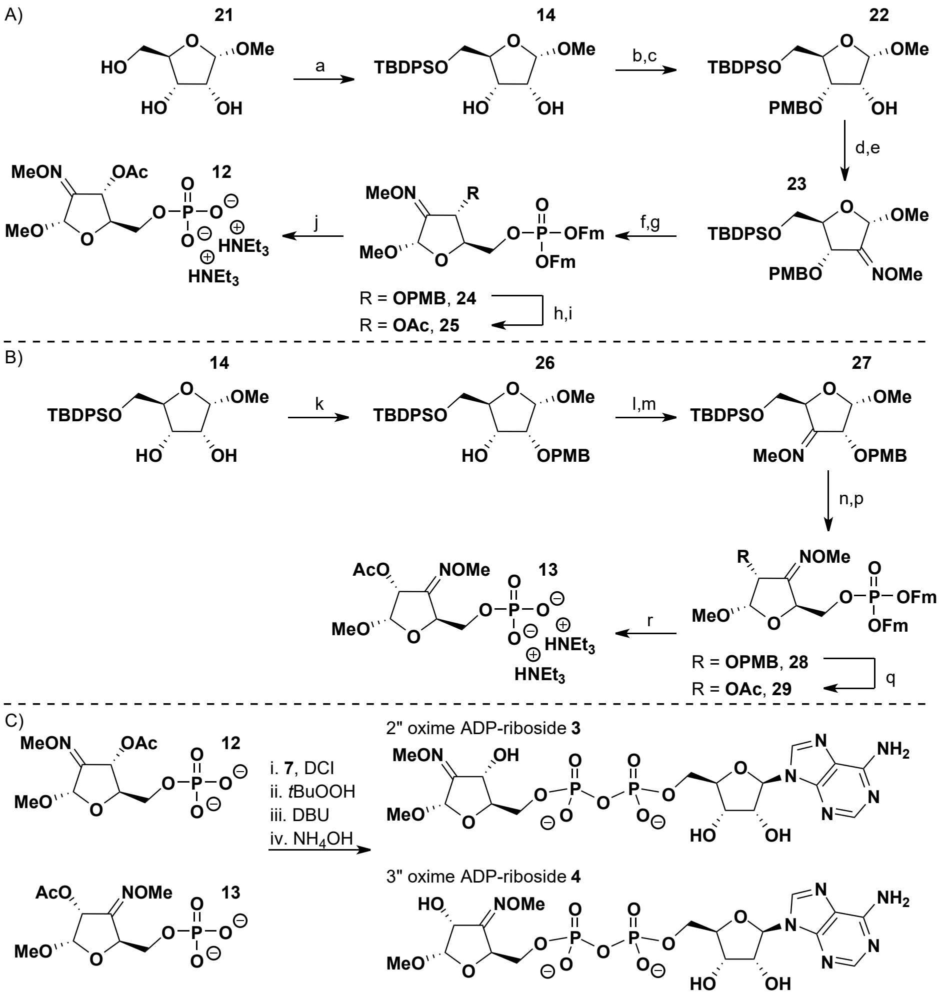
Scheme 2 Synthesis of the benzylated ribosyl phosphates. A) Synthesis of the 2” benzyl α-*O*-methyl ribosyl phosphate **6**. Reagents and conditions. a) NaOMe, MeOH, 0 ˚C, 3.5 hrs, 82%; b) $TIPDSCl_2$, pyr., -20 ˚C, 3.5 hrs, 48%; c) BnBr, NaH, DMF, 0 ˚C, 40 min., 64%; d) HF in pyr., pyr., RT, 1 hr, 95%; e) TBDPS-Cl, pyr., RT, o.n., 81%; f) Ac_2O, DMAP, pyr., RT, o.n., quant.; g) HF in pyr., pyr., 0 ˚C, 2 hrs, 64%; h) (i) **18**, DCI, ACN, RT, 90 min. (ii) *t*BuOOH, ACN, RT, 35 min., 74% (over two steps); i) Et_3N, ACN, RT, 48 hrs. B) Synthesis of the 2” benzyl α-azide ribosyl phosphate **9**. Reagents and conditions. j) BnBr, NaH, DMF, RT, 2 hrs, 37%; k) Ac_2O, DMAP, pyr., RT, o.n., 49%; l) (i) $Ir(COD)(PPh_2Me)PF_6$, H_2, THF, o.n. (ii) aq. $NaHCO_3$, I_2, THF, 0 ˚C, 30 min., 69% (over two steps); m) PTFAI-Cl, cesium carbonate, acetone/water (50/1), 0 ˚C, 20 hrs, 96%; n) TMS-N_3, TMS triflate, DCM, -60 ˚C, 20 hrs, 71%; o) HF in pyr., pyr., RT, 60 min., 65%; p) (i) **18**, DCI, ACN, RT, 90 min. (ii) *t*BuOOH, ACN, RT, 50% (over two steps); q) Et_3N, ACN, RT, 72 hrs. C) Final steps in the synthesis of the 2” benzylated ADP-ribose analogues. Reagents and conditions. (i) DCI, ACN, RT (ii) *t*BuOOH, ACN, RT (iii) DBU, ACN, RT iv) aq. NH_4OH (30 wt%), ACN, RT, (**1**: 15%; **2**: 7%, over five steps);

phenyltrifluoroacetimidoyl chloride (PTFAI-Cl) in wet acetone in the presence of cesium carbonate (96%). Donor **10** was then used in a glycosylation reaction with TMS azide as the nucleophile,[12,13] which furnished α-azide **20** as a single anomer in 71% yield. Finally, the TBDPS protective group in azide **20** was removed using HF in pyridine (65%), whereafter phosphorylation using **18** and oxidation gave the corresponding protected phosphate. This phosphate was then deprotected using triethylamine in acetonitrile to afford benzylated α-azide monophosphate **9** as the triethylammonium salt.

Finally, both monophosphates (*O*-methyl **6** and azide **9**) were coupled to adenosine phosphoramidite **7** (Scheme 2C) utilizing the previously described P(III)-P(V) methodology,[6–8] followed by one pot oxidation of the intermediate P(III)-P(V) species, deprotection of the generated pyrophosphate and global deprotection to afford benzylated α-*O*-methyl ADP-ribose analogue **1** and α-azide ADP-ribose analogue **2** in 15% and 7% (over five steps), respectively.

Synthesis of 2” O-methyl oxime monophosphate **12** commenced from previously produced anomerically pure triol **21** which was regioselectively protected with a bulky silyl ether (TBDPS) to afford the intermediate diol **14** in 73% yield (Scheme 3A). Diol **14** was then outfitted with a bridging *para*-methoxy benzylidene group to produce the bicyclic intermediate (83%). The *para*-methoxy benzylidene acetal was then regioselectively opened using di-*iso*-butyl aluminium hydride (DIBAL-H) which gave **22** with a PMB ether at the 3-hydroxyl in adequate yield (57%). Next, oxidation of the free secondary hydroxyl in compound **22** using 2-iodobenzoic acid (IBX) afforded the ketone (77%), which was then treated with methoxyamine hydrochloride in a basic and protic solvent system to afford *O*-methyl oxime **23** in 79% yield. To achieve a one-step global deprotection, the 3” PMB had to be exchanged for an acetyl group. Notably, all efforts to remove the PMB in compound **23** through oxidative cleavage[14] or subjection to acid were unsuccessful, generating the desired secondary alcohol in very low yields or not at all. Therefore, it was decided to postpone this manipulation to a later stage. To this end, PMB ether **23** was treated with HF in pyridine to selectively remove the silyl ether (84%) and the liberated primary hydroxyl was then phosphorylated using $(FmO)_2PN(i\text{Pr})_2$ **18**, followed by one-pot oxidation of the generated phosphite into protected phosphate **24**. Next, the PMB in protected phosphate **24** was successfully removed using DDQ, and an acetyl was installed on the generated hydroxyl using acetic anhydride in pyridine to furnish the desired 3” position ester **25** in 34% over two steps. Finally, the phosphate was deprotected using triethylamine in acetonitrile which provided 2” *O*-methyl oxime monophosphate **18** as the triethylammonium salt.

With the 2” *O*-methyl oxime monophosphate **12** in hand, attention was turned to the synthesis of **13** (Scheme 3B). Starting from common intermediate **14**, this compound was subjected to Williamson etherification conditions using 1.1 equivalents of both PMB chloride and sodium hydride to regioselectively afford the C2”-*O*-PMB ether **26** in 63% yield. Similar to the previously described methodology, the oxime was installed by oxidation of the remaining hydroxyl (68%) followed by protection of the generated ketone with an *O*-methyl oxime moiety, which afforded compound **27** in 82% yield.



Scheme 3 A) Synthesis of the 2” oxime α-*O*-methyl ribosyl phosphate **12**. Reagents and conditions. a) TBDPS-Cl, DMAP, imidazole, DMF, RT, 24 hrs, 73%; b) anisaldehyde dimethyl acetal, PPTS, DMF, 30 ˚C, 5.5 hrs, 83%; c) DIBAL-H, DCM, -78 ˚C → -20 ˚C, 3 hrs, 57%; d) IBX, ACN, reflux, 1 hr, 77%; e) $MeONH_2$•HCl, pyr., ACN/methanol (1/6), RT, 2 hrs, 79%; f) HF in pyr., pyr., RT, 2 hrs, 84%; g) (i) **18**, DCI, ACN, RT, 90 min. (ii) *t*BuOOH, ACN, RT, 64% (over two steps); h) DDQ, phosphate buffer (pH = 7.4), DCM, RT, 6 hrs, 82%; i) Ac_2O, DMAP, pyr., RT, 2 hrs, 41%; j) Et_3N, ACN, RT, 24 hrs. B) Synthesis of the 3” oxime α-*O*-methyl ribosyl phosphate **13**. Reagents and conditions. k) PMB-Cl, TBAI, NaH, DMF, RT, 20 hrs, 63%; l) Dess-Martin periodinane, DCM, RT, 17 hrs, 68%; m) $MeONH_2$•HCl, pyr., ACN/methanol (2/5), RT, 2 hrs, 82%; n) HF in pyr., pyr., RT, 2 hrs, 74%; p) (i) **18**, DCI, ACN, RT, 90 min. (ii) *t*BuOOH, ACN, RT, 71% (over two steps); q) (i) TFA, TES-H, DCM, RT, 4.5 hrs. (ii) Ac_2O, pyr., RT, 3 hrs, 24% (over two steps); r) Et_3N, ACN, RT, 60 hrs. C) Final steps in the synthesis of the 2” and 3” oxime ADP-ribose analogues. Reagents and conditions. (i) DCI, ACN, RT (ii) *t*BuOOH, ACN, RT (iii) DBU, ACN, RT. iv) aq. NH_4OH (30 wt%), ACN, (**3**: 21%; **4**: 32%, over five steps).

Again, in order to install the acetyl group, the C-5” silyl ether had to be switched to the protected phosphate. Accordingly, silyl ether **27** was first treated with HF in pyridine to regenerate the primary alcohol (74%), whereafter this position was phosphorylated using $(FmO)_2PN(i\text{Pr})_2$ **18** and then oxidized to provide protected phosphate **28** in 71% yield. Next, the 2” position protective group was switched for an acetyl group, through acidic PMB removal followed by acetylation to afford the desired ester **29** in 24% yield over two steps. Finally, the phosphate was deprotected using triethylamine in acetonitrile to afford the 3” *O*-methyl oxime monophosphate **13** as the triethylammonium salt.

Now, with both monophosphates **12** and **13** in hand, the desired ADP-ribose analogues could be produced (Scheme 3C). Both monophosphates were first co-evaporated with mixtures of dry acetonitrile and pyridine to exchange triethylammonium for the pyridinium, whereafter the phosphates were coupled to the adenosine phosphoramidite **7** to generate the P(III)-P(V) intermediates. These intermediates were then oxidized to afford the protected pyrophosphates, which were subsequently deprotected using DBU to afford the free pyrophosphates. The constructs, still protected at the hydroxyl and amine groups, were then fully deprotected using ammonium hydroxide which, after purification and lyophilization, afforded both the 2” and 3” *O*-methyl oxime ADP-ribose analogues **3** and **4** in 21% and 32% yield over five steps, respectively.

3.3 Evaluation of the ADPr-analogues as potential inhibitors of Mac-1

With all the ADP-ribose analogues (**1** – **4**) successfully synthesized, testing of their potency for the SARS-CoV-2 Mac1 was performed using the HTRF assay described in Chapter 2.[15] As Table 1 shows, benzylation of the C2”-OH diminished binding affinity, with the *O*-methyl **1** showing no binding ($IC_{50} > 200\ \mu M$) while its azide counterpart **2** showed some binding ($IC_{50} = 80\ \mu M$), a decrease of almost twenty-fold compared to the parent C2”-OH compound. This loss in binding shows that the C2” benzyl ether does not have favourable interactions with phenylalanine 132 and may indicate that the C2” hydroxyl is necessary for binding of the ADP-ribose analogues. In contrast the C2” oxime **3** was found to bind relatively well ($IC_{50} = 9.4\ \mu M$) but slightly worse than its C2”-OH counterpart ($IC_{50} = 7.0\ \mu M$). Installation of the oxime at the 3” position greatly diminished binding affinity ($IC_{50} > 200\ \mu M$).

