Synthetic Study on ADP-ribosylation

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8 Summary and future prospects

This Thesis focuses on the design and synthesis of ADP-ribosylated compounds that can be applied in biological studies. **Chapter 1** reviews the majority of the synthetic work in the field of ADP-ribosylation which includes methods toward ADP-ribosylated amino acids, ADP-ribosylated peptides/proteins, linear and branched ADPr oligomers. The target molecules of the following experimental chapters are outlined in Figure 1.

Synthetic mono-ADP-ribosylated oligopeptides are useful tools to investigate biological ADP-ribosylation events at a molecular level. Although several ADP-ribosylated oligopeptides have been synthesized previously,[1-3] synthetic mono-ADP-ribosylated amino acids are relatively scarce. **Chapter 6** deals with the synthesis of α-configured Asn-ADPr **1**, a more stable bioisostere of ADPr-Asp and the structure elucidation of the co-crystals of Asn-ADPr **1** with a macrodomain of a bacterial protein. The presented synthesis of **1**, using suitably protected ribose donors and P(V)-P(III) chemistry for the introduction of the pyrophosphate can be transferred to other mono-ADP-ribosylated amino acids. A general procedure to prepare analogs of mono-ADPr peptides and even a protein (**2**) is the subject of **Chapter 7**. A newly designed propargyl-ADPr building block reacted efficiently via CuAAC reaction with an oligopeptide having either an azidoalanine or an azidohomoalanine residue, to afford several triazole linked mono-ADPr peptides. In a similar way, triazole linked ubiquitin was generated, which is the first reported artificial ADPr-protein. Importantly, the synthetic ADPr-Ub possessed similar bioactivity as the native counterpart in the auto-ubiquitination assay, suggesting a potential application of triazole linked mono-ADPr-peptides/proteins in biological studies.

