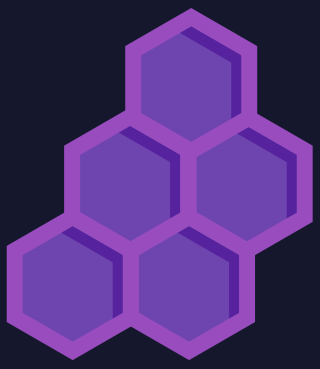
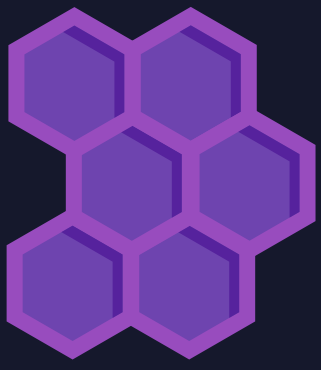
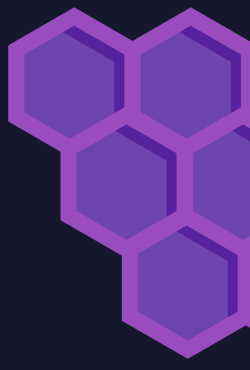
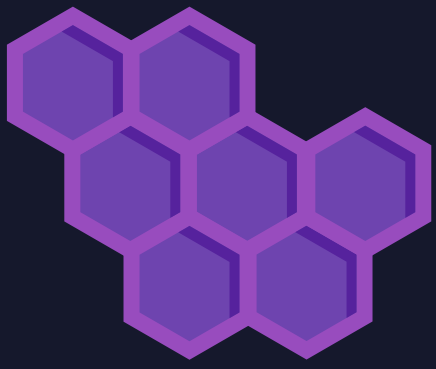
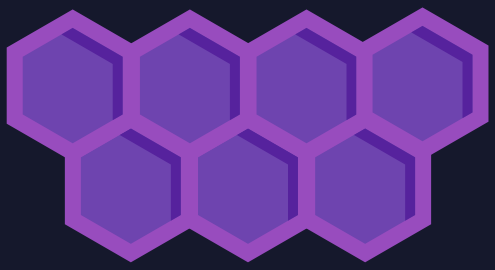
Thiosugars: reactivity, methodology and applications

Madern, J.M.

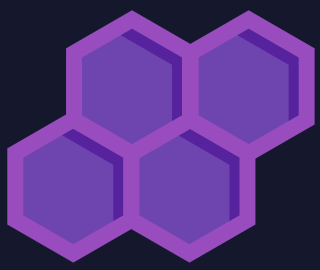



CHAPTER 4



THE DEVELOPMENT OF A SOLID-PHASE SYNTHESIS PROCEDURE FOR MONO-4-THIO-ADP-RIBOSYLATED PEPTIDES



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4.1 Introduction

Post-translational modifications (PTMs) are a wide variety of enzymatic alterations in the late stage of the biosynthesis of proteins, changing their function and activity. Most common among PTMs are phosphorylation, acetylation and glycosylation, resulting in the covalent attachment of a phosphate, an acetate or a mono- or polysaccharide moiety to a specific amino acid in a target protein, respectively.[1] The diversity among PTMs involving glycosylation of an acceptor protein is exemplified by adenosine diphosphate ribosylation (ADP ribosylation). This PTM is mediated by ADP-ribosyl transferases (ARTs) and *poly*-ADP-ribosyl polymerases (PARPs), which are responsible for adding a single adenosine diphosphate ribose (ADPr) unit or by stepwise elongation *poly*-ADPr chain to the side chain of a specific amino acid, respectively. Although initially glutamate and aspartate were regarded as the prime acceptors for ADPr, recent studies have revealed arginine[2], lysine[3], cysteine[4], histidine[5,6], tyrosine[7,8] and serine[9,10] as acceptors as well. As shown in Figure 1, the ADP ribosylation reaction to provide a *mono*-ADPr protein entails the enzymatic transfer of an ADPr unit from the co-enzyme NAD+ (nicotinamide adenosine dinucleotide) to a nucleophilic (e.g OH) function in the side chain of a specific amino acid of the protein with the concomitant release of nicotinamide. Linear ADP-ribosyl polymers are formed by a similar process using the 2'-hydroxyl group of the adenosine in the *mono*-ADP-ribosylated protein as a nucleophilic function. In addition, branching of the ADP-ribosyl polymers occurs when the 2-hydroxyl of a ribose residue in the ADPr chain acts as a nucleophile (not shown). ADP-ribosylation is a reversible PTM and the linear ADPr chains can be degraded by a *poly*-ADP ribose glycohydrolase (PARG) to provide the mono-ADPr protein. Cleavage of the mono-ADPr modification occurs under the action of glycohydrolases that are specific for the amino acid the ADPr moiety is attached to, for example, ARH1 and ARH3 for arginine and serine, respectively.[11–13]

