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

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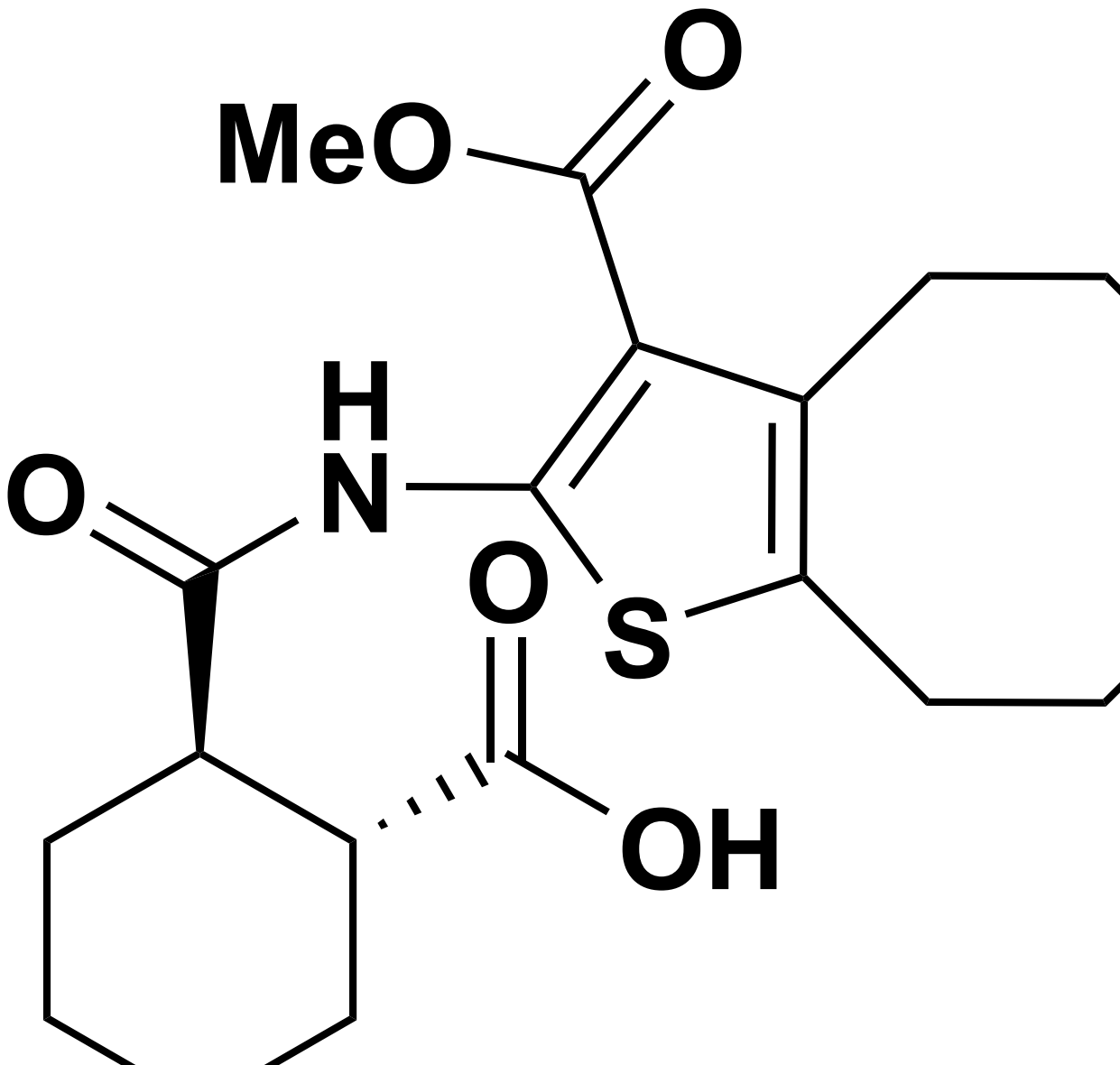
Chapter 5

Adenosine Part 2:

Ψ-Nucleosides



Introduction

ADP-ribose is a molecule consisting of three structural elements: the adenosine, the pyrophosphate and the distal ribose.[1–4] Work described in Chapter 4 demonstrates that by substituting the adenosine with Remdesivir metabolite GS-441524 (Figure 1A),[5–7] the potency inhibitors for the SARS-CoV-2 macrodomain 1 (Mac1) can be substantially improved.[7] With this in mind, other compounds that mimic adenosine within the Mac1 binding site were sought out.

Recent examples of compounds that have been shown to bind the adenosine-binding pocket are the 2-amide-3-methylester thiophenes **3** – **6**, published by Wazir *et al.* (Figure 1B).[8] These molecules were derived from structurally similar leads first identified by the high-throughput screening of 30.000 different compounds.[8] Upon inspection of the co-crystal structure of lead compound **6** and Mac1 (Figure 2), it becomes apparent that the cyclooctaaminothiophene takes the role of the hydrophobic adenine, functioning as a

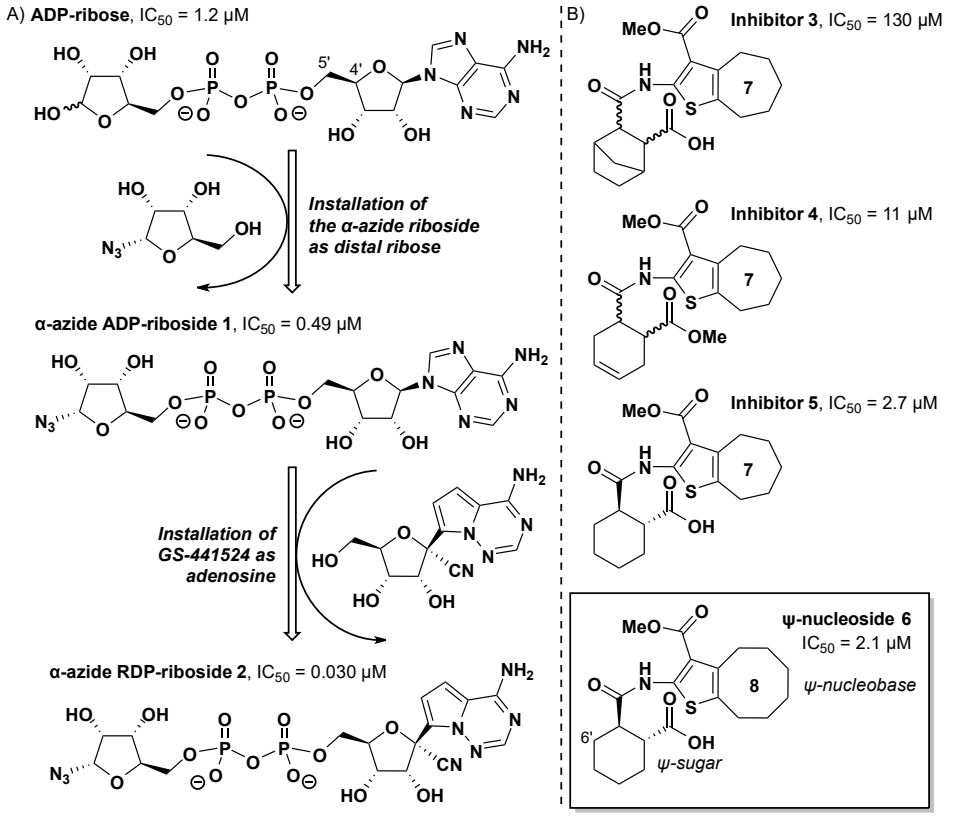


Figure 1 A) ADP-ribose analogues **1** and **2**, discussed in Chapters 2 – 4. B) Structures of thiophene-based inhibitors **3** – **6** reported by Wazir *et al*. in 2024.[8]