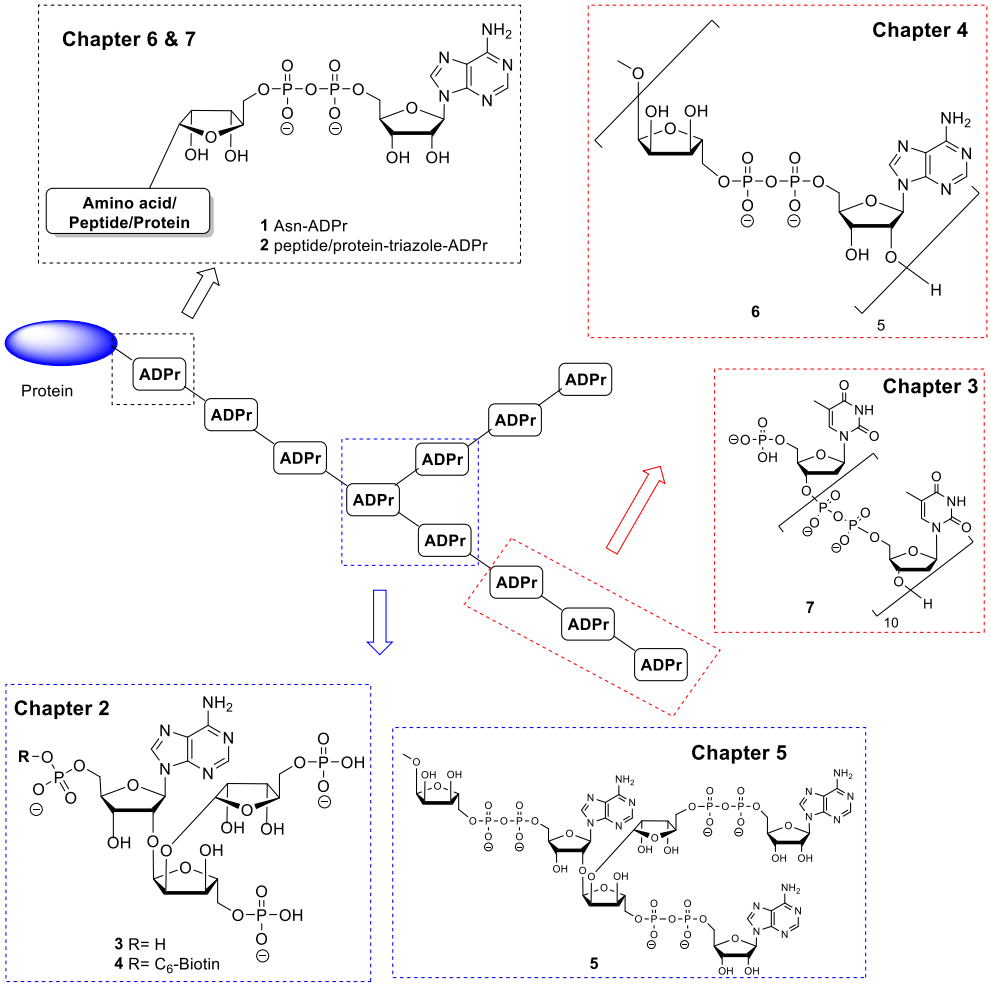


Figure 1. Target ADPr molecules of this thesis

Although it was discovered 40 years ago, branched poly-ADPr (PAR) has always been an enigma in the ADPr biology.[4, 5] This Thesis describes the synthesis of various branched fragments of PAR able for application in biological studies to investigate the function of the branch points in the structure of PAR. **Chapter 2** deals with the synthesis of branched fragment **3** (Figure 1) with two sequential 1,2-cis glycosylations, Vorbrüggen coupling to install the adenine and phosphorylation of three primary alcohols as key transformations. ^{1}H-NMR comparison of the obtained synthetic compound with its enzymatically obtained natural counterpart showed identical structure, indicating that the regio- and stereochemistry of branch point of PAR was correctly elucidated by Miwa.[4] Furthermore, this synthetic methodology was also utilized for the synthesis of biotinylated linear or branched PAR fragments like **4**, as valuable tools for future discovery of proteins that bind to branched PAR. An even more ambitious synthetic target, branched ADPr trimer **5**, containing three full ADPr units (Figure 1), is described in **Chapter 5**. The introduction of the three pyrophosphate linkages with P(V)-P(III) chemistry, as described in Chapter 2, is adapted for solid-phase synthesis. A highly advanced branched

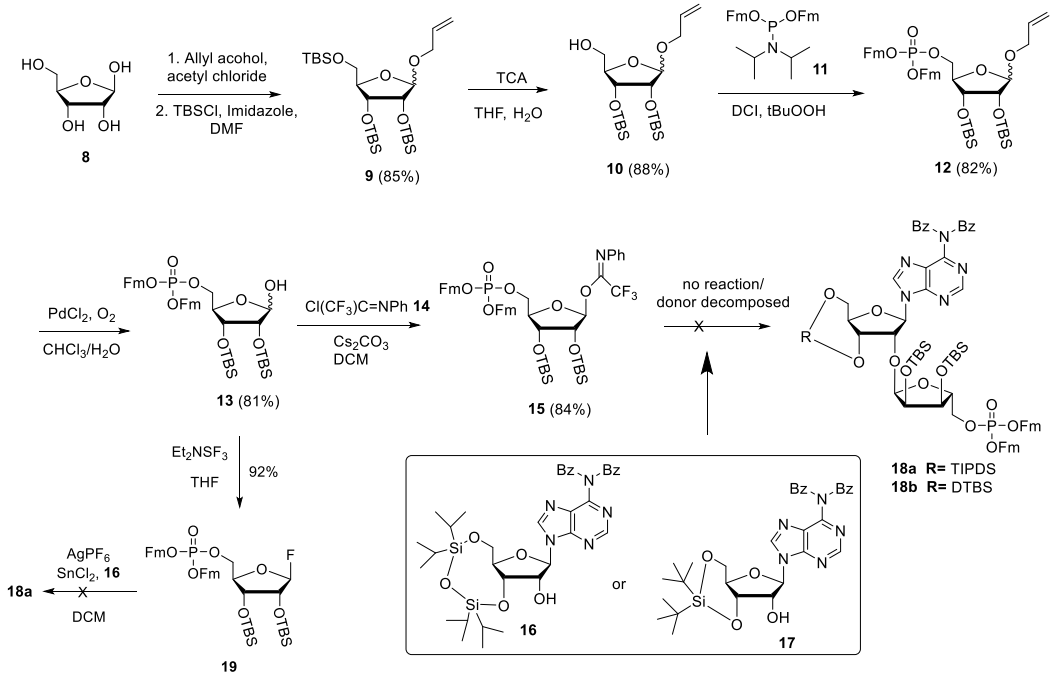
phosphoramidite with two phosphotriesters was synthesized in 14 steps and applied for the introduction of the first pyrophosphate. The two remaining pyrophosphates were then simultaneously installed with the aid of an adenosine phosphoramidite. The binding of branched compounds **4** and **5** with "reader" proteins of interest[6] is presently under investigation. The modular synthesis of **5** is a valuable asset for future synthesis of other branched ADPr.