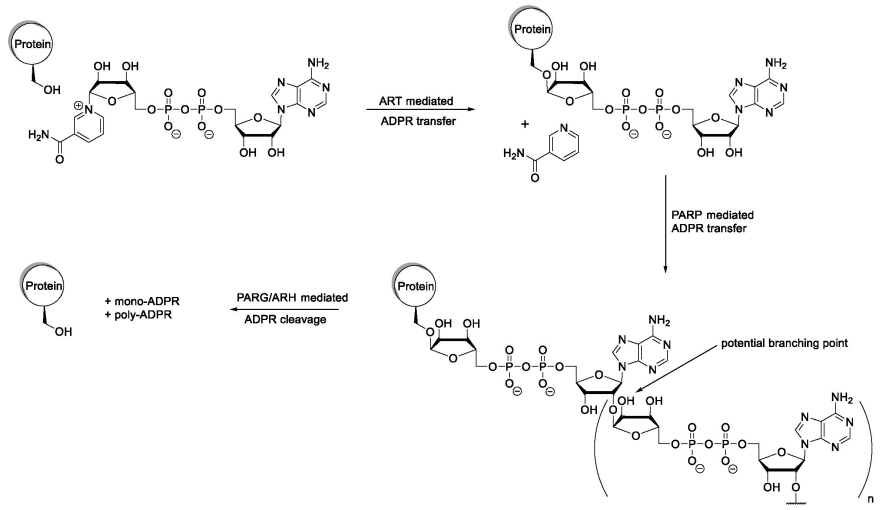


Figure 1. Schematic representation of *mono*- and *poly*-ADP ribosylation and hydrolysis.

ADP-ribosylation plays a role in a wide variety of cellular and biological processes such as DNA repair, mitosis, apoptosis, transcription and metabolism, cellular stress and immune response. Although the vital role of ADP-ribosylation in health and disease has been recognized, knowledge of ADP-ribosylation lags far behind our understanding of other PTMs. The dynamic character and the chemical lability of the ADPr modifications contribute to the lack of knowledge at the molecular level of the processes regulated by ADP-ribosylation. Sufficient quantities of structurally well-defined ADP-ribosylated peptide fragments are difficult to isolate from natural sources. In the last decade, organic synthesis has begun to provide ADPr fragments, as well as analogues and mimics equipped with a tag or fluorescent label, for biological testing.[14–16] For instance, relevant peptides, in which asparagine (Asn), glutamine (Gln) and citrulline (Cit) are provided with one ADPr moiety have been synthesized by solid phase peptide synthesis (SPPS).[17–19] as stabilized analogues for the natural aspartic acid (Asp), glutamic acid (Glu) and arginine (Arg) ADPr residues, respectively. Up to now, the only native ADP-ribosylated amino acids that have been incorporated with SPPS in an oligopeptide are ADPr-serine (Ser-ADPr), ADPr-threonine (Thr-ADPr) and ADPr cysteine (Cys-ADPr)[20], serine being the most biologically significant.[20] Ser-ADPr can undergo acidic cleavage or anomerization, although it is relatively stable in comparison to Asp-, Glu- and Arg-ADPr. Ser-ADPr can undergo β-elimination when exposed to alkaline conditions resulting in the formation of dehydroalanine and free ADPr.[21]

In all ADPr-proteins the glycosidic bond represents a labile functionality that is potentially vulnerable to cleavage or anomerization. By substituting the endocyclic

oxygen atom of the ribose with a sulfur atom, conceivably a bioisostere with resistance towards chemical or enzymatic cleavage is created. As it has recently been established that serine is the most frequent amino acid to be ADP-ribosylated in the DNA damage response pathway,[9,10,22,23] it was decided to explore the synthesis of an oligopeptide having a 4-thio-ribosylated serine to function as a stabilized mimic that can be used for functional and structural studies.