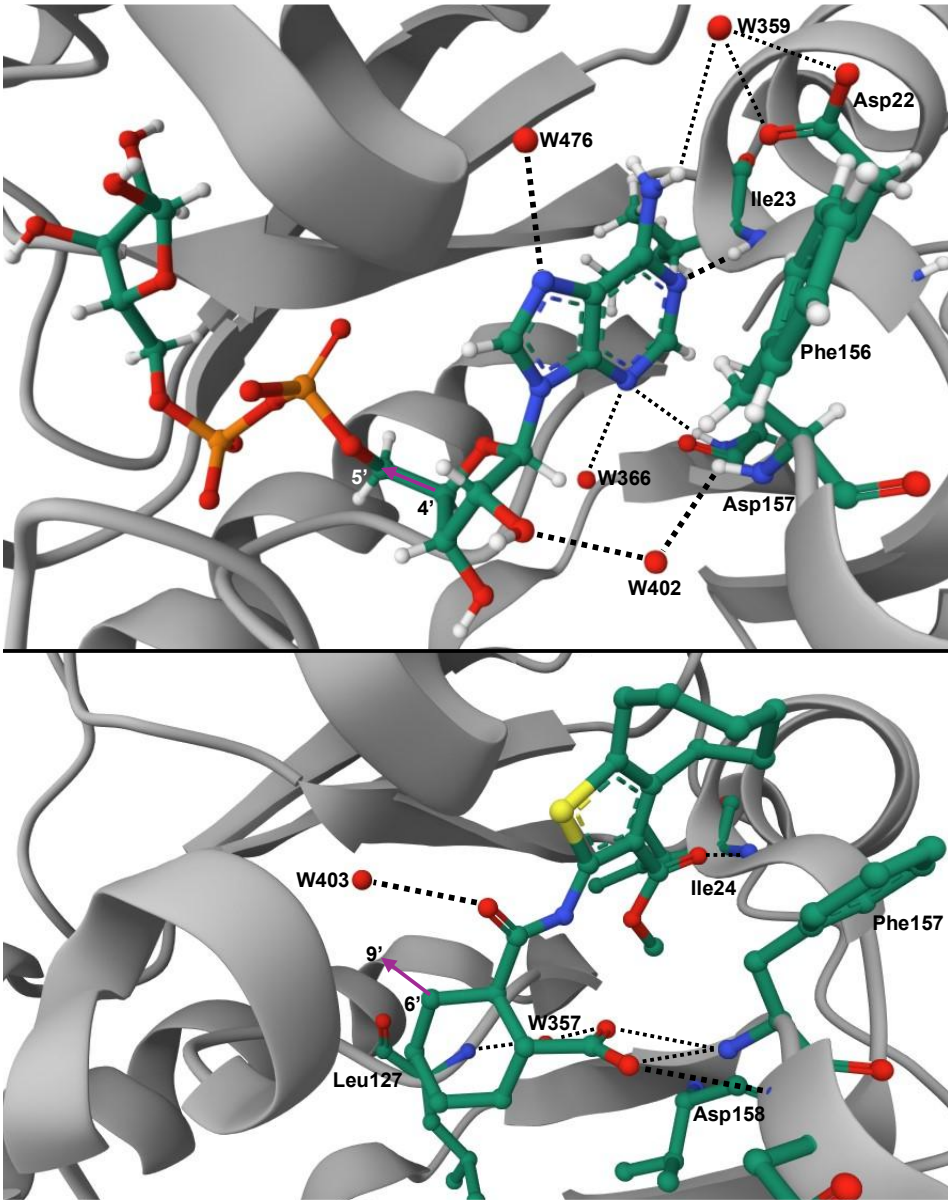


Figure 2 Co-crystal structures of ADP-ribose and ψ-nucleoside **6** with SARS-CoV-2 macrodomain 1 (Mac1). Similar binding modes can be observed between the adenine and ψ-nucleobase, and between the proximal ribose and ψ-sugar. Top: ADP-ribose and Mac1, (PDB: 7KQP).[9] Bottom: ψ-nucleoside **6** and Mac1, (PDB: 8TV7).[8] Note: the bottom co-crystal structure has the corresponding amino acid as +1 due to a difference in Mac1 production techniques between studies.

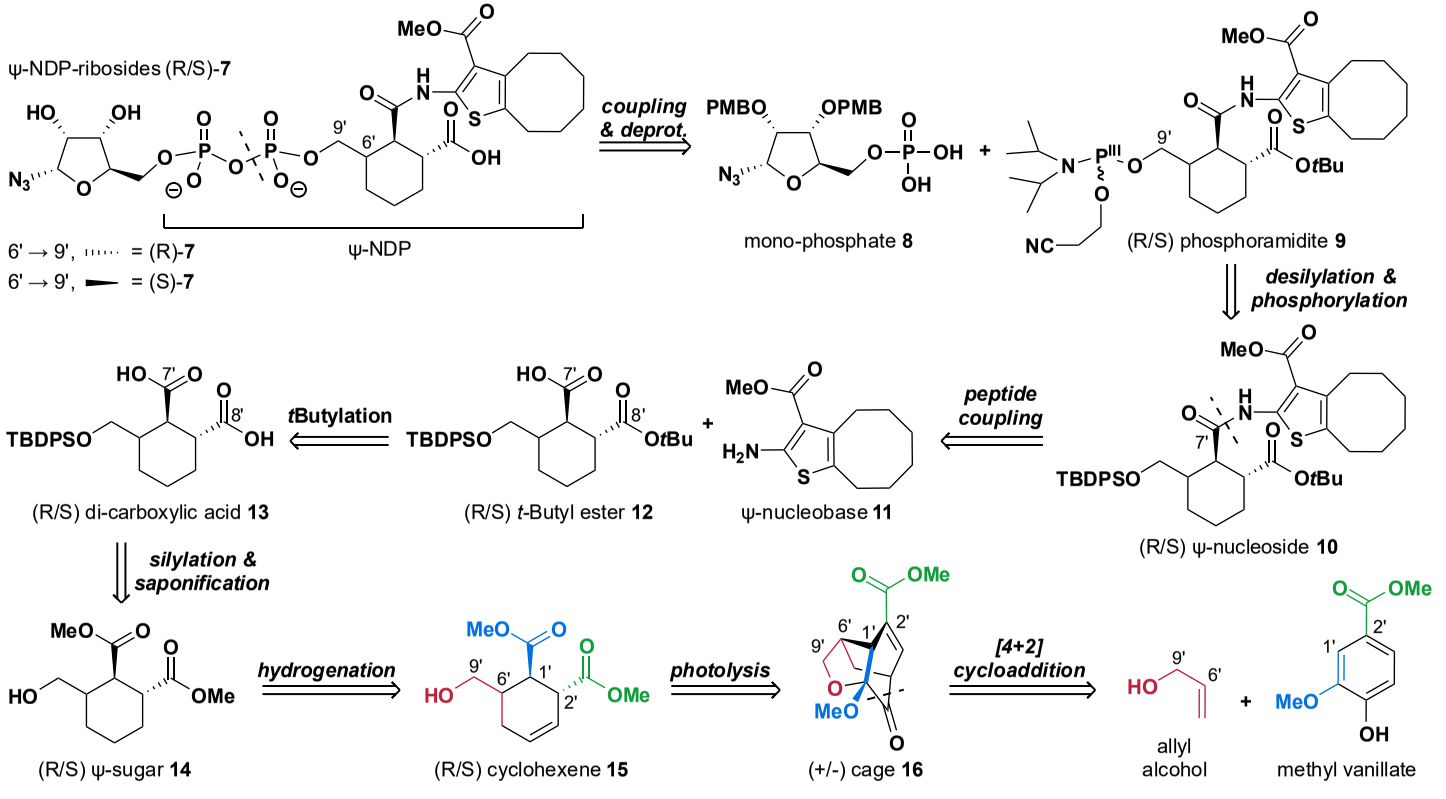
pseudonucleobase (ψ-nucleobase) and interacting with Phe157 and Ile24, while the cyclohexylcarboxylate stands in for the more polar proximal ribose, functioning as a pseudosugar (ψ-sugar) and interacting with Asp158 and waters 357 and 403, making compound **6** a pseudonucleoside (ψ-nucleoside).[8,9] Notably, the high potency of ψ-nucleoside **6** (IC_{50} = 2.1 μM), which is just short of that established for ADP-ribose (IC_{50} = 1.2 μM), makes this motif attractive for incorporation into the structure of an ADP-ribose mimetic.