Poly-ADPr plays a pivotal role in various physiological and pathological processes such as DNA repair, apoptosis, inflammation and neurodegeneration disease.[7-10] Synthetic ADPr oligomers would facilitate a better understanding of the involved processes at a molecular level and eventually contribute to the discovery of new drug targets.[11, 12] The challenges in the synthesis of ADPr oligomers are many. The difficulties begin with the preparation of advanced building blocks in sufficient quantities with the stereoselective ribosylation and regioselective adenine introduction as key steps. On top of this comes the notoriously difficult construction of multiple pyrophosphate linkages that are intrinsic labile and the anionic properties of the intermediates and target ADPr oligomers that restrict its assembly on the solid phase and hamper the monitoring of the involved reactions. The synthetic procedure to the pyrophosphate linkages, that is used throughout the research described in this thesis, is performed with P(V)-P(III) chemistry. **Chapter 3** describes the solid-phase synthesis of 3',5'-pyrophosphate-linked thymidine oligomers, using a new phosphoramidite thymidine building block with an Fm protected 5'-phosphotriester. The successful construction of 3',5'-pyrophosphate-linked thymidine decamer was an incentive to expand the Fm-based chemistry towards the preparation of ADPr oligomers, described in **Chapter 4**, which describes the first synthesis of ADPr pentamer.

Toward an alternative synthesis of the phosphoramidite building block for the assembly of ADPr oligomers

The solid phase assembly of linear ADPr oligomers, such as described in Chapter 4, requires the repeated use of a building block, the synthesis of which is rather lengthy and time-consuming.[13] The number of steps toward this building block can potentially be reduced by changing the applied protecting groups. The use of benzyl groups enables selective 1,2-cis ribosylation but also requires intermediate removal by hydrogenolysis which proved to be troublesome and tedious on a number of occasions. Besides, the introduction of adenine moiety in a late stage by Vorbrüggen-type reaction also increases the number of protective group manipulations.[13, 14] To circumvent these issues an alternative route of synthesis was explored with the condensation of *N*-phenyl-trifluoroacetimidate donor **15** and adenosine acceptor **16** or **17** as a key step (Scheme 1). In this way the route of synthesis could be shortened by avoiding both the use of benzyl groups and the Vorbrüggen coupling. The synthesis of donor **15** started with Fischer glycosylation of ribose **8** with allyl alcohol, followed by

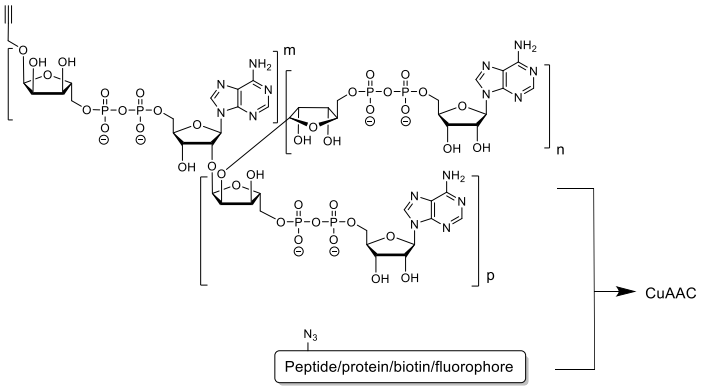
silylation of the remaining hydroxyls to obtain **9** in good yield. TBS group on the primary OH in **9** was selectively removed with TCA in THF/H_2O,[12] allowing for the phosphitylation of **10** and subsequent oxidation to give phosphotriester **12**. $PdCl_2$ catalyzed deallylation (see Chapter 5) yielded hemiacetal **13** which could be used for the synthesis of various donors. The introduction of 2,2,2-trifluoro-*N*-phenylacetimidoyl group with Cs_2CO_3 in acetone failed due to the concomitant cleavage of Fm groups.[15, 16] Fortunately the use of DCM to suppress the solubility of Cs_2CO_3, furnished *N*-phenyl-trifluoroacetimidate donor **15** in good yield. The attempted couplings of **15** with adenosine acceptors **16** and **17** to get desired α-ribosylated adenosine **18,** using activators, like TMSOTf, DTBS(OTf)$_2$, TfOH and Tf_2NH, have failed. The low reactivity of these adenosine acceptors could hamper glycosylation, as the glycosylation between **15** and other acceptors was successful determined by TLCMS.



Scheme 1. Toward an alternative synthetic route to key linear phosphoramidite

Inspired by the report of Lambrecht *et al*[12] who coupled a ribosyl fluoride with an adenosine acceptor under activation of $AgPF_6/SnCl_2$, donor **19** was prepared in good yield from alcohol **13** using Et_2NSF_3 in THF (Scheme 1). However, the subsequent coupling of **16** with **19** using the same procedure was unsuccessful. The difficulties with the direct ribosylation of adenosine acceptor described above testify for the low reactivity of 2'-OH of adenosine in glycosylation reactions. Other methods like gold-catalyzed glycosylation using *O*-alkynylbenzoates as donors should be attempted in the future.[17] Once the glycosylation is successful, **18a**/**b** could be easily converted into the phosphoramidite building block for the assembly of ADPr oligomers.[13]

Conjugation of oligo-ADPr with peptides, proteins and molecular probes



Scheme 2. Proposed CuAAC click chemistry of ADPr oligomer (branched or linear) with biomolecules and molecular probes relevant for research in biology