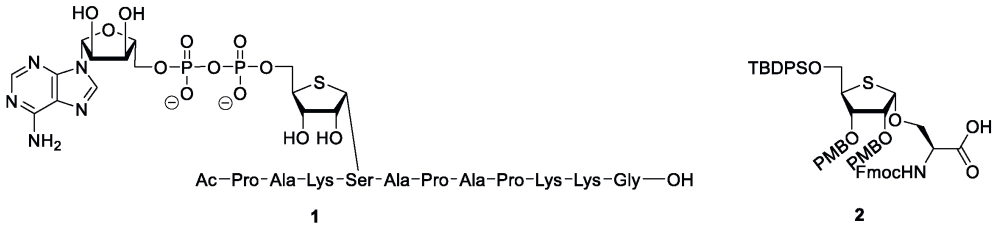


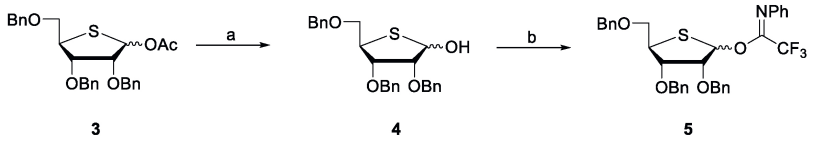
Figure 2. A) Mono-ADP-thioribosylated peptide **1**, B) Thioribosylated serine SPPS building block **2**.

Mono-ADP-thioribosylated peptide **1**, the sequence of which originates from H2B histone, was chosen as the target oligopeptide as it is known to be naturally ADP-ribosylated at the incorporated Ser (Figure 2). With the objective to synthesize **1** with solid-phase peptide synthesis (SPPS) using standard Fmoc chemistry, protected serine building block **2** carrying a suitably protected 4-thioribose was designed and pursued. Guided by the SPPS of the native Ser-ADPr-peptide the protective group pattern of the ribose in **2** entails *p*-methoxybenzyl (PMB) groups for the *cis*-diol system and a TBDPS-ether for the 5-OH function. It was expected that the PMB and TBDPS groups in a 4-thioribose donor could steer the ribosylation with a suitably protected serine acceptor to provide the desired α-ribose linkage,[24] while the orthogonality of the TBDPS group allows the on resin-introduction of the pyrophosphate function by the established P^{III} – P^{V} methodology that is applied for most ADPr-peptides.[17,20]

4.2 Results

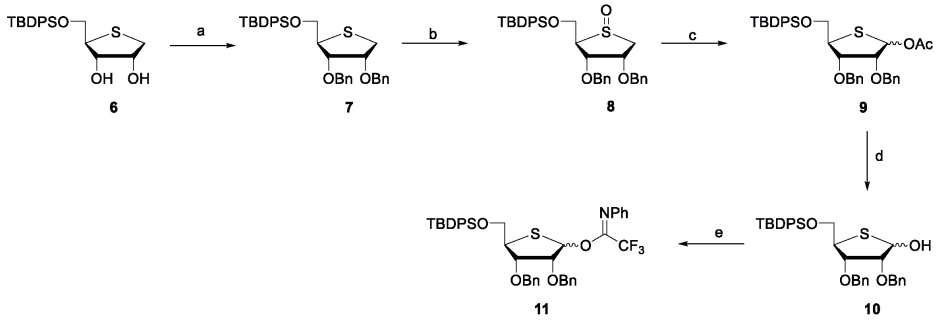
The glycosylation properties of 4-thioribofuranose donors, have been scarcely investigated. In Chapter 2 the reactivity and stereoselectivity of all diastereoisomeric 4-thiofuranosides in the reduction with TES-*d* have been described. It turned out that in these S_N1 type glycosylations, all 4-thio donors react in a 1,2-*cis*-selective manner. It has also become apparent that the ribo-configured 4-thiofuranoside was less reactive than the parent 4-O-furanoside. Guided by these results 1-O-acetyl-2,3,5-tri-O-benzyl-4-thio-α,β-D-ribofuranose donor **3** (for the synthesis see Chapter 2) was initially used to investigate the