An inhibitor was designed that would combine a ψ-nucleoside similar to **6** and the 5-*O*-pyrophosphate of α-azide riboside (Scheme 1). To transform ψ-nucleoside **6** into an ADP-ribose analogue, a point of attachment for the pyrophosphate had to be established. As can be observed from Figure 2, the C6' position of compound **6** seems to overlap with the proximal ribose C4' position. Accordingly, the C6' position of compound **6** should be equipped with a hydroxymethyl substituent (C9') (Figure 2) to which the pyrophosphate ribosyl could be attached, affording a ψ-nucleoside diphosphate-riboside (ψ-NDP-riboside).

Mounting the hydroxymethyl group at the C6' of **6** creates a new stereogenic center leading to the design of the corresponding ψ-NDP-ribosides **7R** and **7S** (Scheme 1). Amide **10** could be established through a peptide coupling between cyclooctaaminothiophene **11** and cyclohexyl carboxylic acid **12**. The synthesis of ψ-nucleobase **11** has previously been described,[8] and it was projected that **12** could be prepared from the corresponding protected methyl ester (**14**) through a saponification reaction followed by a selective *tert*-butylation.[10] Some precedent for the formation of the desired cyclohexane dicarboxylic acid core motif can be found in literature.[11–14] Chu *et al.* have shown that a 4+2 cycloaddition between methyl vanillate and allyl alcohol can provide compound **16**, which can undergo a fragmentation using radical photolysis to afford cyclohexene **15**.[11,12] Hydrogenation of this alkene could furnish the desired core motif,[15] ψ-sugar **14**. Because this procedure would provide **14** as a racemic mixture,[12] chiral resolution was envisioned to occur either during the ψ-nucleoside synthesis, for example through the use of a chiral auxiliaries,[16–19] or through chiral column chromatography at a later stage.[20,21]

Results and discussion

The ψ-nucleobase **11** was synthesized using the procedure of Wazir *et al.*,[8] a one-step Gewald amino thiophene reaction (Scheme 2A),[22,23] which is a variation of the Knoevenagel condensation.[24] In this reaction, methyl cyanoacetate was condensed with cyclooctenone in the presence of pyrrolidine and sulphur which, after microwave irradiation induced cyclization and tautomerization, afforded ψ-nucleobase **11** in 84%



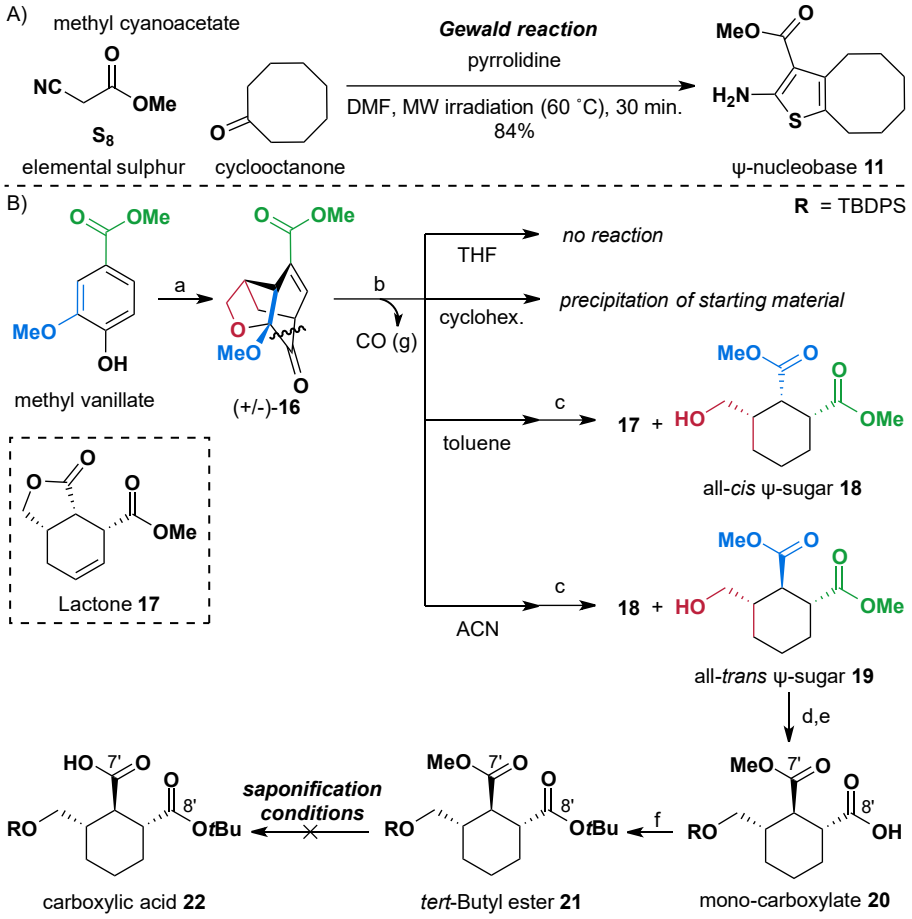
Scheme 1 Retrosynthesis of ψ-NDP-ribosides **7R** and **7S.** Given here is the retrosynthetic analysis towards ψ-nucleoside-based ADP-ribose analogues (ψ-NDP-ribosides) **7**. Key steps are the pyrophosphate establishment, amide formation, anhydride establishment, photolysis and a [4+2] cycloaddition.