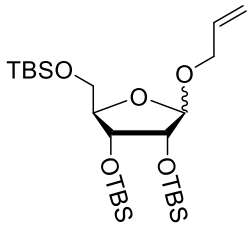
Using the methods described in **Chapter 4** and **Chapter 5**, more complicated synthetic targets like branched (m, n, p ≥ 1) or linear ADPr oligomers (n = 0, m, p ≥ 2) with a propargyl at the anomeric center could be envisaged (Scheme 2). These precious oligomers could be further conjugated via CuAAC chemistry with either natural substrates (like peptides or proteins) or tags (such as biotin or fluorophorescent labels), containing an azide functionality (see **Chapter 7**). With these ADPr conjugates in hand, many biological experiments are possible, such as the use of biotinylated branched ADPr oligomers for searching corresponding binding proteins[6] and the use of a series of linear ADPr oligomers, varying in length, to study the binding of these oligomers with "reader" and "eraser" proteins.[18-20]

Acknowledgement

Liming Wang, Zhen Wang, Dr. Sizhe Li and Yongzhen Zhang are kindly acknowledged for the nice discussion on glycosylation reactions in this chapter.

Experimental section

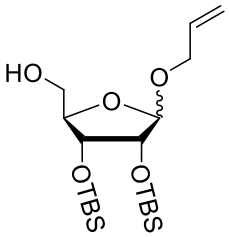
The general procedure is the same as described in Chapter 2.



1-*O*-Allyl-2,3,5-tri-*O*-tert-butyldimethylsilyl-α,β-D-ribofuranoside (9)

D-Ribose **8** (3 g, 20 mmol), allyl alcohol (50 mL) and acetyl chloride (1 mL, 14 mmol) were added into the flask and stirred at room temperature for 2 hours before it was quenched upon addition of pyridine (2.5 mL). The mixture was concentrated, co-evaporated with toluene (2 x). To the residue, DMF (30 mL), imidazole (6.8 g, 100 mmol) and TBSCl (34.65 mL, 100 mmol) was added and stirred at room temperature for 16 hours after which the reaction was quenched by addition of H_2O (50 mL) and Et_2O (50 mL). The organic layer was

separated, washed with additional H_2O (2 x) and brine (1 x), dried ($MgSO_4$), filtered, concentrated and purified by silica gel chromatography (pentane/EtOAc, 100/0 – 100/2) to afford **9** as a colorless oil (9.01 g, 16.93 mmol, 85%). ^{1}H NMR (500 MHz, chloroform-*d*) δ 5.97 – 5.81 (m, 1H, *CH*=CH_2), 5.37 – 5.07 (m, 2H, CH=*CH_2*), 4.92 (d, *J* = 4.1 Hz, 0.25H, H1-α), 4.82 (d, *J* = 1.6 Hz, 0.75H, H1-β), 4.32 – 3.88 (m, 5H, H3, H2, O*CH_2*CH=CH_2, H4), 3.82 – 3.58 (m, 2H, H5), 0.96 – 0.82 (m, 27H, TBS), 0.14 – 0.00 (m, 18H, TBS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 135.12, 134.56 (*CH*=CH_2), 117.19, 116.14 (CH=*CH_2*), 106.31, 101.99 (C1), 85.12, 83.22 (C4), 76.69, 74.10 (C2), 71.66, 71.54 (C3), 68.77, 68.56 (O*CH_2*CH=CH_2), 63.29, 63.02 (C5), 26.17, 26.15, 26.06, 26.04, 25.97 (CH_3, TBS), 18.64, 18.61, 18.49, 18.34, 18.27, 18.22 (Cq. TBS), -4.12, -4.21, -4.30, -4.34, -4.44, -4.47, -4.72, -4.77, -5.09, -5.19, -5.26, -5.34 ($SiCH_3$, TBS). IR (film): 2953, 2929, 2896, 2857, 1472, 1463, 1252, 1129, 1004, 835, 775 cm^{-1}. HRMS (ESI^+) calcd for $C_{26}H_{56}O_5Si_3Na$ (M+Na) 555.3328. Found 555.3327. $[\alpha]_D^{20}$ +9.2 (c = 1, in DCM)



1-*O*-Allyl-2,3-di-*O*-tert-butyldimethylsilyl-α,β-D-ribofuranoside (10)

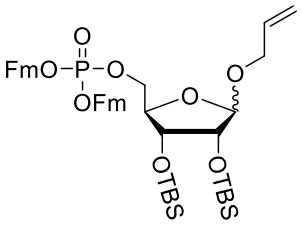
TCA solution (12.8 g, 79 mmol, in 6 mL H_2O) was added dropwise to a flask containing **9** (909 mg, 1.71 mmol) and THF (24 mL) at 0°C. After complete addition, the reaction was stirred at 0°C for 2 hours after which it was quenched by aq. $NaHCO_3$ (sat.). DCM extracted this mixture (3 x) and the organic layers are combined, dried ($MgSO_4$), filtered, concentrated, and purified by silica gel chromatography (pentane/EtOAc, 100/0 – 90/10) to afford **10** (α+β) as a colorless oil. (α anomer: 510 mg, 1.23 mmol, 72%; β anomer: 117 mg, 0.28 mmol, 16%)