Besides the new analogues synthesized in this chapter, the fluorinated ADP-ribose analogue **5** was also probed for binding to show that replacing the 3” hydroxyl for a fluorine atom leads to an inactive compound ($IC_{50} > 200\ \mu M$). Fluorine atoms, in contrast to hydroxyl groups, cannot donate hydrogen bonds, but can serve as a weak

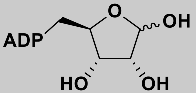
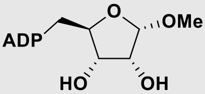
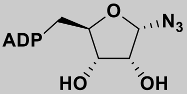
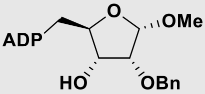
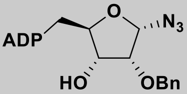
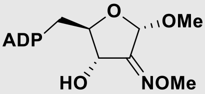
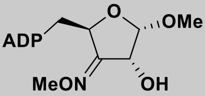
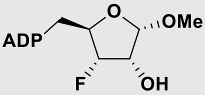
Compound	Structure	IC_{50}
ADP-ribose		1.2 μM
α-OMe-ADP-riboside		7.0 μM
α-N_3-ADP-riboside		0.49 μM
2” benzyl α-OMe-ADP-riboside 1		>200 μM
2” benzyl α-N_3-ADP-riboside 2		80 μM
2” oxime α-OMe-ADP-riboside 3		9.4 μM
3” oxime α-OMe-ADP-riboside 4		>200 μM
3” fluorine α-OMe-ADP-riboside 5		>200 μM

Table 1 ADP-ribose analogues described in Chapters 2 and 3.

hydrogen bond acceptor.[16,17]Taken together, these results consolidate that loss of hydrogen bonding capability is what diminished inhibitor potency in the produced C3” position analogues.

Discussion and concluding remarks

In this chapter, the modification of the distal ribose at the 2” and 3” position was explored. First, a benzyl group was installed on the 2” position, which was envisioned to have favourable interactions with Mac1 phenylalanine residue 132. However, binding affinity of the inhibitors greatly diminished compared to the unmodified parent compounds. The other inhibitors, with an oxime installed on either the 2” or 3” position also showed diminished activity compared to the parent compounds, although the C2" oxime retained most of the binding affinity. Finally, the previously synthesized fluorinated compound **5** also showed greatly diminished binding affinity compared to its C3-hydroxyl counterpart.

Taken together, these results indicate that the hydroxyls at 2" and 3" positions of distal ribose are essential for effective binding. Modification of the 2" position such as in the 2"-benzylated α-azide derivative **2**, results in a drop of potency of two orders of magnitude in comparison with the unmodified parent compound. Notably, the modification of the 3" position impacts the analogue's binding affinity more than the modification at the 2" position, as evidenced by the difference in potency between the two oxime analogues. This observation aligns with the co-crystal structure of Mac1 with native ADP-ribose, where the 3" position hydroxyl appears sitting deeper within the binding cleft compared to the 2" position, in addition to the C3"-OH favourable hydrogen bonding interactions with Asn40. While modification of the C2" and/or C3" position hydroxyls with groups other reported here might prove fruitful, these results prompted a shift away from this avenue of structural exploration for now, and with the distal ribose appropriately examined, our focus turned toward modifying a different domain of the ADP-ribose molecule: the adenosine.

Acknowledgements

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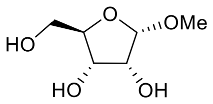
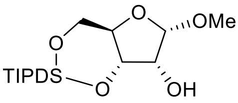
Experimental Section

General experimental procedures

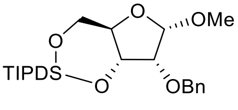
All chemicals were used as received unless stated otherwise. HF in pyridine and *t*BuOOH (5.5 M in nonane or decanes) were purchased at Sigma Aldrich. Molecular sieves were flamedried in vacuo before use. Solvents were dried over activated 4Å molsieves for 24 h except for MeCN and MeOH which were dried over 3Å molsieves. Reactions were performed under N_2 or argon atmosphere unless stated otherwise. A Julabo FT902 cryostat was used for low temperature glycosylation reactions. Reaction mixtures were concentrated under reduced pressure using rotary evaporators at 40-45 ˚C unless state otherwise. Reactions were monitored by thin layer chromatography (TLC) analysis using silica gel 60 F254 coated aluminium sheets from Merck. TLC plates were visualized with ultraviolet light (254 nm) or sprayed with H_2SO_4 (20% v/v in MeOH), potassium permanganate (1 gram $KMnO_4$, 5 grams K_2CO_3, in 200 ml H_2O) or ceric ammonium molybdate (1 gram $Ce(NH_4)_4(SO_4)_4 \bullet 2H_2O$, 2.5 grams $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$, 10 mL H_2SO_4 in 90 mL H_2O). Infrared (IR) values are reported in cm^{-1}. Analytical LC-MS was performed on a LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI+) coupled to a surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 5 µm particle size, phenomenex) in combination buffers A: H_2O, B:acetonitrile and C: 1% aq. TFA. Alternatively, LC-MS analysis was performed on a JASCO HPLC system (detection simultaneously at 214 and 254 nm) coupled to a PE/SCIEX API 165 single quadrupole mass spectrometer (Perkin-Elmer) equipped with a C18 column (Gemini, 4.6 x 50 mm, 3 µm particle size, Phenomenex) in combination with buffers A: H_2O, B:acetonotrile and C: 0.1 M aq. NH4OAc. High resolution mass spectra were recorded by direct injection on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250°C) with resolution R = 60000 at m/z 400 (mass range m/z 150 – 4000) and dioctylpthalate (m/z = 391.28428) as a ‘lock mass". For compounds 10 and 11 HRMS data were recorded on Sciex X500B QTOF mass spectrometer calibrated as recommended by the manufacturer. ^1H NMR, ^{13}C NMR, ^{19}F NMR and ^{31}P NMR spectra were recorded on Bruker AV-300 (300 MHz), AV-400 (400 MHz) or AV-500 (500 MHz) spectrometer. ^{13}C NMR spectra are acquired via the attached proton test (APT) experiment and are presented with even signals S-3 (Cq and CH_2) pointing upwards and odd signals (CH and CH_3) pointing downwards. The chemical shifts are noted as δ-values in parts per million (ppm) relative to the tetramethylsilane signal ($\delta = 0$ ppm) or solvent signal of D_2O ($\delta = 4.79$ ppm) for ^1H NMR and relative to the solvent signal of $CDCl_3$ ($\delta = 77.16$ ppm) for ^{13}C NMR. Phosphorylation reactions were monitored with ^{31}P NMR using an acetone-d6 insert for a locking signal and the resulting spectra were indirectly calibrated with H_3PO_4. HRMS samples were prepared in either MeOH, acetonitrile or MilliQ grade water with an approximate concentration of 1 mM and measured on a Thermo Scientific LTQ Orbitrap XL. Size exclusion chromatography (SEC), here sometimes referred to as “gel filtration”, was performed by constant elution (1 ml/min) with an aqueous NH_4OAc (0.15 M) + 10% acetonitrile buffer system over an HW-40-S resin (16x 600 mm) from TOYOPEARL. Purification by preparative high pressure liquid chromatography (HPLC) if carried out is performed on a Gilson-preparative-system equipped with a Phenomenex-Gemini-NX C18 column (5µm, 10x250 mm) using Buffer A (25 mM NH_4OAc in water) and Buffer B (MeCN) (0 → 20% A/B). Yields for the ADPr analogues after size exclusion chromatography were calculated assuming its obtained as NH_4 salt.



1-α-*O*-methyl-D-ribofuranoside (21) 1-α-*O*-methyl-2,3,5-tri-*O*-acetyl-D-ribofuranoside (8.6 grams, 29 mmol) was dissolved in methanol (190 mL), cooled to 0 °C and was brought under a nitrogen atmosphere. Additionally, sodium methoxide (NaOMe) (0.41 grams, 7.6 mmol, 0.40 eq) was slowly added and the resulting solution was allowed to stir at 0 °C for 3.5 hours while regularly checking reaction progress via TLC analysis. Upon completion, the mixture was quenched by the addition of solid carbon dioxide until pH 7 was reached. After addition of celite, the mixture was concentrated *in vacuo*. Purification by column chromatography (methanol/dichloromethane 0/1 → 1/4, v/v) gave compound **21** (3.89 grams, 23.7 mmol, 82%) as a light-yellow oil. TLC: R_f 0.5 (methanol/dichloromethane, 1/4, v/v). ^{1}H NMR (400 MHz, MeOD) δ 4.85 (d, *J* = 4.3 Hz, 1H), 4.03 – 3.96 (m, 2H), 3.94 (dd, *J* = 6.5, 3.1 Hz, 1H), 3.70 – 3.57 (m, 2H), 3.43 (s, 3H), 3.35 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 104.6, 86.7, 73.1, 71.3, 63.3, 55.5. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_6H_{12}O_5Na$ 187.0577; Found 187.0577.

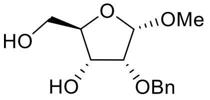


1-α-*O*-methyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-ribofuranoside (15) Compound **21** (1.30 grams, 7.92 mmol) was co-evaporated thrice using dry pyridine whereafter it was dissolved in dry pyridine (12 mL, 0.65 M) and stirred under a nitrogen atmosphere at -20 ºC. To the stirring and cooled solution is added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2) (2.8 mL, 8.7 mmol, 1.1 eq.) dropwise over a period of 1 hour after which the solution was allowed to stir at -20 ºC for another 2.5 hours. At this point TLC analysis indicated no further reaction progress and the reaction was quenched by the addition of water. The quenched mixture was then diluted with diethyl ether and the organic phased was washed with water multiple times to remove the pyridine. The organic phase dried over Na_2SO_4, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (ethyl acetate/pentane, 0/1 → 1/9, v/v) gave compound **15** (1.53 grams, 3.77 mmol, 48%) as a colourless oil. R_f 0.5 (pentane/ethyl acetate, 19/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.87 (d, *J* = 4.2 Hz, 1H), 4.24 – 4.17 (m, 1H), 4.11 – 3.98 (m, 3H), 3.79 – 3.68 (m, 1H), 3.44 (s, 3H), 3.03 (d, *J* = 8.7 Hz, 1H), 1.12 – 1.02 (m, 28H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 102.7, 83.2, 71.4, 71.1, 64.1, 55.2, 17.7, 17.6, 17.52, 17.49, 17.2, 17.1, 17.0, 16.9, 13.7, 13.5, 13.2, 12.6. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{18}H_{38}O_6Si_2Na$ 429.2099; Found 429.2099.

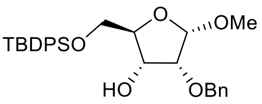


1-α-*O*-methyl-2-*O*-benzyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-ribofuranoside (16) Compound **15** (2.71 grams, 6.66 mmol) was co-evaporated thrice using dry and distilled toluene, whereafter it was dissolved in dry dimethylformamide (DMF) (40 mL, 0.17 M). The resulting solution was flushed with nitrogen gas and left under nitrogen gas while stirring, after which it was cooled to 0 ºC. Once cooled, benzyl bromide (2.4 mL, 20 mmol, 3.0 eq.) was added whereafter sodium hydride (60 w/w % dispersion in mineral oil) (0.32 grams, 8.0 mmol, 1.2 eq.) was added. The reaction mixture was allowed to stir at 0 ºC for 40 minutes, whereafter TLC-analysis indicated full consumption of the starting material. The reaction was quenched by the addition of ice-water and subsequently extracted using diethyl ether. The organic phase was washed with brine and dried over Na_2SO_4, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (diethyl ether/pentane, 1/99 → 1/3, v/v) gave compound **16** (2.11 grams, 4.24 mmol, 64%). R_f 0.4 (pentane/ethyl acetate, 19/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 –

7.38 (m, 2H), 7.37 – 7.23 (m, 3H), 4.94 (d, *J* = 3.9 Hz, 1H), 4.84 (d, *J* = 12.8 Hz, 1H), 4.77 (d, *J* = 12.8 Hz, 1H), 4.25 (dd, *J* = 7.1, 5.6 Hz, 1H), 4.19 – 4.10 (m, 1H), 3.98 (dd, *J* = 12.5, 3.0 Hz, 1H), 3.92 – 3.83 (m, 2H), 3.48 (s, 3H), 1.13 – 0.95 (m, 28H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 128.4, 127.8, 127.6, 103.0, 81.8, 77.1, 72.8, 70.8, 61.9, 56.0, 17.6, 17.5, 17.4, 17.3, 17.1, 17.0, 13.7, 13.4, 13.0, 12.7. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{25}H_{44}O_6Si_2Na$ 519.2569; Found 519.2567.

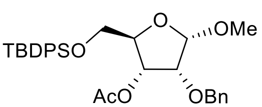


1-α-*O*-methyl-2-*O*-benzyl-D-ribofuranoside (30) Compound **16** (2.11 grams, 4.24 mmol) was dissolved in dry pyridine (43 mL, 0.10 M) and brought under a nitrogen atmosphere where it was stirred. While stirring, added to the solution was HF in pyridine (70% w/w) (4.3 mL) and the corresponding reaction mixture was allowed to stir at room temperature for 1 hour after which TLC analysis indicated full consumption of the starting material. The reaction was then quenched by the addition of aq. sat. sodium bicarbonate solution, and the product was extracted using dichloromethane. The combined organic phases were dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (methanol/dichloromethane, 1/99 → 1/20, v/v) gave compound **30** (1.02 grams, 4.02 mmol, 95%). R_f 0.4 (dichloromethane/methanol, 19/1, v/v). ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 5H), 4.89 (d, *J* = 4.1 Hz, 1H), 4.75 (d, *J* = 11.8 Hz, 1H), 4.61 (d, *J* = 11.8 Hz, 1H), 4.20 – 4.13 (m, 1H), 4.08 (s, 1H), 3.82 (dd, *J* = 6.2, 4.1 Hz, 1H), 3.75 (dd, *J* = 12.0, 3.2 Hz, 1H), 3.65 (dd, *J* = 12.0, 3.8 Hz, 1H), 3.43 (s, 3H), 3.04 (s, 1H), 2.26 – 2.05 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.2, 128.6, 128.33, 128.27, 102.7, 86.5, 78.1, 72.5, 69.6, 62.9, 55.2. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{13}H_{18}O_5Na$ 277.1046; Found 277.1047.