yield. Following literature precedent, microwave irradiation was used as opposed to conventional heating, as it had been reported that this greatly benefits reaction time and yield.[8,22]

Synthesis of the ψ-sugar cyclohexane core commenced from methyl vanillate, which was activated using bis(acetoxy)iodobenzene (BAIB) and treated with allyl alcohol to undergo a 4+2 cycloaddition reaction,[14] affording cage **16** in 50% yield (Scheme 2B). Opening of this cage was effected by Chu *et al*. in benzene using a radical photolysis induced excision of carbon monoxide to stereoselectively afford the desired cyclohexene.[11] However, to facilitate upscaling an alternative to the use of benzene was sought. Test reactions using tetrahydrofuran (THF), acetonitrile, cyclohexane, and toluene were performed using a 254 nm wavelength photoreactor (See appendix for the used reaction set-up). Interestingly, neither the reaction performed in THF nor cyclohexane afforded the desired cyclohexenes, but the experiment using toluene did provide a cyclohexene in 26% yield, although the formation of a lactone (**17**) side product was significant (42%). The experiment in acetonitrile furnished the desired cyclohexenes with the highest yields: 48% of one cyclohexene and 23% of another stereo isomer were obtained. After hydrogenation of the cyclohexene double bond the relative stereochemistry of the products could be conclusively determined using the Karplus relation and coupling constants of the ring protons.[25,26] Thus, the cyclohexenes were reduced using platinum (IV) oxide and hydrogen gas in yields ranging from 85 – 97%, delivering the all-*cis* ψ-sugar **18** and all-*trans* ψ-sugar **19**, the latter being one of the target compounds.

With ψ-sugar **19** in hand, the synthesis of the ψ-nucleoside was continued (Scheme 2B). To this end, **19** was treated with TBDPS chloride in the presence of imidazole to furnish the silyl ether in 82% yield. Subsequent saponification using lithium hydroxide gave a mono-carboxylate, even when the reaction was allowed to run for a prolonged time. Interestingly, the product proved to be selectively demethylated at the C8' position, furnishing ψ-sugar carboxylate **20** in 92% yield. Increasing the temperature or equivalents of lithium hydroxide did not lead to the formation of the dicarboxylate product but only led to more degradation. Thus, the synthetic route was adapted to furnish the desired C7' position carboxylic acid through protecting the generated C8' carboxylic acid with a *tert*-butyl group, followed by saponification of the remaining methyl ester.[27–29]

To this end, ψ-sugar carboxylic acid **20** was treated with different *tert*-butylating reagents. Initial attempts using a DCC-mediated coupling [30] of *tert*-butanol in the presence of *N*,*N*-dimethylaminopyridine (DMAP) were unsuccessful, affording ester **21** in a mere 2% yield. Treatment of **20** with *tert*-butyl bromide in the presence of a phase-transfer catalyst[31] also did not furnish the desired *tert*-butyl ester. Installation of the *tert*-butyl was then attempted via treatment with freshly generated and distilled *iso*-butene at



Scheme 2 A) Synthesis of ψ-nucleobase **11**. Synthesis adapted from literature.[8] B) Synthetic work towards the ψ-nucleoside. Reagents and conditions. a) Allyl alcohol, BAIB, dichloromethane, RT, o.n., 50%; b) *hv* (λ = 254 nm). For toluene: RT, argon, 6 days, (lactone **17**, 42%) (all-*cis* cyclohexene **23**, 26%). For ACN: RT, argon, 7 days, (all-*cis* cyclohexene **23**, 48%) (all-*trans* cyclohexene **24**, 23%); c) cat. PtO_2, H_2 (g), ethyl acetate, RT. For all-*cis* ψ-sugar **18**: 2 hrs, 97%. For all-*trans* ψ-sugar **19**: o.n., 85%; d) TBDPS-Cl, imidazole, DMF, RT, o.n., 82%; e) lithium hydroxide, water/THF (1/1, v/v), RT, o.n., 92%; f) *O*-*tert*-butyl-*N,N*-di-*iso*-propylimidocarbamate, DCM, RT, o.n., 56% (64% brsm.).

high pressures in the presence of *p*TsOH,[32,33] but no *tert*-butyl ester formation was observed. Finally, treatment of **20** with commercially available *tert*-butyl *N,N,*-di-*iso*-propylimidocarbamate, the species usually formed *in situ* in the reaction of DIC and *tert*-butanol,[34–36] afforded the desired *tert*-butyl ester **21** in 56% yield.

With the *tert*-butyl ester installed, compound **21** was treated with lithium hydroxide, but no conversion to the carboxylate was observed, even upon increasing the reaction time, temperature or the equivalents of lithium hydroxide. Microwave irradiation at 150 ˚C did not afford any product but only etched the inside of the microwave tube, indicating that the lithium hydroxide (1.0 eq.) was not being consumed through saponification of the construct. Also enzymatic generation of carboxylic acid **22** from methyl ester **21** using several esterases and lipases (amano lipase, porcine liver esterase, porcine pancreas lipase)[37–39] proved unsuccessful and only the starting material could be isolated from the reaction mixtures.

Summary and concluding remarks

This Chapter described efforts toward the synthesis of a ψ-nucleoside to be used in the generation of novel ADP-ribose analogues. Inspired by the work of Wazir *et al*., who reported a co-crystal structure of Mac1 with a thiophene-based adenosine mimic, an ADP-ribose analogue based on this thiophene was designed. The cyclohexene scaffold required for the thiophene ψ-nucleoside was generated in a [4+2] cycloaddition between activated methyl vannilate and allyl alcohol, which afforded a molecular cage that upon photofragmentation and hydrogenation furnished the desired cyclohexane. Protecting group manipulations were envisioned to afford a dicarboxylate, but saponification of the dimethylester regioselectively provided a monocarboxylate, which was reprotected with a *tert*-butyl group to allow for installation of the thiophene moiety using a peptide coupling after hydrolysis of the remaining methyl ester.

Although the ψ-nucleoside-based inhibitor remains unfinished, the core cyclohexane motif of such an inhibitor was produced in just three synthetic steps. Future efforts in removal of the methyl ester should afford the carboxylate necessary for ψ-nucleobase installation. Alternatively, a different protective group strategy, for example by exchanging the bulky TBDPS group for an equally orthogonal yet less bulky protective group, could allow for the formation of a dicarboxylate and its corresponding anhydride, which could furnish the ψ-nucleoside through nucleophilic attack of the aminothiophene.

Acknowledgements

Bas van den Berg is kindly acknowledged for his synthetic work. Bob van Puffelen is kindly acknowledged for his contributions to the installation of the *tert*-butyl protective group.

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 (3x) 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 $Ce(NH_4)_4(SO_4)_4$•$2H_2O$, 2.5 grams $(NH_4)_6Mo_7O_{24}$•$4H_2O$, 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 Adventage 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, phenomex) 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. ^{1}H 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 (δ = 0 ppm) or solvent signal of D_2O (δ = 4.79 ppm) for ^{1}H NMR and relative to the solvent signal of $CDCl_3$ (δ = 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.