α anomer:

^{1}H NMR (500 MHz, chloroform-*d*) δ 5.90 (dddd, *J* = 17.2, 10.6, 5.9, 4.9 Hz, 1H, *CH*=CH_2), 5.31 (dq, *J* = 17.2, 1.8 Hz, 1H, CH=*CH_2*), 5.13 (dq, *J* = 10.4, 1.6 Hz, 1H, CH=*CH_2*), 4.96 (d, *J* = 3.8 Hz, 1H, H1), 4.27 (ddt, *J* = 13.0, 4.9, 1.6 Hz, 1H, O*CH_2*CH=CH_2), 4.15 – 3.91 (m, 4H, O*CH_2*CH=CH_2, H4, H3, H2), 3.80 (AB, *J* = 12.0 Hz, 1H, H5), 3.58 (AB, *J* = 11.6, 3.3 Hz, 1H, H5), 1.89 (s, 1H, OH), 0.90 (d, *J* = 10.6 Hz, 18H, TBS), 0.11 – 0.02 (m, 12H, TBS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.71 (*CH*=CH_2), 116.66 (CH=*CH_2*), 102.10 (C1), 83.78 (C4), 74.05 (C2), 71.19 (C3), 68.97 (O*CH_2*CH=CH_2), 62.04 (C5), 26.12, 26.02 (CH_3, TBS), 18.60, 18.28 (Cq. TBS), -4.11, -4.19, -4.45, -4.80 ($SiCH_3$, TBS). HRMS (ESI^+) calcd for $C_{20}H_{42}O_5Si_2Na$ (M+Na) 441.2463. Found 441.2469.

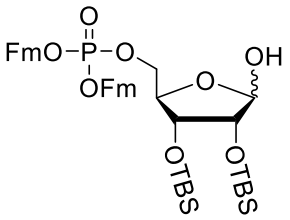
β anomer:

^{1}H NMR (500 MHz, chloroform-*d*) δ 5.90 (dddd, *J* = 17.2, 10.4, 6.1, 5.2 Hz, 1H, *CH*=CH_2), 5.34 – 5.17 (m, 2H, CH=*CH_2*), 4.81 (s, 1H, H1), 4.27 (dd, *J* = 7.5, 4.1 Hz, 1H, H3), 4.20 (AB, *J* = 13.0, 5.3, 1.5 Hz, 1H, O*CH_2*CH=CH_2), 4.09 – 3.98 (m, 2H, O*CH_2*CH=CH_2, H4), 3.94 (d, *J* = 4.1 Hz, 1H, H2), 3.82 (AB, *J* = 12.1, 2.5 Hz, 1H, H5), 3.56 (AB, *J* = 12.0 Hz, 1H, H5), 1.87 (s, 1H, OH), 0.94 – 0.83 (m, 18H, TBS), 0.12 – 0.06 (m, 12H, TBS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.14 (*CH*=CH_2), 117.86 (CH=*CH_2*), 106.77 (C1), 82.79 (C4), 76.90 (C2), 70.95 (C3), 69.12 (O*CH_2*CH=CH_2), 61.61 (C5), 26.00, 25.92 (CH_3, TBS), 18.25, 18.21 (Cq. TBS), -4.12, -4.42, -4.46, -4.90 ($SiCH_3$, TBS). IR (film): 2929, 2858, 1472, 1253, 1161, 834, 774cm^{-1}. HRMS (ESI^+) calcd for $C_{20}H_{42}O_5Si_2Na$ (M+Na) 441.2463. Found 441.2465. $[\alpha]_D^{20}$ -2.1 (c = 1, in DCM)



1-*O*-Allyl-2,3-di-*O*-tert-butyldimethylsilyl-5-O-(di-O-flourenylmethyl)-phosphoryl-α,β-D-ribofuranoside (12)