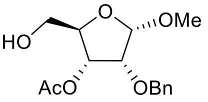


1-α-*O*-methyl-2-*O*-benzyl-5-*O*-(*tert*-butyl-di-phenylsilyl)-D-ribofuranoside (31) Compound **30** (51 mg, 0.20 mmol) was dissolved in dry pyridine (1.0 mL, 0.20 M) and the resulting solution was stirred under a nitrogen atmosphere. While stirring, to the solution was added *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) (62 µL, 0.24 mmol, 1.2 eq.) and the resulting mixture was stirred at room temperature for 21 hours at which point TLC analysis indicated full consumption of the starting material. The reaction was quenched by the addition of water, after which it was diluted with ethyl acetate. The organic phase was washed thrice with water and once with brine, after which it was dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (ethyl acetate/pentane) gave compound **31** (80 mg, 0.16 mmol, 81%). R_f 0.6 (pentane/ethyl acetate, 7/3, v/v). ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.58 (m, 4H), 7.48 – 7.28 (m, 11H), 4.92 (d, *J* = 4.1 Hz, 1H), 4.77 (d, *J* = 12.1 Hz, 1H), 4.63 (d, *J* = 12.1 Hz, 1H), 4.23 (ddd, *J* = 9.0, 5.8, 1.5 Hz, 1H), 4.18 (td, *J* = 2.9, 1.4 Hz, 1H), 4.01 (dd, *J* = 5.9, 4.1 Hz, 1H), 3.79 – 3.64 (m, 2H), 3.44 (s, 3H), 3.03 (d, *J* = 9.1 Hz, 1H), 0.96 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.4, 135.8, 135.7, 133.2, 133.0, 130.0, 129.9, 128.7, 128.3, 127.9, 127.8, 102.7, 87.1, 78.4, 72.5, 70.1, 64.2, 55.3, 26.9, 19.3. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{29}H_{36}O_5SiNa$ 515.2224; Found 515.2224.

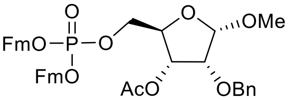
1-α-*O*-methyl-2-*O*-benzyl-3-*O*-acetyl-5-*O*-(*tert*-butyl-di-phenylsilyl)-D-ribofuranoside (17)



Compound **31** (72 mg, 0.15 mmol) was co-evaporated thrice with dry and distilled toluene, after which it was dissolved in dry pyridine (1.5 mL, 0.10 M) and stirred under a nitrogen atmosphere at 0 °C. To the stirring mixture was then added acetic anhydride (30 µL, 0.30 mmol, 2.0 eq.) and DMAP (2.0 mg, 15 µmol, 0.10 eq.) after which the resulting mixture was allowed to warm to room temperature and stirred for 20 hours. At this point TLC analysis indicated full consumption of the starting material and the reaction was quenched by the addition of water. The mixture was then diluted with diethyl ether after which the organic phase was washed with water and then brine. The organic phase was dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (diethyl ether/pentane, 1/9 → 1/1, v/v) gave compound **17** (78 mg, 0.15 mmol, quant.). R_f 0.4 (pentane/diethyl ether, 3/2, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.59 (m, 4H), 7.47 – 7.27 (m, 11H), 5.33 (dd, *J* = 6.8, 2.4 Hz, 1H), 4.87 (d, *J* = 4.5 Hz, 1H), 4.69 (d, *J* = 12.6 Hz, 1H), 4.60 (d, *J* = 12.6 Hz, 1H), 4.12 (q, *J* = 2.8 Hz, 1H), 3.99 (dd, *J* = 6.8, 4.5 Hz, 1H), 3.83 (dd, *J* = 11.1, 2.8 Hz, 1H), 3.73 (dd, *J* = 11.1, 3.0 Hz, 1H), 3.46 (s, 3H), 2.14 (s, 3H), 0.96 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 137.6, 135.8, 135.7, 133.2, 133.1, 129.9, 129.8, 128.6, 128.1, 128.1, 127.9, 127.8, 102.5, 83.3, 77.4, 73.2, 70.5, 64.0, 55.6, 26.8, 21.4, 19.3. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{38}O_6SiNa$ 557.2330; Found 557.2330.

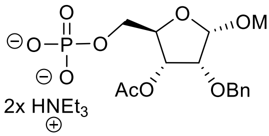


1-α-*O*-methyl-2-*O*-benzyl-3-*O*-acetyl-D-ribofuranoside (32) Compound **17** (78 mg, 0.15 mmol) was dissolved in dry pyridine (1.5 mL, 0.1 M) and stirred at 0 °C under a nitrogen atmosphere when HF in pyridine (70% w/w) (0.15 mL) was added. The resulting mixture was allowed to stir for 2 hours when TLC analysis indicated full consumption of the starting material. The reaction was quenched by the careful addition of sat. aq. sodium bicarbonate solution after which it was diluted by the addition of ethyl acetate. The organic phase was washed with sat. aq. sodium bicarbonate solution twice and with brine once. The organic phase was then dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (ethyl acetate/pentane, 3/7 → 4/1, v/v) gave compound **32** (28 mg, 0.094 mmol, 64%). R_f 0.3 (pentane/ethyl acetate, 2/3, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.28 (m, 5H), 5.10 (dd, *J* = 7.1, 3.2 Hz, 1H), 4.85 (d, *J* = 4.4 Hz, 1H), 4.67 (d, *J* = 12.3 Hz, 1H), 4.58 (d, *J* = 12.4 Hz, 1H), 4.12 (q, *J* = 3.4 Hz, 1H), 3.85 (dd, *J* = 7.1, 4.4 Hz, 1H), 3.81 – 3.68 (m, 2H), 3.45 (s, 3H), 2.14 (s, 3H), 2.08 (bs, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 137.41, 128.6, 128.2, 102.4, 82.9, 77.2, 73.3, 70.2, 62.6, 55.5, 21.3. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{15}H_{20}O_6Na$ 319.1152; Found 319.1154.

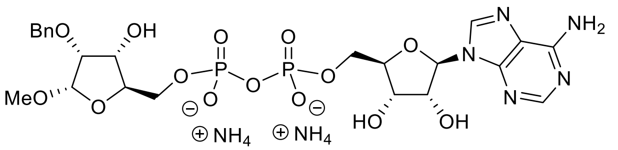


1-α-*O*-methyl-2-*O*-benzyl-3-*O*-acetyl-5-*O*-(di(9*H*-fluoren-9-yl))-phosphoryl-D-ribofuranoside (33) To compound **32** (28 mg, 0.094 mmol) was added DCI (33 mg, 0.28 mmol, 3.0 eq.) after which the mixture was co-evaporated thrice with dry and distilled toluene. Next, the mixture was dissolved in dry acetonitrile (0.62 mL, 0.15 M) and stirred under a nitrogen atmosphere. In another flask, a solution of $(FmO)_2PN(i\text{Pr})_2$ (**15**) was prepared (0.32 M in acetonitrile) and some of this (0.44 mL, 0.14 mmol, 1.5 eq.) was added to the stirring mixture dropwise. The resulting reaction mixture was then allowed to stir at room temperature for 1.5 hours. Upon completion, to the stirring mixture was added *t*BuOOH (5.5 M in decanes) (85 µL, 0.47 mmol, 5.0 eq.) and the resulting solution was stirred at room temperature for 35 minutes, after which the reaction was quenched by the addition of water. The solution was diluted using dichloromethane, and the organic phase was washed with brine, after which it was dried over Na_2SO_4, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (ethyl acetate/pentane, 3/7 → 3/1, v/v) gave compound **33** (51 mg, 0.069 mmol, 74%). R_f 0.6 (pentane/ethyl acetate, 3/7, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 – 7.68 (m, 4H), 7.58 – 7.44 (m, 4H), 7.42 – 7.31 (m, 4H), 7.30 – 7.19 (m, 9H), 4.97 (dd, *J* = 7.1, 3.2 Hz, 1H), 4.68 (d, *J* = 4.4 Hz, 1H), 4.53 (d, *J* = 12.1 Hz, 1H), 4.47 (d, *J* = 12.1 Hz, 1H), 4.32 – 4.18 (m, 4H), 4.17 – 4.00 (m, 5H), 3.71 (dd, *J* = 7.2, 4.5 Hz, 1H), 3.36 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 143.1, 143.0, 143.02, 143.00, 141.48, 141.46, 141.43, 137.2, 128.6, 128.3, 128.2, 128.1, 127.3, 125.20, 125.18, 125.15, 120.2, 120.1, 102.3, 80.7 (d, *J* = 7.3 Hz), 77.0, 73.4, 69.6 (d, *J* = 2.5 Hz), 69.51, 69.49, 69.48, 67.2 (d, *J* = 5.8 Hz), 55.5, 48.0 (d, *J* = 3.3 Hz), 47.9 (d, *J* = 3.3 Hz), 21.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.9. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{43}H_{41}O_9PNa$ 755.2380; Found 755.2383.

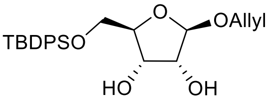
1-α-*O*-methyl-2-*O*-benzyl-3-*O*-acetyl-5-*O*-phosphate-D-ribofuranoside (6) Compound **33** (51 mg, 0.069 mmol) was dissolved in dry acetonitrile (1.6 mL, 0.044 M) and brought under a nitrogen atmosphere. To the stirring mixture was then added triethylamine (0.17 mL, 1.2 mmol, 17 eq.) and the mixture was allowed to stir at room temperature for 48 hours, after which it was directly concentrated in vacuo. The crude product was used directly in the next reaction without any further purification.



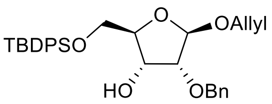
1”-α-*O*-methyl-2”-*O*-benzyl-ADPr (1) Crude compound **6** (~0.069 mmol) was co-evaporated thrice using a solvent system consisting of 1/1 dry acetonitrile/dry pyridine after which it was co-evaporated once using dry acetonitrile and to it was added DCI (21 mg, 0.17 mmol, 2.5 eq.). The mixture was co-evaporated thrice with dry acetonitrile after which it was dissolved in dry acetonitrile (1.3 mL, 0.055 M) and stirred vigorously under a nitrogen atmosphere. In another flask, phosphoramidite **7** was co-evaporated thrice with dry and distilled toluene after which it was dissolved in dry acetonitrile to create a stock solution (0.22 M in acetonitrile). A part of this stock solution (0.32 mL, 0.069 mmol, 1.0 eq.) was then added dropwise to the solution containing the phosphate. The resulting mixture was stirred at room temperature for 1 hour and 20 minutes at which point TLC analysis indicated full consumption of the phosphoramidite. Subsequently, *t*BuOOH (5.5 M in decanes) (26 μL, 0.15 mmol, 2.1 eq.) was added to the mixture and the solution was stirred at room temperature for 1 hour, at which point another and equal batch of *t*BuOOH (26 μL, 0.15 mmol, 2.1 eq.) was added to the mixture. After another hour and 15 minutes, ^{31}P-NMR indicated full conversion of the phosphite and to the reaction mixture was added DBU (0.52 μL, 0.35 mmol, 5.0 eq.). The resulting solution was stirred at room temperature for 1 hour and 10 minutes at which point aqueous ammonium hydroxide (28% ammonia, w/w) (1.3 mL) was added. The resulting yellow clear solution was stirred at room temperature for 48 hours, at which point LCMS indicated full deprotection of the construct. The solution was then diluted with water and ethyl acetate and the aqueous phase was washed multiple times with ethyl acetate until the organic phase returned clear. The aqueous phase was directly concentrated *in vacuo*. Purification by gel filtration (HW-40, NH_4OAc buffer) afforded compound **1** (11.4 mg, 16.3 μmol, 24% (over 5 steps)) as the ammonium salt as a white foam. ^{1}H NMR (500 MHz, D_2O) δ 8.52 – 8.46



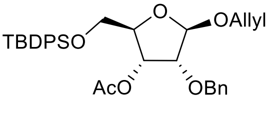
(m, 1H), 8.16 (m, 1H), 7.39 – 7.27 (m, 5H), 6.10 – 6.05 (m, 1H), 4.78 – 4.74 (m, 1H), 4.73 – 4.67 (m, 1H), 4.56 (d, *J* = 11.6 Hz, 1H), 4.50 (dd, *J* = 5.1, 3.8 Hz, 1H), 4.42 (d, *J* = 11.6 Hz, 1H), 4.37 (s, 1H), 4.30 – 4.17 (m, 4H), 4.05 – 3.92 (m, 2H), 3.88 (dd, *J* = 6.1, 4.5 Hz, 1H), 3.29 (s, 3H). ^{13}C NMR (126 MHz, D_2O) δ 155.3, 152.6, 148.9, 139.7, 136.7, 128.5, 128.3, 128.2, 118.5, 102.3, 86.9, 83.9 (d, *J* = 7.1 Hz), 83.7 (d, *J* = 4.8 Hz), 77.8, 74.3, 72.4, 70.2, 68.5, 65.6 (d, *J* = 3.9 Hz), 65.2 (d, *J* = 4.3 Hz), 55.1. ^{31}P NMR (202 MHz, D_2O) δ -11.3. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{23}H_{32}N_5O_{14}P_2$ 664.1416; Found 664.1415.