glycosylation of serine. As shown in Table 1 (Entry 1) the expected glycoside **29** was isolated in 50% yield as a 60/40 α/β-mixture. Coupling of 4-thioribose **3** with **A5** using similar conditions proved to be unsuccessful (Entry 2). Therefore, a shift was made to the N-phenyltrifluoroacetimidate donor **5,** obtained by anomeric deacetylation of **3** and subsequent reaction of the released hydroxyl with 2,2,2-trifluoro-N-phenylacetimidoyl chloride (Scheme 1). Condensation of donor **5** with both **A1** and **A5** gave respective ribosides **29** and **33** in low yield with moderate stereoselectivity (Table 1, entries 3 and 4). As it is known that the presence of a bulky protective group at the 5-OH of a ribofuranose donor favors the formation of 1,2-*cis*-ribosides,[24] donors **9** and **11**, having 5-O-*tert*-butyldiphenylsilyl (TBDPS) protection, were prepared and evaluated. The synthesis of these donors is shown in Scheme 2 and commenced with thioribitol **6** (see Chapter 3 for its synthesis), which was benzylated using benzyl bromide and sodium hydride leading to fully protected thioribitol **7** in 51% yield. Next **7** was oxidized using m-CPBA in DCM at -40 °C, followed by a Pummerer rearrangement of sulfoxide **8** using acetic anhydride at 100 °C to yield donor **9** in 77% yield over two steps. Donor **11** was synthesized by deacetylation of the anomeric acetate using sodium methoxide in methanol, followed by introduction of an imidate group using 2,2,2-trifluoro-N-phenylacetimidoyl chloride and cesium carbonate resulting in N-phenyltrifluoroacetimidate donor **11** in 96% yield over 2 steps.



3 **4** **5**

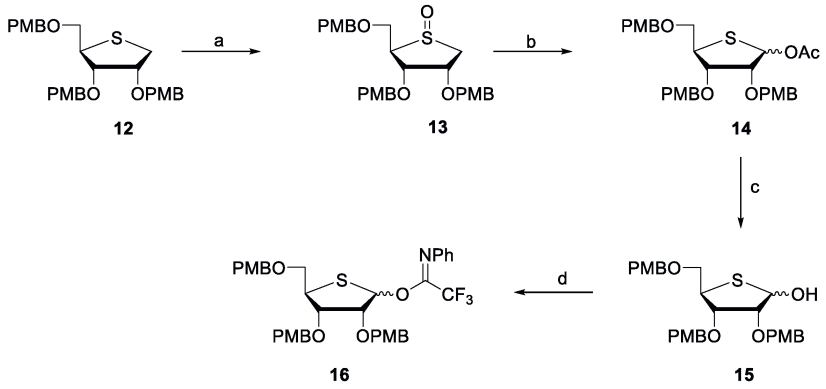
Scheme 1. Reagents and conditions: a) NaOMe, MeOH, 0 °C; b) ClC(NPh)CF_3, Cs_2CO_3, acetone, H_2O



6 **7** **8** **9** **10** **11**

Scheme 2. Reagents and conditions: a) BnBr, NaH, DMF (51%); b) DCM, m-CPBA, -40 °C; c) Ac_2O, 100 °C (77% over 2 steps); d) NaOMe, MeOH, 0 °C; e) ClC(NPh)CF_3, Cs_2CO_3, acetone, H_2O (96% over 2 steps)