Compound **10** (4.72 g, 11.29 mmol), DCI activator (4,5-dicyanoimidazole solution 0.25 M in ACN, 90 mL, 22.58 mmol) and freshly activated 3Å molecular sieves were added in to the flask. 8 mL DMF was added into the flask and then **11** (0.4 M in ACN, 31 mL, 12.42 mmol) were added. The reaction was stirred for 10 minutes at r.t. after which *t*BuOOH (5.5 M in decane, 16.42 mL, 90.32 mmol) was added at 0°C. The reaction was stirred at same temperature for 30 minutes and quenched by aq. $NaHCO_3$ (sat.). The mixture was filtered and EtOAc was added to the filtration. The mixture was washed by H_2O (1 x) and brine (2 x) and the organic layer was dried (Na_2SO_4), filtered, concentrated, and purified by silica gel column chromatography (pentane/acetone, 100/5 – 100/15) to obtain **12** (α+β mixture) as a colorless oil (7.82 g, 9.25 mmol, 82%). ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.78 – 7.67 (m, 4H), 7.60 – 7.50 (m, 4H), 7.44 – 7.31 (m, 4H), 7.31 – 7.21 (m, 4H, Ar), 5.76 (dddd, *J* = 17.0, 10.4, 6.3, 5.0 Hz, 1H, *CH*=CH_2), 5.23 – 5.00 (m, 2H, CH=*CH_2*), 4.81 – 4.69 (m, 1H, H1), 4.35 – 3.73 (m, 13H, H2, H3, H4, H5, CH-Fm, CH2-Fm, O*CH_2*CH=CH_2), 0.94 – 0.79 (m, 18H, TBS), 0.11 – -0.04 (m, 12H, TBS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.30, 143.25, 141.48, 141.46 (Cq, Ar), 134.57, 134.15, 128.05, 128.02, 127.97, 127.24, 125.40, 125.37, 125.33, 125.31, 125.27, 120.17, 120.13, 120.12, 120.09, 120.08 (Ar), 117.49 (β, CH=*CH_2*), 116.51 (α, CH=*CH_2*), 106.08 (H1- β), 101.93 (H1-α), 81.57 (H4-α), 80.39 (H4-β), 76.38 (H2-β), 73.59 (H2-α), 71.80 (H3-β), 71.13 (H3-α), 69.51, 69.49, 69.47, 69.44 (CH_2-Fm), 68.98 (O*CH_2*CH=CH_2, α), 68.25 (O*CH_2*CH=CH_2, β), 67.75, 67.71 (C5-β), 66.87, 66.82 (C5-α), 48.10, 48.03 (CH-Fm), 26.09, 25.99 (TBS, α), 25.96, 25.90 (TBS, β), 18.20, 18.10 (Cq. TBS), -4.09, -4.19, -4.37, -4.49, -4.82, -4.90 ($SiCH_3$, αβ). ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.85 (β), -0.88 (α). IR (film): 2928, 2893, 2856, 1450, 1254, 1127, 1076, 1014, 991, 837, 776, 756 cm^{-1}. HRMS (ESI$^+$) calcd for $C_{48}H_{63}O_8PSi_2Na$ (M+Na) 877.3691. Found 877.3695. $[\alpha]_D^{20}$ +10.3 (c = 1, in DCM)

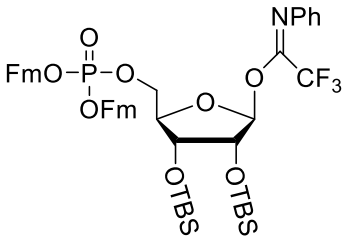


2,3-di-*O*-Tert-butyldimethylsilyl-5-O-(di-O-flourenylmethyl)-phosphoryl-α,β-D-ribofuranoside (13)

Compound **12** (440 mg, 0.51 mmol), $CHCl_3/H_2O$ (v/v, 2.1 mL/1.4 mL) and $PdCl_2$ (18 mg, 0.10 mmol) were added into a flask and the mixture was stirred vigorously under O_2 at 45°C for 48 hours after which it was quenched by $NaHCO_3$ (aq. sat.). DCM extracted the mixture (2 x) and the organic layers are combined, dried ($MgSO_4$), filtered, concentrated. Purification by silica gel column chromatography (pentane/acetone, 100/5 – 100/10) afforded **13** (α+β mixture) as a white foam (341 mg, 0.42 mmol, 82%).

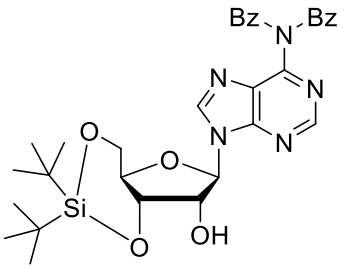
^{1}H NMR (500 MHz, chloroform-*d*) δ 7.79 – 7.64 (m, 4H, Ar), 7.62 – 7.46 (m, 4H, Ar), 7.45 – 7.21 (m, 8H, Ar), 5.16 – 5.08 (m, 0.35H, H1-β), 5.03 (dd, *J* = 11.5, 4.1 Hz, 0.65H, H1-α), 4.63 (d, *J* = 4.4 Hz, 0.35H, OH-β), 4.51 – 4.32 (m, 0.7H, CH_2-Fm-β, H3-β), 4.32 – 4.06 (m, 7H, CH2-Fm-αβ, CH-Fm-αβ, H4-α, OH-α, H5-β), 4.06 – 3.97 (m, 1.35H, H4-β, H3-α, H5-β), 3.95 – 3.90 (m, 1H, H2), 3.87 (dd, *J* = 5.9, 4.4 Hz, 1.3H, H5-α), 0.96 – 0.81 (m, 18H, CH_3, TBS), 0.15 – 0.00 (m, 12H, $SiCH_3$, TBS). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.08, 143.05, 141.46, 141.44 (Cq. Ar), 128.00, 127.95, 127.94, 127.91, 127.22, 127.18, 127.16, 125.27, 125.17, 125.14, 125.13, 125.11, 120.14, 120.09, 120.07, 120.05, 120.02 (Ar), 102.71 (C1-β), 97.70 (C1-α), 82.73, 82.66 (C4-α), 80.00, 79.97 (C4-αβ), 77.25 (C2-β), 73.52 (C3-α),