1-β-*O*-allyl-5-*O*-(*tert*-butyl-diphenylsilyl)-D-ribofuranoside (11) Compound **11** was prepared following a procedure previously described.[18] All spectra are in full accordance with literary precedence. ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.64 (m, 4H), 7.47 – 7.34 (m, 6H), 5.81 (dddd, *J* = 17.2, 10.4, 6.2, 5.2 Hz, 1H), 5.20 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.13 (dq, *J* = 10.4, 1.4 Hz, 1H), 4.98 (d, *J* = 1.0 Hz, 1H), 4.39 – 4.27 (m, 1H), 4.19 – 4.10 (m, 1H), 4.08 (dd, *J* = 4.8, 2.6 Hz, 1H), 4.03 (dd, *J* = 6.1, 5.1 Hz, 1H), 3.92 (ddt, *J* = 12.8, 6.2, 1.4 Hz, 1H), 3.86 – 3.73 (m, 2H), 2.81 (d, *J* = 3.3 Hz, 1H), 2.46 (d, *J* = 5.5 Hz, 1H), 1.07 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.7, 134.1, 133.34, 133.30, 130.0, 129.9, 127.91, 127.90, 117.5, 106.3, 83.2, 75.5, 72.9, 68.6, 65.4, 27.0, 19.4. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{24}H_{32}O_5SiNa$ 451.1911; Found 451.1909.

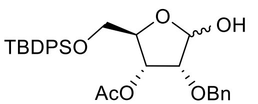


1-β-*O*-allyl-2-*O*-benzyl-5-*O*-(*tert*-butyl-diphenylsilyl)-D-ribofuranoside (34) Compound **11** (14.2 grams, 33.2 mmol) was co-evaporated with anhydrous toluene and then dissolved in anhydrous DMF (200 mL, 0.166 M) and subsequently cooled to 0 °C. Benzyl bromide (7.9 mL, 66 mmol, 2.0 eq.) was slowly added to the solution, followed by sodium hydride (60% w/w dispersion in mineral oil) (1.5 grams, 37 mmol, 1.1 eq.). The resulting solution was stirred for two hours at room temperature when TLC analysis indicated full conversion of the starting material into a mixture of both mono-benzylated constructs as well as the di-benzylated construct, after which the reaction mixture was quenched using water (100 mL) and extracted with ethyl acetate (100 mL). The combined organic fractions were dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification via silica gel chromatography (pentane/diethyl ether, 19/1 → 7/3, v/v) yielded compound **34** (6.40 grams, 12.3 mmol, 37%) as a colourless clear oil. R_f 0.4 (pentane/diethyl ether, 4/1, v/v). ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.66 (m, 4H), 7.45 – 7.29 (m, 11H), 5.82 (dddd, *J* = 16.8, 10.4, 6.2, 5.0 Hz, 1H), 5.21 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.14 (dq, *J* = 10.4, 1.4 Hz, 1H), 5.08 (d, *J* = 1.6 Hz, 1H), 4.74 (d, *J* = 11.7 Hz, 1H), 4.63 (d, *J* = 11.7 Hz, 1H), 4.30 (dt, *J* = 8.1, 5.4 Hz, 1H), 4.18 (ddt, *J* = 12.9, 5.1, 1.6 Hz, 1H), 4.04 (q, *J* = 4.9 Hz, 1H), 4.00 – 3.89 (m, 2H), 3.82 (dd, *J* = 11.0, 4.1 Hz, 1H), 3.76 – 3.65 (m, 1H), 2.55 (d, *J* = 8.1 Hz, 1H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.3, z135.8, 134.2, 133.5, 129.8, 129.8, 128.7, 128.3, 128.1, 127.82, 127.80, 117.3, 104.1, 84.9, 82.5, 72.9, 71.4, 68.7, 64.9, 26.9, 19.4. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{38}O_5SiNa$ 541.2381; Found 541.2377.



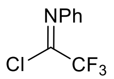
1-β-*O*-allyl-2-*O*-benzyl-3-*O*-acetyl-5-*O*-(*tert*-butyl-diphenylsilyl)-D-ribofuranoside (19) Compound **34** (2.6 grams, 5.0 mmol) was co-evaporated with anhydrous toluene and then dissolved in anhydrous pyridine (30 mL, 0.17 M). Acetic anhydride (1.4 mL, 15 mmol, 3.0 eq.) and 4-(dimethylamino)pyridine (DMAP) (0.12 grams, 1.0 mmol, 0.20 eq.)

were added and the resulting solution was allowed to stir overnight at room temperature. The reaction mixture was then cooled to 0 °C and diluted with water (30 mL) and washed with diethyl ether (50 mL) and an aqueous solution of HCl (1.0 M) (50 mL). The combined organic fractions were dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification via silica gel chromatography (pentane/diethyl ether, 19/1 → 17/3, v/v) yielded compound **19** (1.37 grams, 2.45 mmol, 49%) as colourless clear oil. Rf 0.55 (pentane/diethyl ether, 4/1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.61 (m, 4H), 7.45 – 7.26 (m, 11H), 5.82 (dddd, *J* = 17.3, 10.4, 6.0, 5.0 Hz, 1H), 5.27 (t, *J* = 5.1 Hz, 1H), 5.22 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.13 (dq, *J* = 10.4, 1.4 Hz, 1H), 5.05 (d, *J* = 2.3 Hz, 1H), 4.58 (dd, *J* = 12.3, 1.0 Hz, 2H), 4.24 (q, *J* = 5.0 Hz, 1H), 4.20 – 4.14 (m, 2H), 3.95 (ddt, *J* = 12.9, 6.0, 1.4 Hz, 1H), 3.73 (dd, *J* = 4.9, 1.2 Hz, 2H), 2.07 (s, 3H), 1.03 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 137.8, 135.79, 135.76, 134.1, 133.4, 133.3, 129.9, 129.8, 128.6, 128.0, 127.9, 127.8, 127.8, 117.2, 105.2, 81.8, 81.3, 73.6, 73.2, 68.9, 64.8, 26.9, 21.0, 19.3. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{33}H_{40}O_6SiNa$ 583.2486; Found 583.2484.

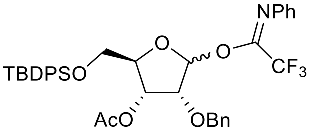


2-*O*-benzyl-3-*O*-acetyl-5-*O*-(*tert*-butyl-diphenylsilyl)-D-ribofuranoside (35) Compound **19** (0.56 grams, 1.0 mmol) was co-evaporated with anhydrous toluene and dissolved in anhydrous tetrahydrofuran (5.0 mL, 0.20 M) under an argon atmosphere. In another flask, $Ir(COD)(PPh_2Me)PF_6$ (4.2 mg, 5.0 µmol, 0.0050 eq.) was dissolved in anhydrous tetrahydrofuran (5.0 mL, 0.0010 M) and flushed with argon. The solution containing the catalyst was first flushed with H_2 until the solution became completely colourless and was subsequently flushed with argon. This process was repeated until the solution stayed completely clear after flushing with argon. The solution containing the catalyst was then added dropwise to the solution of compound **16** after which the resulting mixture was allowed to stir at room temperature. Isomerization of the starting material was followed via TLC analysis. When the starting material had fully isomerized, aq. sat. sodium bicarbonate solution (7 mL) and solid iodine (0.31 grams, 1.2 mmol, 1.2 eq.) were added to the reaction mixture, respectively. After stirring for 30 minutes at room temperature the reaction was quenched using a solution of sodium thiosulphate (20 mL) and diluted with dichloromethane (30 mL) which was washed with a solution of sodium thiosulphate until the organic phase had fully discoloured. The organic phase was dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification via silica gel chromatography (pentane/ethyl acetate, 9/1 → 7/3, v/v) yielded compound **35** (0.357 grams. 0.686 mmol, 69%) as a slightly yellow clear oil as 1:1 mixture of anomers. Rf 0.25 (pentane/ethyl acetate, 4:1, v/v). Both anomers are reported here. As a consequence, the total integral for e.g. δ 7.70 – 7.59 is doubled and given as 8H, as opposed to the expected 4H for a single anomer due to the mixture being 1:1. With this in mind, ^{13}C-NMR also reports both anomers; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.59 (m, 8H), 7.47 – 7.27 (m, 22H), 5.47 – 5.34 (m, 3H), 5.32 (dd, *J* = 7.1, 1.9 Hz, 1H), 4.62 (m, 2x2H), 4.24 (dt, *J* = 5.6, 3.0 Hz, 2H), 4.20 (dd, *J* = 5.8, 4.5 Hz, 1H), 4.15 – 4.06 (m, 1H), 3.82 (dd, *J* = 11.3, 3.3 Hz, 1H), 3.77 – 3.72 (m, 2H), 3.68 (dd, *J* = 11.2, 2.9 Hz, 1H), 3.60 (d, *J* = 8.8 Hz, 1H), 3.26 (d, *J* = 7.2 Hz, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 1.05 (s, 9H), 0.98 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 170.3, 137.7, 137.2, 135.9, 135.74, 135.72, 135.67, 130.2, 130.1, 130.0, 129.9, 128.7, 128.6, 128.3, 128.0, 127.9, 127.9, 100.9, 96.2, 82.4, 82.4, 82.1, 77.2, 73.3, 73.2, 72.7, 72.2, 64.0, 63.9, 26.9, 26.9, 21.1, 21.0, 19.3, 19.2. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{30}H_{36}O_6SiNa$ 543.2173; Found 543.2173.

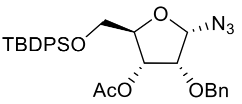
PTFAI-Cl To a 500 mL two-neck round-bottom flask equipped with a condenser was first added THF (100 mL) whereafter this solvent was stirred vigorously. To the stirring solvent was then added TFA (15.3 mL, 200 mmol), aniline (20 mL, 0.22 mol, 1.1 eq.), triethylamine (31 mL, 0.22 mol, 1.1 eq.) and $CBrCl_3$ (50 mL, 0.50 mol, 2.5 eq.). To the resulting reaction mixture was then added PPh_3 (130 grams, 500 mmol, 2.50 eq.) while stirring and in portions as to sustain a gentle reflux utilizing the heat generated from the exothermic reaction. After final PPh_3 addition, the reaction mixture was allowed to cool down to room temperature whereafter the reaction mixture was poured directly into a large beaker containing *n*-pentane (2 Liters). The resulting slurry was then stirred vigorously at room temperature whereafter all solids were removed via vacuum filtration. The filtrate was concentrated *in vacuo*. The crude product was then loaded onto a column containing a plug of normal phase silica gel material wetted with *n*-pentane whereafter the pure product was eluted from this plug using *n*-pentane. All fractions containing the product were collected and then concentrated *in vacuo* which afforded **PTFAI-Cl** (32.8 grams, 158 mmol, 79%) as a colourless and clear and runny oil that solidifies into a semi-transparent crystalline solid when cooled to -20 ˚C. TLC: R_f 0.8 (*n*-pentane). Notes: When PPh_3 addition is unable to initialize the desired reflux it is recommended that the flask be gently heated with a heat gun until slight bubbling can be observed. At this point, further PPh_3 addition should be able to sustain reflux. It is recommended to store the product in an argon flushed pot outfitted with a septum of some kind, wherafter it can be stored at -20 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.36 (m, 2H), 7.31 – 7.22 (m, 1H), 7.10 – 7.02 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.6, 132.1, 129.3, 127.6, 120.8, 117.0 (q, *J* = 277.0 Hz). The obtained spectra are in full agreement with those reported in literature.



1-α,β-*O*-((*N*-phenyl)-2,2,2-trifluoroacetamido)-2-*O*-benzyl-3-*O*-acetyl-5-*O*-(*tert*-butyl-diphenylsilyl)-D-ribofuranoside (10) Compound **35** (2.65 grams, 5.08 mmol) was dissolved in a mixture of acetone and water (51 mL, 0.1 M, 50/1, acetone/water, v/v) and subsequently cooled to 0 °C. Next, Cs_2CO_3 (2.5 grams, 7.6 mmol, 1.5 eq.) and **PTFAI-Cl** (1.2 mL, 7.6 mmol, 1.5 eq.) were added and the resulting solution was stirred at 0 °C for 20 hours. The reaction mixture was filtered off and concentrated *in vacuo*. Purification via silica gel chromatography (pentane/diethyl ether, 1/0 → 9/1, v/v) (with 1% triethylamine, v/v) yielded compound **10** (3.38 grams, 4.89 mmol, 96%) as a yellow oil. Rf 0.45 (pentane/diethyl ether, 9/1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.54 (m, 4H), 7.46 – 7.36 (m, 6H), 7.35 – 7.25 (m, 7H), 7.10 (td, *J* = 7.4, 1.0 Hz, 1H), 6.86 – 6.79 (m, 2H), 6.30 (bs, 1H), 5.28 (t, *J* = 5.2 Hz, 1H), 4.63 (q, *J* = 12.2, 8.9 Hz, 2H), 4.50 (dd, *J* = 5.2, 2.2 Hz, 1H), 4.38 (q, *J* = 4.5 Hz, 1H), 3.80 (qd, *J* = 11.3, 4.3 Hz, 2H), 2.09 (s, 3H), 1.03 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 143.8, 137.3, 135.8, 135.7, 135.7, 133.1, 132.9, 130.0, 129.9, 128.8, 128.7, 128.6, 128.2, 127.99, 127.95, 127.93, 127.90, 127.87, 127.5, 124.4, 119.6, 102.9, 83.5, 80.3, 73.3, 72.3, 63.9, 46.3, 26.84, 26.78, 20.9, 19.3. HRMS (ESI) m/z: [M-imidate+OH+Na]$^+$ Calcd for $C_{30}H_{36}O_6SiNa$ 543.2173; Found 543.2176.