Methyl 2-amino-4,5,6,7,8,9-hexa-hydro-cyclo-octa[b]thiophene-3-carboxylate (11) To four 20 mL microwave tubes were added (individually) cyclooctenone (0.69 grams, 5.5 mmol), methyl cyanoacetate (0.53 mL, 6.1 mmol, 1.1 eq.), elemental sulphur (S_8) (0.19 grams, 6.1 mmol, 1.1 eq.), pyrrolidine (0.45 mL, 5.5 mmol, 1.0 eq.), DMF (6.5 mL) and a stirring bar. The tubes containing a yellow suspension were then purged using argon gas while stirring before being capped off. The tubes were then transferred to a Biotage® Initiator+ microwave where they were successively queued. The microwave was then programmed to pre-stir the vials for 2 minutes, whereafter heating (very high absorption) to 60˚C for 30 minutes would commence. After 2.5 hours the tubes containing to now amber solution were collected and combined, whereafter the reaction mixture was filtered over a silica gel plug. The silica material was washed with more DMF whereafter the filtrates were combined and concentrated *in vacuo*. The residue was then taken up in ethyl acetate, whereafter celite was added and the resulting suspension was concentrated *in vacuo*. Purification by silica column chromatography (solid loading) (diethyl ether/pentane, 1/99 → 1/3, v/v) afforded compound **11** (4.41 grams, 18.5 mmol, 84%) as a yellow solid. TLC: R_f 0.45 (diethyl ether/pentane, 1/4, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 (s, 2H), 3.81 (s, 3H), 2.85 – 2.77 (m, 2H), 2.64 – 2.56 (m, 2H), 1.68 – 1.50 (m, 3H), 1.50 – 1.43 (m, 2H), 1.37 – 1.23 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5, 161.6, 134.9, 120.3, 106.2, 50.8, 32.2, 29.9, 26.8, 26.6, 25.9, 25.8. HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{12}H_{17}NO_2SH$ 240.1053; Found 240.1053.

(+/-)-methyl (4aR,7S,8aR)-3-methoxy-9-oxo-3,4,4a,7,8,8a-hexahydro-1H-3,7-methanoisochromene-5-carboxylate (16) To a 1.0 L round bottom flask were added bis(acetoxy)iodobenzene (BAIB) (77.3 grams, 240 mmol, 1.20 eq.), allyl alcohol (68 mL, 1.0 mol, 5.0 eq.), dichloromethane (285 mL) and a stirring bar. The flask was then outfitted with a 500 mL dropping funnel and the system was brought under an argon atmosphere, whereafter the solution was stirred vigorously. In another flask, methyl vanillate (36.4 grams, 200 mmol, 1.0 eq.) was dissolved in dichloromethane (285 mL) and the resulting solution was added to the dropping funnel. The dropping funnel was then opened to allow for addition of the methyl vanillate solution over the period of one hour. The resulting reaction mixture was then allowed to stir overnight. The next morning, TLC analysis indicated full consumption of methyl vannilate and the reaction was quenched by the addition of sat. aq. sodium bicarbonate solution. The quenching mixture was stirred until bubbling seized, whereafter it was diluted with more dichloromethane. The layers were separated and the aqueous phase was washed thrice with dichloromethane. The organic phases were then combined and washed once with brine before being dried over Na_2SO_4, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (ethyl acetate/pentane, 1/4 → 1/1, v/v) afforded compound **16** (23.7 grams, 100 mmol, 50%). TLC: R_f 0.5 (ethyl acetate/pentane, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (dd, J = 7.0, 2.2 Hz, 1H), 4.20 (dd, J = 8.1, 3.5 Hz, 1H), 4.04 (dd, J = 4.6, 2.2 Hz, 1H), 3.83 (d, J = 8.2 Hz, 1H), 3.81 (s, 3H), 3.51 (s, 3H), 3.37 (ddd, J = 7.0, 3.2, 2.0 Hz, 1H), 2.60 (dddd, J = 9.8, 4.9, 3.4, 1.7 Hz, 1H), 1.96 (ddd, J = 13.6, 3.3, 1.7 Hz, 1H), 1.86 (ddd, J = 13.6, 9.9, 2.1 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.4, 164.4, 139.5, 133.0, 99.7, 73.6, 52.2, 50.9, 46.2, 41.2, 35.0, 30.4. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{12}H_{14}O_5Na$ 261.0733; Found 261.0734.

(+/-)-(1S,2R,6S)-6-hydroxymethyl-cyclohex-3,4-ene-1,2-dicarboxylic acid dimethyl ester (23) (left) and (+/-)-(1S,2R,6S)-6-hydroxymethyl-cyclohex-3,4-ene-1,2-dicarboxylic acid-γ-lactone methyl ester (17) (right) Compound **16** (0.96 grams, 4.0 mmol) was dissolved in toluene (0.44 L, 0.0092 M) and transferred to a glass chromatography column of appropriate size containing a greased glass valve, set to closed. The column was then stoppered using a rubber septum and the solution was sonicated for 20 minutes while being purged with argon gas. After this, the column was placed inside the photo-reactor and brought under a (very slight) flow of nitrogen gas as to prevent build-up of carbon monoxide gas. At this point the photo-reactor was turned on and the reaction was allowed to stand for six days. After six days, the reactor was turned off and TLC analysis was performed by (careful!) sampling of the reaction mixture which indicated full consumption of the starting material and the solution was purged with argon gas to remove any remaining carbon monoxide. The solution was then concentrated *in vacuo*. Subsequent purification by silica column chromatography (ethyl acetate/pentane, 3/7 → 1/1, v/v) separately afforded all-*cis* cyclohexene **23** (0.24 grams, 1.0 mmol, 26%) and lactone **17** (0.34 grams, 1.7 mmol, 42%). Notably, none of the cyclohexenes produced provided clear HRMS signals.

For **23**: TLC: R_f 0.30 (ethyl acetate/pentane, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.08 – 5.97 (m, 1H), 5.82 (ddt, J = 10.3, 5.1, 2.8 Hz, 1H), 3.72 (s, 3H), 3.69 – 3.62 (m, 5H), 3.51 (dd, J = 5.5, 3.9 Hz, 1H), 3.38 (dp, J = 8.2, 2.6 Hz, 1H), 2.39 (bs, 1H), 2.33 – 2.22 (m, 1H), 2.09 (dtd, J = 20.4, 5.0, 2.4 Hz, 1H), 1.81 (dddt, J = 17.5, 10.9, 3.9, 2.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 172.6, 127.0, 123.1, 64.9, 52.2, 51.8, 43.8, 41.6, 38.5, 25.4.

For **17**: TLC: R_f 0.35 (ethyl acetate/pentane, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.00 (ddt, J = 7.4, 3.2, 1.9 Hz, 1H), 5.91 (dddd, J = 9.4, 6.8, 2.5, 1.7 Hz, 1H), 4.58 (ddd, J = 12.1, 5.5, 1.5 Hz, 1H), 4.26 (dt, J = 12.1, 1.2 Hz, 1H), 3.77 (s, 3H), 3.46 (dtt, J = 6.4, 1.7, 0.8 Hz, 1H), 2.96 (td, J = 2.6, 1.3 Hz, 1H), 2.78 – 2.63 (m, 2H), 2.39 – 2.29 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 168.7, 130.2, 124.1, 74.0, 52.4, 42.1, 39.3, 33.9, 28.1.