72.49 (C2-α), 70.60, 70.02 (C3-β), 69.97, 69.56, 69.51, 69.47, 69.44, 69.43, 69.39 (CH_2-Fm), 67.37, 67.32 (C5-β), 66.79, 66.74 (C5-α), 48.09, 48.03, 48.00, 47.99, 47.94, 47.92, 47.89, 47.83 (CH-Fm), 25.93, 25.89, 25.81 (CH_3, TBS), 18.34, 18.18, 18.09, 18.04 (Cq. TBS), -4.17, -4.38, -4.52, -4.57, -4.61, -4.68, -4.96, -5.03 ($SiCH_3$, TBS). ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.85 (β), -0.91 (α). IR (film): 2952, 2929, 2893, 1471, 1450, 1253, 1077, 1014, 837, 740, 515 cm^{-1}. HRMS (ESI^+) calcd for $C_{45}H_{59}O_8PSi_2Na$ (M+Na) 837.3378. Found 837.3381. $[\alpha]_D^{20}$ +24.1 (c = 1, in DCM)



1-*O*-((*N*-Phenyl)-2,2,2-trifluoroacetimido)-2,3-di-*O*-tert-butyldimethylsilyl-5-*O*-(di-*O*-flourenylmethyl)-phosphoryl-α,β-D-ribofuranoside (15)

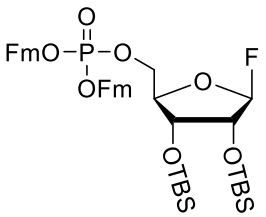
Compound **13** (1.43 g, 1.76 mmol), Cs_2CO_3 (858 mg, 2.63 mmol), DCM (20 mL) and $Cl(CF_3)C$=NPh **14** (0.37 mL, 2.28 mmol) were added into a flask and the mixture was stirred under N_2 for 16 hours. TLC (pentane/acetone=9/1) showed incomplete conversion and more **14** (0.37 mL, 2.28 mmol) was added. The reaction was stirred for 24 hour after which it was filtered, extracted with aq. $NaHCO_3$ (sat. 2 x) and brine (1 x). The organic layer is dried ($MgSO_4$), filtered and concentrated. Purification by silica gel (neutral spherical silica gel is used) column chromatography (pentane/acetone, 98/2 – 90/10) to obtain **15** (α+β mixture) as a colorless oil (1.46 g, 1.48 mmol, 84%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.83 – 7.68 (m, 4H), 7.64 – 7.51 (m, 4H), 7.47 – 7.34 (m, 4H), 7.33 – 7.24 (m, 6H), 7.16 – 7.08 (m, 1H), 6.81 (d, *J* = 7.7 Hz, 2H, Ar), 6.11 (s, 1H, H1), 4.41 – 4.02 (m, 11H, H2, H3, H4, H5, Fm), 0.93 (s, 18H, CH_3, TBS), 0.26 – 0.03 (m, 12H, $SiCH_3$, TBS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.65, 143.15, 143.13, 141.39, 141.36 (Cq. Ar), 128.76, 127.87, 127.85, 127.14, 127.11, 125.27, 125.22, 125.17, 124.24, 120.01, 119.99, 119.97, 119.95, 119.50 (Ar), 103.64 (C1), 81.67 (C4), 81.58 (C2), 75.51 (C3), 70.95, 69.46, 69.40, 69.33 (CH_2-Fm), 66.51, 66.46 (C5), 47.98, 47.96, 47.90, 47.89 (CH-Fm), 25.83, 25.72 (CH_3, TBS), 18.06, 18.03 (Cq. TBS), -4.23, -4.51, -4.75, -5.03 ($SiCH_3$, TBS). IR (film): 2930, 2857, 1717, 1599, 1450, 1259, 1208, 1160, 1014, 939, 867, 741 cm^{-1}. HRMS (ESI^+) calcd for $C_{53}H_{63}F_3NO_8PSi_2Na$ (M+Na) 1008.3674. Found 1008.3668. $[\alpha]_D^{20}$ +21.8 (c = 1, in DCM)