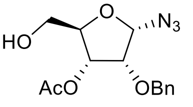


1-α-azido-2-*O*-benzyl-3-*O*-acetyl-5-*O*-(*tert*-butyl-diphenylsilyl)-D-ribofuranoside (20)

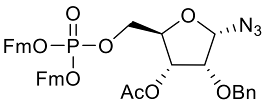


Compound **10** (0.138 grams, 0.200 mmol) was co-evaporated with anhydrous toluene and dissolved in anhydrous dichloromethane (6.0 mL, 0.033 M) in a flame-dried flask containing activated molecular sieves (3Å, rods). Next, trimethylsilyl azide (TMS-N_3) (0.10 mL, 0.80 mmol, 4.0 eq.) was added and the resulting solution was stirred for 90 minutes before cooling to -60 °C. Once fully cooled, a stock solution containing trimethylsilyl triflate (TMS-OTf) (0.1 M in dry dichloromethane) (0.50 mL, 0.050 mmol, 0.25 eq.) was added in one go and the resulting solution was stirred for 20 hours at -60 °C. The reaction mixture was then quenched with triethylamine (0.06 mL), filtered off and concentrated *in vacuo*. Purification via silica gel chromatography (pentane/diethyl ether, 1/0 → 9/1, v/v) gave compound **20** (77 mg, 0.14 mmol, 71%) as white cloudy oil. Rf 0.5 (pentane/diethyl ether, 17:3, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (t, *J* = 5.8 Hz, 4H), 7.48 – 7.25 (m, 11H), 5.40 (dd, *J* = 6.1, 2.2 Hz, 1H), 5.28 (d, *J* = 5.2 Hz, 1H), 4.68 (d, *J* = 12.2 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.28 (q, *J* = 2.5 Hz, 1H), 4.24 (dd, *J* = 6.0, 5.3 Hz, 1H), 3.76 (t, *J* = 2.6 Hz, 2H), 2.14 (s, 3H), 0.96 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 137.2, 135.8, 135.7, 135.6, 132.9, 132.7, 130.1, 130.0, 129.9, 128.7, 128.7, 128.3, 128.2, 128.0, 127.94, 127.92, 90.5, 84.8, 78.1, 73.4, 70.9, 63.9, 29.8, 26.8, 21.2, 19.2. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{30}H_{35}N_3O_5SiNa$ 568.2238; Found 568.2236.

1-α-azido-2-*O*-benzyl-3-*O*-acetyl-D-ribofuranoside (36) Compound **20** (0.88 grams, 1.6 mmol) was dissolved in anhydrous pyridine (16 mL, 0.10 M) and to it was added HF-pyridine (70%, w/w) (1.6 mL). The resulting solution was stirred at room temperature for one hour. The reaction mixture was quenched with aq. sat. sodium bicarbonate solution until bubbling seized. The resulting solution was then diluted with ethyl acetate (10 mL) and washed twice with aq. sat. sodium bicarbonate solution (20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification via silica gel chromatography (pentane/ethyl acetate, 7/3 → 1/1, v/v) afforded compound **36** (0.322 grams, 1.05 mmol, 65%) as a slightly cloudy oil. Rf 0.45 (pentane/ethyl acetate, 1/1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.27 (m, 5H), 5.28 (d, *J* = 5.0 Hz, 1H), 5.19 (dd, *J* = 6.4, 3.4 Hz, 1H), 4.66 (d, *J* = 12.0 Hz, 1H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.29 (q, *J* = 3.2 Hz, 1H), 4.09 (dd, *J* = 6.4, 5.0 Hz, 1H), 3.87 – 3.66 (m, 2H), 2.13 (s, 3H), 2.09 (bs, *J* = 8.4, 3.1 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 137.1, 128.7, 128.3, 128.1, 90.5, 84.2, 77.9, 73.6, 70.3, 62.2, 21.1. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{14}H_{17}N_3O_5Na$ 330.1060; Found 330.1058.

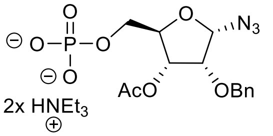


1-α-azido-2-*O*-benzyl-3-*O*-acetyl-5-*O*-(di(9*H*-fluoren-9-yl))-phosphoryl-D-ribofuranoside (37) Compound **36** (0.32 grams, 1.0 mmol) and DCI (0.37 grams, 3.1 mmol, 3.1 eq.) were combined in a flask and co-evaporated with anhydrous toluene and subsequently dissolved in anhydrous acetonitrile (6.3 mL, 0.15 M). In another flask, $(FmO)_2PN(iPr)_2$ (**15**) (0.82 grams, 1.6 mmol, 1.5 eq.) was dissolved in anhydrous acetonitrile (5.0 mL, 0.32 M) and was added dropwise to the stirring solution of compound **36** and DCI. The resulting mixture was stirred at room temperature for 90 minutes. Then, to it was added *t*BuOOH (5.5 M in decanes) (1.0 mL, 5.5 mmol, 5.5 eq.) and the reaction was monitored via ^{31}P-NMR. After complete oxidation of the phosphite into the corresponding phosphate, the reaction mixture was quenched with water (10 mL) and diluted dichloromethane (20 mL). The organic phase was separated and dried over $MgSO_4$,

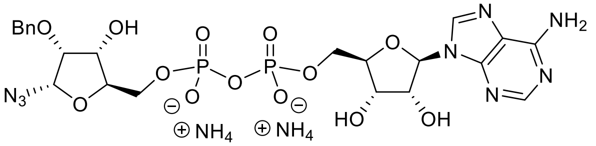


filtered off and concentrated *in vacuo*. Purification via silica gel chromatography (pentane/ethyl acetate, 7/3 → 3/7, v/v) yielded an inseparable 3:2 (mol:mol) mixture of respectively compound **37** and the FmO H-phosphonate (0.520 mmol, 50%) as a clear oil which was used without further purification in the next reaction. Rf 0.55 (pentane/ethyl acetate, 3/3, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.66 (m, 4H), 7.57 – 7.25 (m, 17H), 5.04 – 4.95 (m, 2H), 4.47 (d, *J* = 1.9 Hz, 2H), 4.34 – 4.18 (m, 5H), 4.16 – 4.06 (m, 4H), 3.86 (dd, *J* = 6.3, 5.0 Hz, 1H), 2.07 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 143.1, 143.05, 143.03, 142.96, 141.51, 141.48, 141.45, 137.0, 128.7, 128.6, 128.3, 128.09, 128.08, 128.06, 127.99, 127.9, 127.3, 127.27, 127.2, 125.18, 125.15, 125.12, 125.10, 120.2, 120.1, 120.0, 90.3, 82.0, 81.9 (d, *J* = 7.3 Hz), 77.6, 73.7, 69.9, 69.4 (t, *J* = 6.2 Hz), 67.1 (d, *J* = 6.2 Hz), 48.0 (d, *J* = 2.7 Hz), 47.9 (d, *J* = 3.4 Hz), 21.0. ^{31}P NMR (122 MHz, acetoncapil) δ 9.7 (H-phosphonate), -1.2. IR: 2110, 1740, 1450; HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{42}H_{38}N_3O_8PNa$ 766.2289; Found 766.2282.

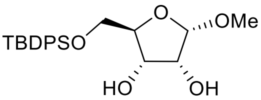
1-α-azido-2-*O*-benzyl-3-*O*-acetyl-5-*O*-phosphate-D-ribofuranoside (9) Compound **37** (0.20 mmol) was dissolved in anhydrous acetonitrile (5.0 mL, 0.044 M) and brought under nitrogen atmosphere. Then, triethylamine (0.47 mL, 3.4 mmol, 17 eq.) was added and the solution was allowed to stir at room temperature for 72 hours. The reaction mixture was then directly concentrated *in vacuo*. The crude product was co-evaporated with 1/1 anhydrous pyridine/anhydrous acetonitrile (1/1, v/v) for a total of three times. The resulting crude product was used directly in the next reaction without any further purification.



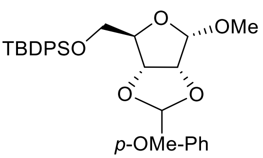
1”-α-azido-2”-*O*-benzyl-ADPr (2) To crude compound **9** (~0.20 mmol) was added DCI (59 mg, 0.50 mmol, 2.5 eq.) and the resulting mixture was co-evaporated thrice with anhydrous acetonitrile after which the mix was suspended in anhydrous acetonitrile (4.0 mL, 0.050 M) and stirred under an argon atmosphere. In another flask, compound **7** (142 mg, 0.200 mol, 1.0 eq.) was co-evaporated twice with anhydrous toluene after which it was dissolved in anhydrous acetonitrile (1.0 mL, 0.20 M) which was added dropwise to the suspension containing compound **9** and DCI. The resulting mixture was allowed to stir for one hour at room temperature after which *t*BuOOH (5.5 M in decanes) (76 μL, 0.42 mmol, 2.1 eq.) was added. The reaction mixture was then allowed to stir at room temperature for one hour, after which a second portion of *t*BuOOH (76 μL, 0.42 mmol, 2.1 eq.) was added and the reaction was monitored via ^{31}P-NMR. Upon complete conversion of the phosphite species to the corresponding protected phosphates, to the mixture was added DBU (0.15 mL, 1.0 mmol, 5.0 eq.) agfetr which the mixture was allowed to stir at room temperature for 30 minutes. At this point to the reaction mixture was added an aqueous solution of ammonium hydroxide (28% NH_3, w/w) (3.6 mL) and the resulting yellow suspension was allowed to stir for 19 hours after which it was diluted with water (10 mL) and the aqueous phase washed with diethyl ether until the organic phase returned clear. The aqueous phase was concentrated *in vacuo* and purification via gel filtration (HW-40, NH_4OAc buffer) and subsequent HPLC purification afforded compound **2** (13.1 mg, 18.5 μmol, 9.3% (over 5 steps)) as the ammonium salt as a white foam. ^{1}H NMR (500 MHz, D_2O) δ 8.40 (s, 1H), 8.08 (s, 1H), 7.35 – 7.12 (m, 5H), 5.99 (d, *J* = 5.8 Hz, 1H),



5.26 (d, *J* = 5.1 Hz, 1H), 4.51 (d, *J* = 11.3 Hz, 1H), 4.45 – 4.37 (m, 2H), 4.31 – 4.17 (m, 4H), 4.14 (bs, 2H), 3.99 (td, *J* = 5.4, 1.7 Hz, 1H), 3.95 – 3.87 (m, 2H). ^{13}C NMR (126 MHz, D_2O) δ 155.3, 152.5, 148.9, 139.8, 136.7, 128.6, 128.3, 118.6, 90.3, 86.9, 84.8 (d, *J* = 7.8 Hz), 83.8 (d, *J* = 9.3 Hz), 78.3, 74.3, 72.9, 70.3, 68.7, 65.3 (d, *J* = 4.0 Hz), 65.2 (d, *J* = 4.2 Hz). ^{31}P NMR (202 MHz, D_2O) δ -11.3. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{22}H_{29}N_8O_{13}P_2$ 675.1324; Found 675.1322.

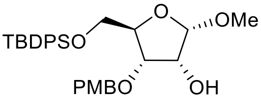


1-α-*O*-methyl-5-*O*-(*tert*-butyl-di-phenylsilyl)-D-ribofuranoside (14) Compound **21** (3.89 grams, 23.7 mmol) was dissolved in anhydrous DMF (0.12 L, 0.20 M) and flushed with nitrogen over a period of 30 minutes. While stirring, imidazole (3.2 grams, 47 mmol, 2.0 eq.), DMAP (0.29 grams, 2.4 mmol, 0.10 eq.) and TBDPS-Cl (7.2 grams, 26 mmol, 1.1 eq.) were added. The reaction mixture was then allowed to stir for 24 hours at room temperature at which point TLC analysis indicated full consumption of the starting material. The reaction was then quenched with water and diluted with diethyl ether. The organic phase was washed three times with water, once with brine and was dried over $MgSO_4$, filtered off concentrated *in vacuo*. Purification by column chromatography (ethyl acetate/pentane, 1/9 → 3/7, v/v) afforded compound **14** (6.97 grams, 17.3 mmol, 73%) as clear colourless oil. TLC: R_f 0.4 (ethyl acetate/pentane, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.62 (m, 4H), 7.47 – 7.34 (m, 6H), 4.94 (d, *J* = 4.4 Hz, 1H), 4.22 (ddd, *J* = 9.5, 5.9, 4.4 Hz, 1H), 4.18 – 4.06 (m, 2H), 3.77 (d, *J* = 3.2 Hz, 2H), 3.47 (s, 3H), 3.07 (d, *J* = 9.5 Hz, 1H), 2.76 (d, *J* = 8.0 Hz, 1H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.7, 135.6, 133.2, 133.0, 129.9, 129.8, 127.9, 127.8, 103.1, 85.8, 72.3, 71.4, 64.2, 55.7, 26.9, 19.3. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{22}H_{30}O_5SiNH_4$ 420.2206; Found 420.2201.



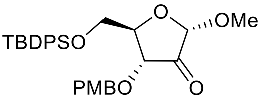
1-α-*O*-methyl-2,3-*O*-(*para*-methoxybenzylidene)-5-O-(*tert*-butyl-di-phenylsilyl)-D-ribofuranoside (38) Compound **14** (6.1 grams, 15 mmol) was dissolved in DMF (30 mL, 0.50 M) containing pyridinium p-toluenesulfonate (PPTS) (26 mg, 0.15 mmol, 0.010 eq.) and anisaldehyde dimethyl acetal (2.87 grams, 15.75 mmol, 1.05 eq.). Subsequently, the resulting clear and colorless solution was slowly concentrated *in vacuo* at 30 °C over a period of 5.5 hours, until methanol ceased to condense. Upon completion, the reaction was quenched with a sat. aq. sodium bicarbonate solution and the resulting mixture was then diluted with diethyl ether. The organic layer was washed three times with water and once with brine. The organic layer was dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by column chromatography (ethyl acetate/pentane, 0/1 → 1/9, v/v) afforded compound **38** (6.47 grams, 12.5 mmol, 83%) as a white solid. TLC: R_f 0.5 (ethyl acetate/pentane, 1/9, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (dddd, *J* = 8.2, 6.4, 2.8, 1.5 Hz, 4H), 7.62 – 7.55 (m, 2H), 7.49 – 7.34 (m, 6H), 7.04 – 6.97 (m, 2H), 6.94 – 6.87 (m, 2H), 6.02 (s, 1H), 5.95 (s, 1H), 5.16 – 5.09 (m, 1H), 4.87 – 4.80 (m, 1H), 4.78 (dd, *J* = 7.1, 4.3 Hz, 1H), 4.38 (q, *J* = 3.1 Hz, 1H), 4.19 – 4.13 (m, 1H), 3.91 (d, *J* = 3.1 Hz, 1H), 3.89 (s, 3H), 3.88 – 3.86 (m, 1H), 3.84 (d, *J* = 3.2 Hz, 1H), 3.81 (d, *J* = 1.9 Hz, 3H), 3.78 (d, *J* = 3.1 Hz, 1H), 3.54 (s, 3H), 3.52 (s, 3H), 1.07 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.9, 135.8, 135.7, 135.7, 133.2, 133.1, 130.0, 129.9, 129.9, 129.1, 128.6, 128.3, 127.9, 127.9, 127.9, 114.4, 113.9, 113.9, 113.8, 108.5, 106.2, 104.0, 103.8, 82.6, 81.2, 81.0, 80.9, 80.2, 79.8, 64.8, 64.4, 56.3, 55.9, 55.7, 55.4, 26.9, 19.4.