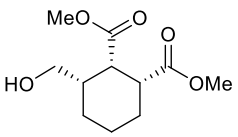
(+/-)-(1S,2R,6S)-6-hydroxymethyl-cyclohex-3,4-ene-1,2-dicarboxylic acid dimethyl ester (23) (left) and (+/-)-(1R,2R,6S)-6-hydroxymethyl-cyclohex-3,4-ene-1,2-dicarboxylic acid dimethyl ester (24) (right) Compound **16** (2.38 grams, 10.0 mmol) was dissolved in acetonitrile (1.0 L, 0.010 M) and transferred to a glass chromatography column of appropriate size. The column was then stoppered off using a rubber septum, whereafter the solution was sonicated for 10 minutes while being purged with argon gas. The column was then placed inside the photo-reactor and its contents were brought under a slight flow of nitrogen gas to prevent the build-up of carbon monoxide gas during the reaction. The photo-reactor was then turned on and the solution was allowed to react for seven days. At this point, TLC analysis via (careful!) sampling of the reaction mixture indicated full consumption of the starting material, and the reactor was turned off. The reaction mixture was then purged with argon gas in order to remove any dissolved carbon monoxide gas, whereafter the solution was concentrated *in vacuo*. Purification by silica column

chromatography (ethyl acetate/pentane, 3/7 → 1/1, v/v) afforded all-*cis* cyclohexene **23** (1.10 grams, 4.84 mmol, 48%) and all-*trans* cyclohexene **24** (0.531 grams, 2.33 mmol, 23%). Notably, none of the cyclohexenes produced provided clear HRMS signals.

For **23**: data as reported.

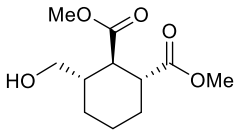
For **24**: TLC: R_f 0.42 (ethyl acetate/pentane, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (ddd, J = 9.3, 4.5, 2.2 Hz, 1H), 5.77 (dtd, J = 10.0, 2.1, 1.2 Hz, 1H), 3.75 (s, 3H), 3.71 (s, 3H), 3.69 – 3.58 (m, 2H), 3.57 – 3.50 (m, 1H), 2.86 (app. t, dd, J = 10.0 Hz, J = 10.0 Hz, 1H), 2.23 – 1.96 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.4, 173.4, 127.9, 122.9, 64.6, 52.4, 52.2, 44.9, 44.0, 38.4, 27.5.

(+/-)-(1S,2R,6S)-6-hydroxymethyl-cyclohexane-1,2-dicarboxylic acid dimethyl ester (18) To



cis-cyclohexene **23** (0.39 grams, 1.7 mmol) was added a catalytic amount of PtO_2 and the resulting mixture was suspended in ethyl acetate (85 mL, 0.020 M). This suspension was then stirred vigorously while being purged with nitrogen gas in order to remove dissolved oxygen, whereafter it was purged with hydrogen gas in order to activate the catalyst. The stirring mixture was then left under a hydrogen atmosphere using a balloon and the reaction mixture was allowed to stir for two hours. At this point, TLC analysis indicated full consumption of the starting material. The ballon was removed and the mixture was purged with nitrogen gas in order to remove dissolved hydrogen. The mixture was then filtered over a celite plug, which was washed with more ethyl acetate. The filtrates were combined and concentrated *in vacuo* which afforded pure compound **18** (0.380 grams, 1.65 mmol, 97%) without any further purification steps. TLC: R_f 0.40 (ethyl acetate/pentane, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (s, 3H), 3.68 (s, 3H), 3.57 (ddd, J = 11.6, 8.0, 5.8 Hz, 1H), 3.46 (ddd, J = 11.6, 9.1, 4.8 Hz, 1H), 3.35 (t, J = 4.6 Hz, 1H), 2.49 (dt, J = 12.4, 4.5 Hz, 1H), 2.27 (dd, J = 8.0, 5.0 Hz, 1H), 2.14 – 1.82 (m, 3H), 1.55 – 1.43 (m, 1H), 1.42 – 1.13 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.4, 173.4, 65.5, 51.9, 51.6, 44.7, 42.4, 42.2, 24.9, 24.2, 23.9. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{11}H_{18}O_5Na$ 253.1046; Found 253.1048.

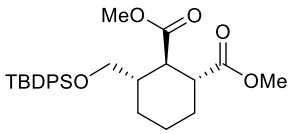
(+/-)-(1R,2R,6S)-6-hydroxymethyl-cyclohexane-1,2-dicarboxylic acid dimethyl ester (19) To



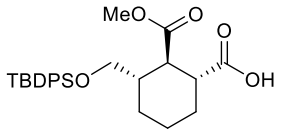
trans-cyclohexene **24** (0.404 grams, 1.77 mmol) was added a catalytic amount of PtO_2 whereafter the mixture was suspended in ethyl acetate (90 mL, 0.020M). The reaction mixture was then stirred vigorously while being purged first with nitrogen gas in order to remove dissolved oxygen and then with hydrogen gas in order to activate the catalyst. The flask was then topped with a hydrogen balloon in order to sustain a hydrogen atmosphere and the reaction mixture was allowed to stir overnight. The next morning, TLC analysis indicated full consumption of the starting material and the reaction mixture was purged with nitrogen gas in order to remove dissolved hydrogen. The Solution was then filtered over a plug of celite, which was subsequently washed with ethyl acetate. The filtrates were combined and concentrated *in vacuo*, whereafter purification by silica column chromatography (ethyl acetate/pentane, 21/29, v/v, isocratic) afforded compound **19** (0.348 grams, 1.51 mmol, 85%). TLC: R_f 0.45 (ethyl acetate/pentane, 1/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 (s, 3H), 3.68 (dd, J = 2.9, 1.7 Hz, 1H), 3.66 (s, 3H), 3.61 – 3.48 (m, 2H), 2.71 (ddd, J = 12.2, 11.2, 3.7 Hz, 1H), 2.51 (app. t, dd, J = 11.3 Hz, 11.3 Hz, 1H), 2.09 (ddt, J = 9.3, 3.8, 2.1 Hz, 1H), 1.94 – 1.76 (m, 3H), 1.75 – 1.62 (m,

1H), 1.48 – 1.32 (m, 2H), 1.32 – 1.17 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.5, 175.0, 65.9, 52.0, 51.9, 47.7, 45.6, 42.3, 28.7, 28.4, 24.8. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{11}H_{18}O_5Na$ 253.1046; Found 253.1047.