N^6-Dibenzoyl-3',5'-di-tert-butylsily-adenosine (17)

N^6-Dibenzoyl adenosine (200 mg, 0.42 mmol), pyridine (2 mL) and TEA (175 uL, 1.26 mmol) were added into a flask and the mixture was cooled down to 0°C after which $DTBS(OTf)_2$ (0.21 mL, 0.63 mmol) was added. The reaction was stirred at room temperature for 20 minutes before it was quenched by $NaHCO_3$ (aq. sat.). DCM (2 x) extracted the mixture and the organic layers were combined, dried (Na_2SO_4), filtered and concentrated. Purification by silica gel column chromatography (DCM/MeOH, 100/0 – 100/5) afforded **17** as a white foam (231 mg, 0.38 mmol, 90%). ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 8.63 (s, 1H, H2), 8.10 (s, 1H, H8), 7.92 – 7.81 (m, 4H, Ar), 7.53 – 7.43 (m, 2H, Ar), 7.36 (t, *J* = 7.7 Hz, 4H, Ar), 6.02 (s, 1H, H1), 4.85 (dd, *J* = 9.0, 5.0 Hz, 1H, H2), 4.71 (d, *J* = 5.0 Hz, 1H, H4), 4.47 (dd, *J* = 8.4, 4.4 Hz, 1H, H3), 4.20 – 4.01 (m, 2H, H5), 2.88 (s, 1H, OH), 1.11 (s, 9H, C(CH_3)$_3$), 1.06 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.38 (CO, Bz), 152.47 (Ar), 152.32, 152.20 (Cq. Ar), 143.88 (Ar), 134.09 (Cq. Ar), 133.18, 129.59, 128.89 (Ar), 128.16 (Cq. Ar), 91.10 (C1), 75.71 (C4), 75.26 (C2), 73.88 (C3), 67.47 (C5), 27.49, 27.34 (C(CH_3)$_3$), 22.88, 20.50

(Cq, *C*(CH_3)$_3$). IR (film): 2935, 2860, 1702, 1598, 1576, 1236, 980, 825, 734 cm^{-1}. HRMS (ESI^+) calcd for $C_{32}H_{38}N_5O_6Si$ (M+H) 616.2586. Found 616.2583. $[\alpha]_D^{20}$ -49.2 (c = 1, in DCM)



1-Fluro-2,3-di-*O*-tert-butyldimethylsilyl-5-O-(di-O-flourenylmethyl)-phosphoryl-β-D-ribofuranoside (19)

Compound **13** (300 mg, 0.37 mmol) was dissolved in THF (2 mL) in a flask and the solution was cooled down to -70°C after which it was added diethylaminosulfur trifluoride (DAST, 58 uL, 0.44 mmol). The reaction was stirred at room temperature for 45 minutes after which it was quenched by $NaHCO_3$ (aq. sat.) at 0°C. The mixture was extracted by EtOAc and the organic layer was washed with H_2O (1 x), brine (1 x), dried, filtered and concentrated. Purification by silica gel column chromatography (pentane/EtOAc, 100/0 – 100/15) to obtain **19** as a colorless oil (277 mg, 0.34 mmol, 92%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.73 (t, *J* = 7.4 Hz, 4H, Ar), 7.56 (dddd, *J* = 8.5, 7.5, 5.5, 1.0 Hz, 4H, Ar), 7.46 – 7.18 (m, 8H, Ar), 5.45 (d, *J* = 64.3 Hz, 1H, H1), 4.46 – 3.95 (m, 11H, H2, H3, H4, H5, Fm), 0.90 (d, *J* = 10.0 Hz, 18H, CH_3, TBS), 0.18 – 0.04 (m, 12H, $SiCH_3$, TBS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.37, 143.30, 143.27, 143.26, 141.47, 141.46, 141.44 (Cq. Ar), 127.94, 127.93, 127.22, 127.21, 127.20, 125.40, 125.36, 125.35, 120.09, 120.08, 120.05 (Ar), 114.80, 112.55 (C1), 81.59, 81.56, 81.51, 81.49 (C4), 75.66, 75.37 (C2), 70.07, 70.05 (C3), 69.58, 69.52 (CH_2-Fm), 65.92, 65.87 (C5), 48.05, 47.97 (CH-Fm), 25.90, 25.79 (CH_3, TBS), 18.15, 18.08 (Cq. TBS), -4.15, -4.48, -4.56, -5.01 ($SiCH_3$, TBS). IR (film): 2929, 2857, 1463, 1259, 1170, 1105, 1011, 838, 756 cm^{-1}. HRMS (ESI^+) calcd for $C_{45}H_{58}FO_7PSi_2Na$ (M+Na) 839.3335. Found 839.3337. $[\alpha]_D^{20}$ +23.6 (c = 1, in DCM)

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