1-*α*-*O*-methyl-3-*O*-(*para*-methoxybenzyl)-5-O-(*tert*-butyl-di-phenylsilyl)-D-ribofuranoside



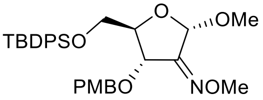
(22) Compound **38** (4.34 grams, 8.33 mmol) was co-evaporated with anhydrous toluene three times, whereafter it was dissolved in anhydrous dichloromethane (28 mL, 0.30 M) and stirred while under a nitrogen atmosphere. The solution was then cooled to -78 °C and to it was added di-*iso*-butyl aluminium hydride (DIBAL-H, 1.0 M in dichloromethane) (42 mL, 42 mmol, 5.0 eq.) after which the resulting mixture was stirred for 10 minutes at this temperature. Then, the reaction was allowed to warm to -20 °C over a period of three hours. Once at -20 °C, the reaction was quenched by the addition of sat. aq. Rochelle's Salt (Tartrate) solution and the product was extracted using dichloromethane. The aqueous layer was back-extracted with dichloromethane three times and the combined organic layers were washed once with water. Then, the combined organic layers was dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by column chromatography (ethyl acetate/pentane, 1/9 → 1/3, v/v) gave compound **22** (2.47 grams, 4.73 mmol, 57%) as a cloudy white oil. TLC: R_f 0.5 (ethyl acetate/pentane, 3/7, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.61 (m, 2H), 7.61 – 7.55 (m, 2H), 7.49 – 7.40 (m, 2H), 7.40 – 7.34 (m, 4H), 7.25 – 7.18 (m, 2H), 6.88 – 6.80 (m, 2H), 4.89 (d, J = 4.7 Hz, 1H), 4.65 (d, J = 12.0 Hz, 1H), 4.52 (d, J = 12.0 Hz, 1H), 4.21 – 4.10 (m, 1H), 4.07 (q, J = 3.5 Hz, 1H), 3.94 (dd, J = 7.1, 2.8 Hz, 1H), 3.79 (s, 3H), 3.62 (dd, J = 11.1, 3.8 Hz, 1H), 3.51 (dd, J = 11.0, 3.4 Hz, 1H), 3.47 (s, 3H), 2.97 (d, J = 11.3 Hz, 1H), 1.02 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.4, 135.7, 135.7, 133.3, 130.0, 129.9, 129.9, 129.7, 127.9, 127.8, 114.1, 103.2, 83.8, 76.0, 72.9, 72.3, 64.1, 55.8, 55.4, 26.9, 19.4. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{30}H_{38}O_6SiNH_4$ 540.2781; Found 540.2776.

1-*α*-*O*-methyl-3-*O*-(*para*-methoxybenzyl)-5-*O*-(*tert*-butyl-diphenylsilyl)-D-erythro-



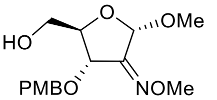
pentofuran-2-uloside (39) Compound **22** (1.21 grams, 2.30 mmol) was co-evaporated three times using anhydrous toluene whereafter it was dissolved in acetonitrile (21 mL, 0.11 M) and stirred under a nitrogen atmosphere. To this mixture was added 2-iodoxybenzoic acid (IBX) (1.3 grams, 4.6 mmol, 2.0 eq.) and the resulting suspension was refluxed for one hour at 82 °C. At this point, TLC analysis indicated full consumption of the starting material and the suspension was allowed to cool to room temperature. Filtration over a glass filter resulted in the convenient removal of IBX-based salts, and the filtrate was concentrated *in vacuo*. Purification by column chromatography (ethyl acetate/pentane, 0/1 → 1/4, v/v) afforded compound **39** (0.920 grams, 1.76 mmol, 77%) as light-yellow oil. TLC: R_f 0-0.7 streak (hydrate) (ethyl acetate/pentane, 3/7, v/v) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (ddd, J = 11.6, 8.0, 1.6 Hz, 4H), 7.49 – 7.41 (m, 2H), 7.39 (d, J = 7.7 Hz, 4H), 7.30 – 7.19 (m, 2H), 6.88 – 6.80 (m, 2H), 4.89 (d, J = 11.4 Hz, 1H), 4.85 (s, 1H), 4.63 (d, J = 11.3 Hz, 1H), 4.28 – 4.18 (m, 2H), 3.97 (dd, J = 11.9, 1.8 Hz, 1H), 3.85 – 3.78 (m, 1H), 3.77 (s, 3H), 3.47 (s, 3H), 1.01 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.6, 135.8, 135.7, 133.2, 133.1, 130.0, 130.0, 129.9, 129.2, 127.9, 127.8, 114.0, 98.8, 79.6, 74.8, 72.3, 62.7, 55.7, 55.4, 26.9, 19.4. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{30}H_{36}O_6SiNH_4$ 538.2625; Found 538.2619.

1-*α*-*O*-methyl-3-*O*-(*para*-methoxybenzyl)-5-*O*-(*tert*-butyl-diphenylsilyl)-D-erythro-

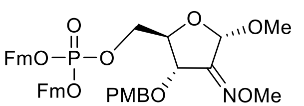


pentofuran-2-uloside *O*-methyl oxime (23) Compound **39** (0.1 grams, 0.2 mmol) was dissolved in a solvent system consisting of methanol (0.6 mL), acetonitrile (0.1 mL) and pyridine (0.09 mL) (0.13 M). While stirring, methoxyamine hydrochloride

($MeONH_2$•HCl) (34 mg, 0.40 mmol, 2.0 eq.) was added to the mixture and the resulting clear solution was allowed to stir at room temperature for a period of two hours. Upon completion, the reaction was quenched with water and the product extracted with ethyl acetate. The organic layer was washed with brine and dried over $MgSO_4$ and filtered off. The filtrate was then concentrated *in vacuo*. Purification by column chromatography (ethyl acetate/pentane, 0/1 → 1/20, v/v) gave compound **23** (87 mg, 0.16 mmol, 80%) as clear and colourless oil. TLC: R_f 0.5 (ethyl acetate/pentane, 1/9, v/v). NMR reports both E and Z oxime. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 – 7.56 (m, 4H), 7.48 – 7.31 (m, 6H), 7.25 (s, 2H), 6.90 – 6.74 (m, 2H), 5.63 (d, *J* = 6.7 Hz, 1H), 5.33 (d, *J* = 6.8 Hz, 1H), 4.76 (d, *J* = 11.6 Hz, 1H), 4.71 – 4.57 (m, 1H), 4.55 – 4.44 (m, 2H), 4.40 (d, *J* = 3.7 Hz, 1H), 4.29 (d, *J* = 3.5 Hz, 1H), 4.12 (q, *J* = 7.2 Hz, 1H), 3.98 (d, *J* = 6.8 Hz, 3H), 3.85 – 3.74 (m, 3H), 3.70 (m, 2H), 3.57 (dd, *J* = 11.2, 3.6 Hz, 2H), 3.51 – 3.35 (m, 3H), 1.04 – 0.95 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0, 136.9, 135.7, 130.1, 129.8, 127.8, 113.9, 98.3, 84.2, 83.7, 75.2, 70.3, 64.2, 63.0, 55.7, 55.2, 26.8, 18.0. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{31}H_{39}NO_6SiNH_4$ 567.2890; Found 567.2885.

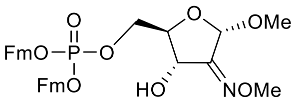


1-α-*O*-methyl-3-*O*-(*para*-methoxybenzyl)-D-erythro-pentofuran-2-uloside *O*-methyl oxime (40) Compound **23** (0.11 grams, 0.20 mmol) was co-evaporated with anhydrous toluene three times and dissolved in pyridine (2.0 mL, 0.10 M). The resulting light-yellow solution was brought under a nitrogen atmosphere and a solution of hydrogen fluoride in pyridine (70% HF, w/w) (0.20 mL, 6.9 mmol, 34 eq.) was carefully added while wearing chemically resistant safety gloves. The solution was allowed to stir at room temperature for two hours. At this point, TLC analysis indicated full consumption of the starting materials and the reaction was quenched with sat. aq. sodium bicarbonate solution. The product was extracted using dichloromethane. The combined organic layers were dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by column (ethyl acetate/pentane, 1/4 → 2/3, v/v) gave compound **40** (52 mg, 0.167 mmol, 84%) as clear colorless oil. TLC: R_f 0.7 (ethyl acetate/pentane, 4/1, v/v). NMR reports both E and Z oxime. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (td, *J* = 6.5, 2.1 Hz, 2H), 6.87 (dd, *J* = 8.7, 1.7 Hz, 2H), 5.52 (s, 1H), 5.24 (s, 1H), 4.83 (d, *J* = 11.6 Hz, 1H), 4.72 (d, *J* = 11.6 Hz, 1H), 4.58 – 4.49 (m, 3H), 4.32 (d, *J* = 5.6 Hz, 1H), 4.28 – 4.19 (m, 2H), 3.98 (d, *J* = 6.6 Hz, 6H), 3.80 (m, 7H), 3.70 (d, *J* = 12.9 Hz, 1H), 3.67 – 3.57 (m, 1H), 3.47 (d, *J* = 8.3 Hz, 6H), 3.44 – 3.34 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.4, 157.3, 157.0, 130.3, 130.0, 129.9, 129.7, 113.9, 113.9, 101.7, 98.1, 82.9, 82.2, 74.2, 72.6, 71.4, 71.0, 63.0, 62.7, 62.0, 61.8, 55.5, 55.4, 55.0. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{15}H_{21}NO_6Na$ 334.1267; found 334.1261.

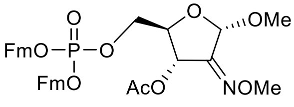


1-α-*O*-methyl-3-*O*-(*para*-methoxybenzyl)-5-*O*-(di(9*H*-fluoren-9-yl))-phosphoryl-D-erythro-pentofuran-2-uloside *O*-methyl oxime (24) Compound **40** (52 mg, 0.17 mmol) was co-evaporated with anhydrous toluene three times and then dissolved in anhydrous acetonitrile (1.1 mL, 0.15M). While stirring, to the mixture was added 4,5-dicyanoimidazole (DCI) (59 mg, 0.51 mmol, 3.0 eq.) and the resulting solution was stirred vigorously while flushing with nitrogen for ten minutes. Next, $(FmO)_2PN(iPr)_2$ (**15**) (0.13 grams, 0.25 mmol, 1.5 eq.) was dissolved in anhydrous acetonitrile (1.1 mL, 0.22 M) and the resulting solution then added to the reaction suspension dropwise whereafter the mixture was stirred at room temperature for 90 minutes. At this point, TLC analysis indicated full consumption of the starting material and to the reaction mixture was added *tert*-butyl hydroperoxide (*t*BuOOH) (5.5 M in decanes) (0.15

mL, 0.84 mmol, 5.0 eq.). Progression of the reaction was followed by ^{31}P-NMR and when this indicated full conversion (~30 minutes), the reaction was quenched by water and the product was extracted using dichloromethane. The combined organic layers were dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by column chromatography (diethyl ether/pentane, 2/3 → 3/1, v/v) afforded compound **24** (79 mg, 0.11 mmol, 64%) as light-yellow oil. TLC: R_f 0.5 (diethyl ether/pentane, 9/1, v/v). NMR reports both E and Z oxime. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.69 (m, 4H), 7.62 – 7.47 (m, 4H), 7.46 – 7.33 (m, 4H), 7.33 – 7.17 (m, 4H), 7.01 (d, *J* = 10.6 Hz, 2H), 6.84 – 6.78 (m, 2H), 5.42 (s, 1H), 5.14 (s, 1H), 4.77 (d, *J* = 11.6 Hz, 1H), 4.65 (d, *J* = 11.4 Hz, 1H), 4.47 (d, *J* = 11.4 Hz, 1H), 4.43 (d, *J* = 5.5 Hz, 1H), 4.34 – 4.18 (m, 9H), 4.18 – 4.10 (m, 8H), 4.10 – 3.93 (m, 2H), 3.92 (app. d, *J* = 1.5 Hz, 6H), 3.72 (app. d, *J* = 8.6 Hz, 6H), 3.40 (app. d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.6, 143.1, 143.1, 141.5, 141.4, 129.9, 129.3, 128.1, 128.0, 128.0, 127.3, 127.3, 127.2, 125.6, 125.2, 125.1, 120.2, 120.1, 113.9, 113.9, 101.6, 98.0, 81.1, 80.3, 80.2, 74.2, 72.6, 70.8, 69.5, 69.5, 67.2, 67.1, 66.6, 63.0, 62.7, 55.5, 55.3, 55.0, 48.1, 48.0, 47.9. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{43}H_{42}NO_9PNH_4$ 765.2941; Found 765.2935.