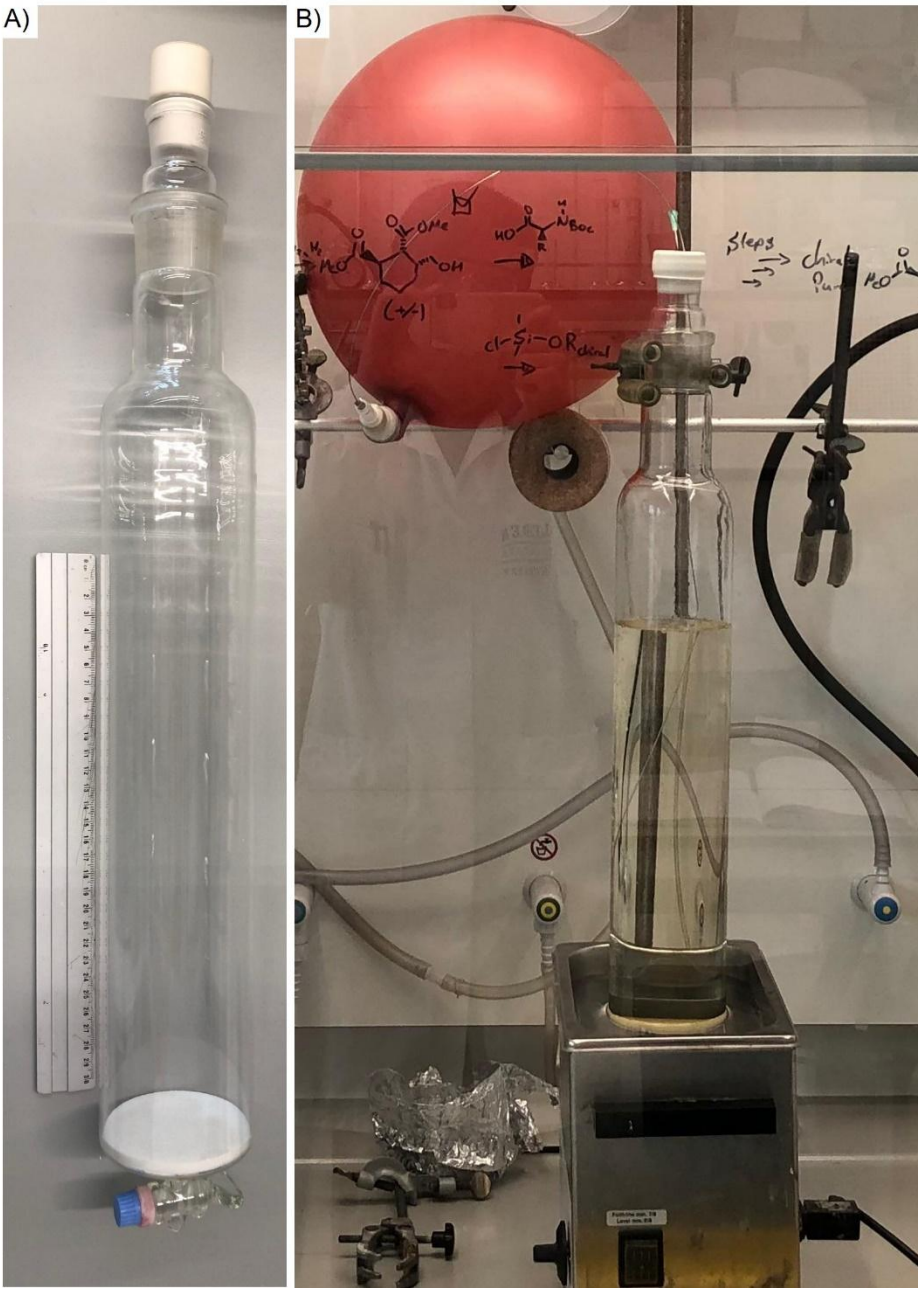
(+/-)-(1R,2R,6S)-6-hydroxymethyl-9-*O*-(*tert*-butyl-di-phenylsilyl)-cyclohexane-1,2-dicarboxylic acid dimethyl ester (25) To a solution of compound **19** (0.329 grams, 1.43 mmol) in dry DMF (15 mL, 0.095 M) was added imidazole (195 mg, 2.86 mmol, 2.00 eq.) whereafter the mixture was cooled to 0 ˚C using an icebath. To the cooled mixture was then added TBDPS chloride (0.56 mL, 2.1 mmol, 1.5 eq.) and the resulting reaction mixture was allowed to warm up to room temperature while stirring overnight. The next morning, TLC analysis indicated full consumption of the starting material and the reaction was quenched via addition of a mixture of water and brine. The mixture was then diluted using diethyl ether, the layers separated and the aqueous phase was washed with diethyl ether for an additional three times. The organic phases were combined and dried over Na_2SO_4, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (diethyl ether/pentane, 9/91, v/v, isocratic) afforded compound **25** (0.549 grams, 1.17 mmol, 82%) TLC: R_f 0.42 (diethyl ether/pentane, 3/17, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.58 (m, 4H), 7.46 – 7.32 (m, 6H), 3.65 (s, 3H), 3.56 – 3.38 (m, 5H), 2.68 (ddd, J = 11.8, 9.3, 3.6 Hz, 1H), 2.56 (app. t, dd, J = 11.3 Hz, 11.3 Hz, 1H), 2.12 – 2.04 (m, 1H), 1.98 – 1.83 (m, 2H), 1.75 (tdd, J = 11.3, 5.5, 3.4 Hz, 1H), 1.48 – 1.21 (m, 3H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.4, 175.0, 135.7, 135.7, 133.6, 133.6, 129.7, 129.6, 127.7, 66.3, 51.9, 51.5, 47.5, 46.0, 42.2, 28.6, 28.5, 26.9, 24.7, 19.4. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{27}H_{36}O_5SiNa$ 491.2224; Found 491.2231.