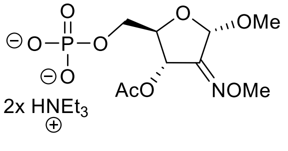


1-α-*O*-methyl-5-*O*-(di(9*H*-fluoren-9-yl))-phosphoryl-D-erythro-pentofuran-2-uloside *O*-methyl oxime (41) Compound **24** (0.24 grams, 0.30 mmol) was dissolved in dichloromethane (6.0 mL, 0.050M) with phosphate buffer (0.30 mL, pH 7.4). The resulting light-yellow solution was brought under a nitrogen atmosphere and cooled on ice. Then, to the stirring solution was added 2,3-di-chloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.41 grams, 1.8 mmol, 6.0 eq.) and the mixture was allowed to warm to room temperature. Once at ambient temperatures, the reaction mixture was stirred for six hours. At this point, TLC analysis indicated absence of the starting material and the reaction was quenched with a mixture of sat. aq. sodium bicarbonate solution and sat. aq. sodium thiosulfate ($Na_2S_2O_3$) solution (1/1, v/v). Then, the aqueous layer was washed multiple times using dichloromethane. The combined organic layers were then dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by column chromatography (acetone/pentane, 1/9 → 3/7, v/v) afforded compound **41** (0.154 grams, 0.250 mmol, 82%) as light-yellow oil. TLC: R_f 0.4 (acetone/pentane, 2/3, v/v). NMR reports both E and Z oxime. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.66 (m, 4H), 7.57 – 7.45 (m, 4H), 7.41 – 7.29 (m, 4H), 7.30 – 7.19 (m, 4H), 5.30 (s, 1H), 5.10 (s, 1H), 4.66 – 4.60 (m, 1H), 4.46 – 4.36 (m, 1H), 4.31 – 4.17 (m, 6H), 4.17 – 4.01 (m, 6H), 3.91 (s, 3H), 3.86 (s, 3H), 3.50 (d, *J* = 5.6 Hz, 1H), 3.36 (app. d, *J* = 5.8 Hz, 6H), 3.22 (d, *J* = 2.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.9, 157.9, 143.1, 143.1, 143.1, 143.1, 143.0, 143.0, 141.4, 141.4, 128.0, 127.2, 125.2, 120.1, 100.7, 97.9, 81.0, 80.7, 77.8, 69.5, 69.5, 69.5, 69.4, 69.3, 67.2, 66.2, 66.1, 66.0, 65.9, 62.9, 62.8, 55.6, 55.1, 48.0, 47.9, 47.9, 47.9, 47.9, 47.8. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{35}H_{34}NO_8PNH_4$ 645.2366; Found 645.2360.



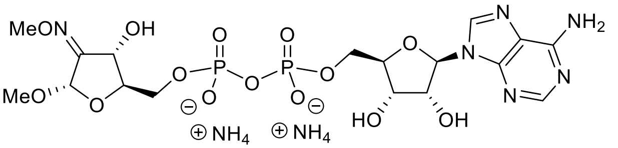
1-α-*O*-methyl-3-*O*-acetyl-5-*O*-(di(9*H*-fluoren-9-yl))-phosphoryl-D-erythro-pentofuran-2-uloside *O*-methyl oxime (25) Compound **41** (0.15 grams, 0.25 mmol) was co-evaporated with anhydrous pyridine three time. Next, it was dissolved in pyridine (0.30 mL, 0.83 M), cooled on ice, and Ac_2O (77 µL, 0.75 mmol, 3.0 eq.) and DMAP (4 mg, 0.03 mmol, 0.01 eq.) were added. The resulting light-yellow solution was allowed to warm to room temperature and stirred for two hours when TLC analysis showed full consumption of the starting material. The reaction was quenched with water and the product was extracted using dichloromethane. The combined organic layers were dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by column chromatography (acetone/pentane, 1/9 → 3/7, v/v) afforded compound **25** (75 mg, 0.11 mmol, 41%) as a clear colorless oil. TLC: R_f 0.5 (acetone/pentane, 3/7, v/v). NMR reports both E and Z oxime. ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.68 (m, 4H), 7.58 – 7.48 (m, 4H), 7.42 – 7.31 (m, 4H), 7.31 – 7.21 (m, 4H), 5.75 (d, *J* = 5.5 Hz, 1H), 5.50 (d, *J* = 5.0 Hz, 1H), 5.39 (s, 1H), 5.29 (s, 1H), 5.17 (bs, 7H), 4.33 – 4.11 (m, 3H), 3.85 (d, *J* = 3.8 Hz, 6H), 3.39 (d, *J* = 4.2 Hz, 6H), 2.17 (s, 3H), 2.08 (d, *J* = 11.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 154.7, 143.2, 143.1, 141.4, 128.0, 127.2, 125.3, 120.1, 101.2, 97.8, 80.5, 80.4, 69.8, 69.6, 66.7, 66.7, 65.3, 63.2, 63.1, 55.75, 55.3, 48.0, 47.9, 20.9. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{37}H_{36}NO_9PNH_4$ 687.2471; Found 687.2466.

1-α-*O*-methyl-3-*O*-acetyl-5-*O*-phosphate-D-erythro-pentofuran-2-uloside *O*-methyl oxime (12)



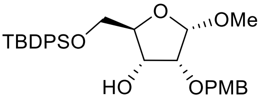
Compound **25** (75 mg, 0.11 mmol) was dissolved in anhydrous acetonitrile (2.3 mL) and to it was added Et_3N (0.8 mL, 5.5 mmol, 50.0 eq.). This solution was then allowed to stir at room temperature for 24 hours, after which TLC analysis indicated full consumption of both the starting material and the mono-deprotected intermediate. The solution was then concentrated *in vacuo* whereafter the resulting crude phosphate was first co-evaporated three times with a mixture of acetonitrile and pyridine (1/1, v/v) and then once with acetonitrile. Crude phosphate **12** was used in the next reaction without any further purification.

1”-α-*O*-methyl-D-erythro-pentofuran-2”-uloside *O*-methyl oxime ADPr (3)



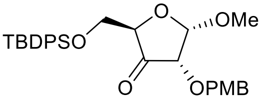
To crude compound **12** was added DCI (32 mg, 0.28 mmol, 2.5 eq.) and the resulting mixture was co-evaporated with anhydrous acetonitrile thrice. The resulting cloudy mixture was suspended in anhydrous acetonitrile (2.8 mL, 0.040 M). In another flask, phosphoramidite **7** (94 mg, 0.13 mmol, 1.2 eq) was co-evaporated thrice with anhydrous toluene whereafter it was dissolved in anhydrous acetonitrile (1.2 mL, 0.11 M). The solution containing phosphoramidite **7** was then added dropwise to the suspension containing DCI and the phosphate, and the resulting mixture was stirred vigorously for one hour at room temperature. After this time, *t*BuOOH (5.5 M in decanes) (0.10 mL, 0.56 mmol, 5.1 eq.) was added to the suspension and the resulting mixture was stirred for an additional hour at room temperature. When ^{31}P NMR showed full consumption of any P(III) species, DBU (84 µL, 0.56 mmol, 5.1 eq.) was added whereafter the resulting suspension was allowed to stir at room temperature for an additional 30 minutes. Finally, ammonium hydroxide (28%, 1.7 mL) was added, a glass stopper was installed and the reaction mixture was then allowed to stir for 72 hours at room temperature. After this time LCMS indicated full deprotection of the construct and the reaction mixture was diluted with water and ethyl acetate. The aqueous phase was then washed thrice with ethyl acetate after which it was concentrated *in vacuo*. Subsequent purification by gel exclusion chromatography on HW40 toyopearl column followed by HPLC and lyophilization afforded compound **3** (25 mg, 0.041 mmol, 38%) as the ammonium salt as a white foam. ^1H NMR (400 MHz, D_2O) δ 8.54 – 8.39 (m, 1H), 8.25 – 8.11 (m, 1H), 6.18 – 5.98 (m, 1H), 5.11 (s, 1H), 4.75 – 4.65 (m, 1H), 4.47 (ddd, *J* = 8.8, 6.2, 2.8 Hz, 2H), 4.34 (p, *J* = 2.8 Hz, 1H), 4.19 (dd, *J* = 4.5, 2.7 Hz, 2H), 4.15 – 3.98 (m, 3H), 3.75 (s, 3H), 3.32 (s, 3H). ^{13}C NMR (101 MHz, D$_2$O) δ 158.5, 152.7, 139.9, 137.1, 100.5, 97.5, 86.8, 83.8, 81.2, 74.3, 70.2, 67.9, 65.6, 65.1, 64.0, 62.4, 55.1. ^{31}P NMR (162 MHz, D$_2$O) δ -10.47 (dd, J = 28.2, 14.1 Hz).

1-α-*O*-methyl-2-*O*-(*para*-methoxybenzyl)-5-*O*-(*tert*-butyl-diphenylsilyl)-D-ribofuranoside (26)



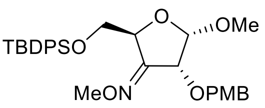
Compound **14** (4.65 grams, 11.5 mmol) was co-evaporated with anhydrous toluene for three times after which it was dissolved in anhydrous DMF (68 mL, 0.17 M) and flushed with nitrogen at 0 °C. To the reaction mixture, tetra-n-butylammonium iodide (TBAI) (0.43 grams, 1.2 mmol, 0.10 eq.) and 4-methoxybenzyl chloride (PMB-Cl) (2.0 grams, 13 mmol, 1.1 eq.) were added and the resulting reaction mixture was stirred for 10 minutes. Subsequently, sodium hydride (60% w/w dispersion in mineral oil) was slowly added (0.31 grams, 13 mmol, 1.1 eq.) and the resulting grey suspension was stirred at room temperature over a period of 20 hours. At this point TLC analysis indicated absence of the starting material and the reaction was quenched with ice and diluted with diethyl ether. The organic phase was washed three times with water and was dried over MgSO$_4$, filtered off concentrated *in vacuo*. Purification by column chromatography (ethyl acetate/pentane, 1/9 → 3/7, v/v) gave a 3/2 (mol/mol) inseparable mixture of compound **26** and anisyl alcohol respectively (3.56 grams, 6.85 mmol, 63%) as cloudy light-yellow oil. TLC: R$_f$ 0.5 (ethyl acetate/pentane, 1/1, v/v). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.72 – 7.59 (m, 4H), 7.47 – 7.34 (m, 6H), 7.33 – 7.28 (m, 2H), 6.93 – 6.78 (m, 2H), 4.89 (d, *J* = 4.1 Hz, 1H), 4.70 (d, *J* = 11.8 Hz, 1H), 4.63 – 4.53 (m, 1H), 4.25 – 4.16 (m, 2H), 3.98 (dd, *J* = 5.8, 4.1 Hz, 1H), 3.79 (s, 3H), 3.72 (qd, *J* = 11.3, 3.0 Hz, 2H), 3.43 (s, 3H), 3.01 (d, *J* = 8.8 Hz, 1H), 0.98 (s, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 159.6, 135.8, 135.7, 135.7, 133.3, 133.1, 130.0, 129.91, 129.86, 129.7, 129.5, 128.8, 127.9, 127.9, 127.83, 127.79, 114.1, 113.9, 113.8, 102.7, 87.0, 78.1, 72.2, 70.1, 65.2, 64.3, 55.4, 55.4, 55.2, 26.9, 19.3. HRMS (ESI) m/z: [M+NH$_4$]$^+$ Calcd for C$_{30}$H$_{38}$O$_6$SiNH$_4$ 540.2781; Found 540.2776.

1-α-*O*-methyl-2-*O*-(*para*-methoxybenzyl)-5-*O*-(*tert*-butyl-diphenylsilyl)-D-erythro-pentofuran-3-uloside (42)

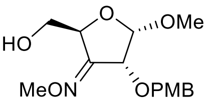


Compound **26** (2.22 grams, 4.25 mmol) was dissolved in dichloromethane (47 mL, 0.090 M) and Dess-Martin Periodinane (DMP) (3.1 grams, 7.2 mmol, 1.7 eq.) was added to the solution. Subsequently, the resulting white suspension was stirred over a period of 17 hours at room temperature. At this point TLC analysis indicated full conversion of the starting material and the reaction was diluted with sat. aq. sodium bicarbonate solution and sat.aq. sodium thiosulphate solution. The product was extracted with dichloromethane and the combined organic phases were dried over MgSO$_4$ filtered off and concentrated *in vacuo*. Purification by column chromatography (diethyl ether/pentane, 0/1 → 1/4, v/v) afforded compound **42** (1.50 grams, 2.88 mmol, 68%) as clear yellow oil. TLC: R$_f$ 0.4 (diethyl ether/pentane, 2/3, v/v). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.61-7.57 (m, 4H), 7.46 – 7.35 (m, 6H), 7.32 (d, *J* = 8.6 Hz, 2H), 6.89 – 6.83 (m, 2H), 5.04 (d, *J* = 4.7 Hz, 1H), 4.84 (d, *J* = 12.0 Hz, 1H), 4.75 (d, *J* = 12.1 Hz, 1H), 4.24 (dd, *J* = 4.7, 0.9 Hz, 1H), 3.98 (t, *J* = 2.2 Hz, 1H), 3.95 – 3.88 (m, 1H), 3.82 (dd, *J* = 11.4, 2.1 Hz, 1H), 3.78 (s, 3H), 3.45 (s, 3H), 0.92 (s, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 210.0, 159.8, 135.7, 135.7, 132.8, 132.6, 130.3, 130.0, 129.9, 128.8, 127.9, 127.9, 127.9, 114.1, 99.8, 79.8, 77.5, 72.6, 62.5, 55.4, 29.8, 26.8, 19.3. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{30}H_{36}O_6SiNH_4$ 538.2625; Found 538.2619.