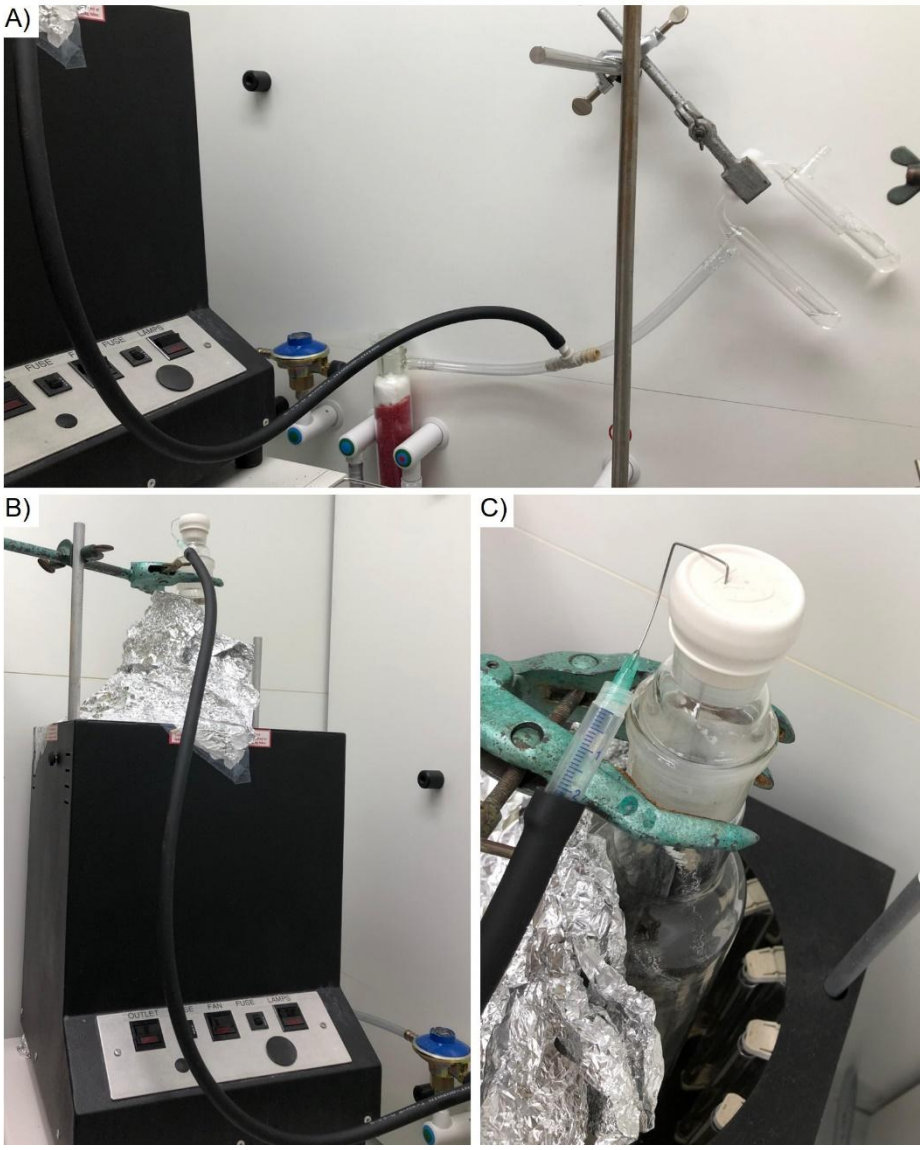
(+/-)-(1R,2R,6S)-6-hydroxymethyl-9-*O*-(*tert*-butyl-di-phenylsilyl)-cyclohexane-1,2-dicarboxylic acid 1-methyl ester (20) Compound **25** (0.472 grams, 1.01 mmol) was dissolved in a solvent system consisting of water and THF (1/1, v/v) (60 mL, 0.017 M), whereafter to it was added lithium hydroxide (anhydrous) (24 mg, 1.0 mmol, 1.0 eq.). The resulting mixture was then stirred vigorously at room temperature and under a nitrogen atmosphere overnight. The next morning TLC analysis indicated full consumption of the starting material and the solution was acidified using aq. hydrochloric acid (1.0 M) until a pH of approximately 2 had been reached. The solution was then washed thrice with dichloromethane and all the organic phases were combined, dried over Na_2SO_4, filtered off and concentrated *in vacuo*. Purification by silica column chromatography (ethyl acetate/pentane/acetic acid, 10/87/3 → 30/69/1, v/v/v) afforded compound **20** (0.416 grams, 0.915 mmol, 92%). TLC: R_f 0.25 (ethyl acetate/pentane, 1/4, v/v, + 3 drops acetic acid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.58 (m, 4H), 7.46 – 7.33 (m, 6H), 3.57 – 3.50 (m, 4H), 3.47 (dd, J = 10.3, 5.5 Hz, 1H), 2.72 (td, J = 11.8, 3.6 Hz, 1H), 2.55 (app. t, dd, J = 11.3 Hz, 11.3 Hz, 1H), 2.22 – 2.04 (m, 1H), 2.02 – 1.83 (m, 2H), 1.81 – 1.64 (m, 1H), 1.49 – 1.16 (m, 3H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.4, 175.3, 135.7, 135.7, 133.6, 133.6, 129.7, 129.7, 127.7, 66.2, 51.6, 47.2, 45.7, 42.2, 28.6, 28.6, 26.9, 24.8, 19.4. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{26}H_{34}O_5SiNa$ 477.2068; Found 477.2074.



(+/-)-(1R,2R,6S)-6-hydroxymethyl-9-*O*-(*tert*-butyl-di-phenylsilyl)-cyclohexane-1,2-dicarboxylic acid 1-methyl-2-(*tert*-butyl) ester (21) To a solution of compound **20** (0.411 grams, 0.906 mmol) in dry dichloromethane (25 mL, 0.036 M) under an argon atmosphere was added *tert*-butyl *N*,*N*,-di-*iso*-propylimidocarbamate (0.71 mL, 3.2 mmol, 3.5 eq.) and the resulting reaction mixture was allowed to stir at room temperature overnight. The next morning, TLC analysis indicated that some starting material remained and to the mixture was added another 5.0 equivalents of *tert*-butylating reagent (totalling 8.5 eq.) and the reaction mixture was stirred for an additional 72 hours. At this point, TLC analysis of the now white suspension indicated no more starting material remained and the mixture was filtered off to remove all solids. The filtrate was then concentrated *in vacuo*. Purification by silica column chromatography (diethyl ether/pentane, 1/19, v/v, isocratic) afforded compound **21** (0.257 grams, 0.504 mmol, 56%, (64% brsm.)). TLC: R_f 0.45 (diethyl ether/pentane, 1/9, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.57 (m, 4H), 7.46 – 7.32 (m, 6H), 3.57 – 3.50 (m, 4H), 3.45 (dd, J = 10.3, 5.6 Hz, 1H), 2.60 – 2.46 (m, 2H), 2.10 – 1.98 (m, 1H), 1.98 – 1.80 (m, 2H), 1.80 – 1.66 (m, 1H), 1.47 – 1.18 (m, 11H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.4, 173.8, 135.8, 135.7, 133.8, 133.7, 129.7, 129.7, 127.7, 80.6, 66.5, 51.4, 47.7, 47.2, 42.3, 28.9, 28.7, 28.1, 27.0, 24.9, 19.4. HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{30}H_{42}O_5SiNa$ 533.2694; Found 533.2700.



Supplementary Figure 1 Large scale synthesis of cyclohexenes **23** and **24,** part one. A) Size of the column used. B) Purging of the starting material in acetonitrile was performed inside a fume hood using a sonicator. To allow for ample time of purging, an extra-large balloon was used.



Supplementary Figure 2 Large scale synthesis of cyclohexenes **23** and **24,** part two. A) Setup to generate a slight overflow of dried nitrogen gas. B) Column placed inside the reactor. Nitrogen flow is attached. Aluminium is added to reflect UV backwards into the fume hood. C) View inside the reactor while off.

Supplementary Figure 3 Distillation of *iso*-butene and the *tert*-Butylation experiment. A) Setup to generate *iso*-butene. To a flask containing *tert*-Butanol (50 mL) and Ac_2O (100 mL) is added a catalytic amount of sulfuric acid (98%, w/w). A condenser is attached, the output of which is first led through a cold-trap at 0 ˚C before being lead to a second cold-trap at -78˚C. The system is then purged with argon gas whereafter the mixture is carefully heated to 73˚C at which point bubbling can be observed. The first cold-trap will collect side products (acetic acid, di-*tert*-butyl ether), while the second cold-trap will condense pure *iso*-butene. B) The *tert*- Butylation experiment. To a solution of carboxylic acid in dry dioxane (25 mL) is added *p*TsOH monohydrate. Liquid *iso*-butene is added (25 mL) and the flask is capped. Upon completion, the flask is first cooled before opening.

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