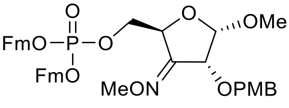
1-α-*O*-methyl-2-*O*-(*para*-methoxybenzyl)-5-*O*-(*tert*-butyl-diphenylsilyl)-D-erythro-pentofuran-3-uloside *O*-methyl oxime (27) Compound **42** (0.52 grams, 1.0 mmol) was dissolved in a solvent system of methanol (2.9 mL), pyridine (0.4 mL) and acetonitrile (0.6 mL) (0.26 M). While stirring, $MeONH_3Cl$ (0.17 grams, 2.0 mmol, 2.0 eq.) was added at room temperature and the resulting solution was allowed to stir over a period of 2 hours. Upon completion, the reaction was quenched with water and diluted with ethyl acetate. The organic phase was washed with brine and dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by column chromatography (ethyl acetate/pentane, 0/1 → 1/4, v/v) gave compound **27** (0.45 grams, 0.82 mmol, 82%) as clear, colourless oil. TLC: R_f 0.5 (ethyl acetate/pentane, 1/4, v/v). ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.58 (m, 4H), 7.47 – 7.28 (m, 8H), 6.90 – 6.82 (m, 2H), 4.86 (d, *J* = 12.1 Hz, 1H), 4.81 – 4.67 (m, 3H), 4.57 (dd, *J* = 4.4, 1.8 Hz, 1H), 4.10 (dd, *J* = 11.0, 2.5 Hz, 1H), 3.91 (s, 3H), 3.78 (s, 3H), 3.76 (dd, *J* = 11.1, 1.9 Hz, 1H), 3.37 (s, 3H), 0.96 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.6, 157.4, 135.80, 135.75, 135.7, 133.3, 133.2, 130.3, 129.9, 129.79, 129.76, 129.4, 127.8, 127.7, 113.9, 113.8, 100.3, 77.1, 75.6, 72.8, 72.6, 62.4, 62.3, 55.4, 54.9, 26.8, 19.3. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{39}NO_6SiNa$ 572.2444; Found 572.2439.



1-α-*O*-methyl-2-*O*-(*para*-methoxybenzyl)-D-erythro-pentofuran-3-uloside *O*-methyl oxime (43) Compound **27** (0.11 grams, 0.20 mmol) was co-evaporated with anhydrous toluene thrice and dissolved in pyridine (2.0 mL, 0.1 M). The resulting solution was brought under a nitrogen atmosphere and while on ice, HF-pyridine (70% w/w) (0.20 mL, 6.9 mmol, 34 eq.) was carefully added. The resulting light-yellow solution was allowed to stir at room temperature for two hours. Upon completion, the reaction was quenched with sat. aq. sodium bicarbonate solution and the product was extracted with dichloromethane. The combined organic phases were dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by column (ethyl acetate/pentane, 2/3 → 1/1, v/v) gave compound **43** (46 mg, 0.15 mmol, 74%) as clear colourless oil. TLC: R_f 0.5 (ethyl acetate/pentane, 7/3, v/v). ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, *J* = 8.6 Hz, 2H), 6.92 – 6.84 (m, 2H), 4.85 (d, *J* = 12.0 Hz, 1H), 4.76 (td, *J* = 3.3, 1.9 Hz, 1H), 4.73 – 4.64 (m, 2H), 4.47 – 4.38 (m, 1H), 4.03 – 3.91 (m, 4H), 3.83 – 3.79 (m, 4H), 3.36 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.7, 157.1, 130.2, 130.0, 129.3, 113.9, 113.8, 102.0, 100.4, 77.0, 75.7, 75.6, 72.9, 72.7, 62.7, 61.4, 55.4, 55.0. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{15}H_{21}NO_6Na$ 334.1267; Found 334.1261.

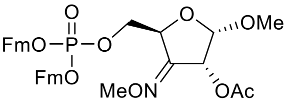


1-α-*O*-methyl-2-*O*-(*para*-methoxybenzyl)-5-*O*-(di(9*H*-fluoren-9-yl))-phosphoryl-D-erythro-pentofuran-3-uloside *O*-methyl oxime (28) Compound **43** (46 mg, 0.15 mmol) was co-evaporated three times with anhydrous toluene and dissolved in anhydrous acetonitrile (1.0 mL, 0.15 M). While stirring, DCI (52 mg, 0.44 mmol, 3.0 eq.) was added and the resulting white suspension was stirred vigorously while flushing with nitrogen for ten minutes. $(FmO)_2PN(iPr)_2$ (**15**) (0.12 grams, 0.22 mmol, 1.5 eq.) was co-evaporated thrice with anhydrous toluene and dissolved in anhydrous acetonitrile (0.7 mL, 0.31 M). This solution was added dropwise to the stirring suspension containing compound **43**. After final addition, the mixture was allowed

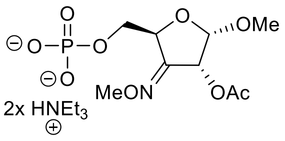


to stir at room temperature over a period of 90 minutes. At this point TLC analysis indicated full conversion of the starting material and *t*BuOOH (5.5 M in decanes) (67 mg, 0.74 mmol, 5.0 eq.) was added and the progression of the reaction was followed by ^{31}P NMR. When this indicated full conversion, the reaction was quenched with water and the product was extracted using dichloromethane. The combined organic phases were dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification by column chromatography (acetone/pentane, 0/1 → 1/4, v/v) gave compound **28** (78 mg, 0.11 mmol, 71%) as light-yellow oil. TLC: R_f 0.4 (acetone/pentane, 3/7, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 – 7.69 (m, 4H), 7.57 – 7.46 (m, 4H), 7.45 – 7.32 (m, 4H), 7.31 – 7.22 (m, 4H), 7.22 – 7.16 (m, 2H), 6.78 – 6.73 (m, 2H), 4.78 – 4.70 (m, 2H), 4.53 (d, *J* = 4.3 Hz, 1H), 4.45 (d, *J* = 11.8 Hz, 1H), 4.35 – 4.07 (m, 9H), 3.83 (d, *J* = 1.7 Hz, 3H), 3.70 (s, 3H), 3.26 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.6, 155.5, 143.2, 143.1, 143.0, 141.5, 141.43, 141.40, 130.1, 129.9, 129.2, 128.06, 128.04, 127.99, 127.91, 127.88, 127.3, 127.24, 127.20, 127.17, 125.31, 125.28, 125.25, 125.1, 125.0, 120.14, 120.11, 120.08, 120.0, 113.8, 100.3, 77.0, 73.1 (d, *J* = 8.2 Hz), 72.8, 69.5 (d, *J* = 6.0 Hz), 65.6 (d, *J* = 5.4 Hz), 62.7, 55.3, 55.1, 53.9, 48.0 (d, *J* = 2.5 Hz), 47.9 (d, *J* = 2.6 Hz), 29.8, 29.4. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{43}H_{42}NO_9PNH_4$ 765.2941; Found 765.2935.

1-α-*O*-methyl-2-*O*-acetyl-5-*O*-(di(9*H*-fluoren-9-yl))-phosphoryl-D-erythro-pentofuran-3-uloside *O*-methyl oxime (29) Compound **28** (75 mg, 0.10 mmol) was dissolved in anhydrous dichloromethane (1.8 mL, 0.055 M) and cooled on ice. The mixture was brought under a nitrogen atmosphere and while stirring, triethyl silane hydride (TES-H) (0.16 mL, 1.0 mmol, 10 eq.) and TFA (0.2 mL, 10% v/v) were added. Subsequently, the light-yellow solution was allowed to stir for 4.5 hours at room temperature. Upon completion, the reaction was quenched with pyridine until a neutral pH was obtained. Subsequently, the solution was carefully concentrated *in vacuo*. Purification by flash column chromatography (acetone/pentane, 1/4 → 1/1, v/v) gave the corresponding secondary alcohol which was co-evaporated three times with pyridine and was dissolved in pyridine (0.10 mL, 1.0 M). The mixture was cooled on ice and acetic anhydride (14 µL, 0.15 mmol, 3.0 eq.) was added. The resulting colourless solution was stirred at room temperature for three hours after which full consumption of the intermediate was observed via TLC analysis. The reaction was quenched with water and the product was extracted using dichloromethane. The combined organic phases were dried over $MgSO_4$, filtered off and concentrated *in vacuo*. Purification via silica column chromatography (acetone/pentane, 1/4 → 2/3, v/v) gave compound **29** (16 mg, 0.024 mmol, 24%) as clear yellow oil. TLC: R_f 0.5 (acetone/pentane, 2/3, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.66 (m, 4H), 7.58 – 7.47 (m, 4H), 7.43 – 7.32 (m, 4H), 7.31 – 7.22 (m, 4H), 5.35 (dd, *J* = 4.5, 2.0 Hz, 1H), 5.14 (d, *J* = 4.5 Hz, 1H), 4.81 (p, *J* = 3.9, 2.2, 2.1 Hz, 1H), 4.39 – 4.19 (m, 6H), 4.16 (t, *J* = 6.9 Hz, 2H), 3.82 (s, 3H), 3.35 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 152.9, 143.3, 143.2, 141.5, 128.1, 128.0, 127.3, 127.3, 125.4, 125.31, 125.27, 120.2, 120.1, 99.8, 73.3 (d, *J* = 8.5 Hz), 71.8, 69.5 (t, *J* = 6.2 Hz), 65.4 (d, *J* = 5.5 Hz), 62.9, 55.4, 48.03 (no C-P coupling observed), 47.96 (no C-P coupling observed), 20.8. HRMS (ESI) m/z: $[M+NH_4]^+$ Calcd for $C_{37}H_{36}NO_9PNH_4$ 687.2471; Found 687.2466.

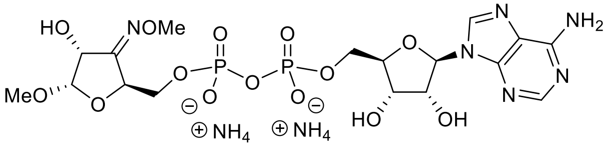


1-α-*O*-methyl-2-*O*-acetyl-5-*O*-phosphate-D-erythro-pentofuran-3-uloside *O*-methyl oxime (13)



Compound **29** (16 mg, 0.024 mmol) was dissolved in a mix of triethylamine (0.17 mL, 1.2 mmol, 50 eq.) and anhydrous acetonitrile (0.5 mL, 0.05 M) and the resulting light-yellow solution was allowed to stir at room temperature for 60 hours. At this point TLC analysis indicated absence of the starting material or its mono-deprotected counterpart and the solution was directly concentrated *in vacuo* and subsequently co-evaporated thrice with pyridine and one with anhydrous acetonitrile. Crude compound **13** was used in the next reaction without any further purification.

1”-α-*O*-methyl-D-erythro-pentofuran-3”-uloside *O*-methyl oxime ADPr (4)



To crude compound **13** was added DCI (7.1 mg, 0.060 mmol, 2.5 eq.) and the mixture was co-evaporated thrice with anhydrous acetonitrile. The resulting white mixture was suspended in anhydrous acetonitrile (0.6 mL, 0.04 M). In another flask, compound **7** (21 mg, 0.029 mmol, 1.2 eq.) was co-evaporated thrice with anhydrous toluene and dissolved in anhydrous acetonitrile (0.3 mL, 0.1 M), before it was added dropwise to the suspension containing the phosphate **13**. The mixture was brought under an atmosphere of argon gas and stirred vigorously for one hour at room temperature. At this point *t*BuOOH (5.5 M in decanes) (22 µL, 0.12 mmol, 5.1 eq.) was added dropwise to the suspension which was then allowed to stir for 90 minutes at room temperature. At this point ^{31}P NMR showed full conversion of all phosphite species into their corresponding oxidated counterparts and to the reaction mixture was added DBU (18 µL, 0.12 mmol, 5.1 eq.). The resulting suspension was allowed to stir at room temperature for half an hour after which ammonium hydroxide (28% NH_3 w/w, 0.4 mL) was added and the resulting solution was allowed to stir at room temperature for six days when LCMS analysis indicated full deprotection of the construct. The reaction mixture was diluted with water and the aqueous phases were washed thrice with ethyl acetate after which the aqueous phase was concentrated *in vacuo.* Subsequent purification by size exclusion chromatography (HW-40, NH_4OAc buffer) and HPLC afforded compound **4** (1.1 mg, 1.8 µmol, 7.5% (over 5 steps)) as the ammonium salt as a white foam. ^{1}H NMR (500 MHz, D_2O) δ 8.56 – 8.45 (m, 1H), 8.29 – 8.18 (m, 1H), 6.15 – 6.11 (m, 1H), 5.01 (d, *J* = 4.6 Hz, 1H), 4.89 – 4.84 (m, 1H), 4.82 (dd, *J* = 4.7, 1.8 Hz, 1H), 4.78 – 4.75 (m, 1H), 4.53 (dd, *J* = 5.2, 3.6 Hz, 1H), 4.39 (q, *J* = 3.4 Hz, 1H), 4.27 – 4.19 (m, 3H), 4.13 (dq, *J* = 11.3, 2.5 Hz, 1H), 3.84 (s, 3H), 3.38 (s, 3H). ^{13}C NMR (126 MHz, D_2O) δ 158.6, 155.4, 152.6, 149.0,139.9, 118.6, 101.0, 86.9, 83.8 (C-P coupling unresolved), 74.2, 73.8 (ap. t, *J* = 4.4 Hz), 70.5, 70.3, 65.1 (C-P coupling unresolved), 63.9 (C-P coupling unresolved), 62.1, 54.8. ^{31}P NMR (202 MHz, D_2O) δ -11.4. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{17}H_{27}N_6O_{14}P_2$ 601.1055; Found 601.1060.

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