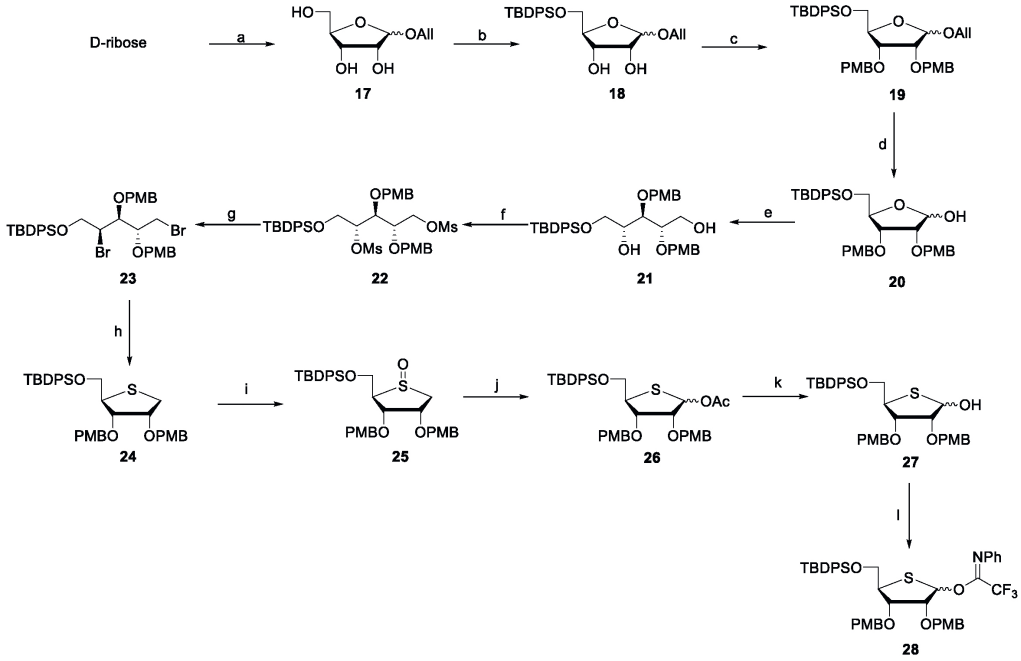
As coupling of N-phenyltrifluoroacetimidate donor **11** with **A1** (Entry 15) outperformed the corresponding reaction of 1-O-acetyl donor **5** (Entry 13), further test glycosylations were conducted with donor **11** (Entries 14, 16, 17, 18). Varying both the conditions and the type of acceptor resulted in the isolation of 4-thioribosylated serine (**29**, **30**), glutamine (**32**) and glutamic acid (**31**) building blocks as well as disaccharide (**33**, **34**) in good yield and satisfactory stereoselectivity. Interestingly, the condensation with glutamic acid (**A3**, Entry 16) provided an anomeric mixture, in which the β-product prevailed. At this stage protective group manipulations were undertaken to replace the benzyl ethers for the more acid labile PMB groups. Unfortunately, debenzylation by hydrogenation or acidic cleavage was not possible or cumbersome. Subsequent *p*-methoxybenzylation comes with new challenges such as the stability of the Fmoc protective group under the alkylation conditions, which would decrease the overall yield of the SPPS building block and therefore this route was abandoned.



Scheme 3. Reagents and conditions: b) DCM, m-CPBA, -40 °C; c) Ac_2O, 100 °C); d) NaOMe, MeOH, 0 °C; e) $ClC(NPh)CF_3$, Cs_2CO_3, acetone, H_2O

Next, attention was directed to the preparation and evaluation of thioribofuranose donors (**14**, **16**, **26**, **28**), having pre-installed PMB protecting groups. The synthesis of **12** is described in chapter 3, which was transformed into donors **14** and **16**, as shown in Scheme 3. The synthesis of donor **26** and **28** is shown in Scheme 4. D-ribose was converted into allyl-**α**,**β**-D-ribofuranoside **17** by a Fischer-glycosylation using allylic alcohol and acetyl chloride. Selective silylation of the 5-OH in **17** using TBDPS-Cl and DMAP in DCM, was followed by *p*-methoxybenzylation of the *cis*-diol system yielding fully protected ribose **19**. Anomeric deallylation of **19** using $[Ir(cod)(PPh_2Me)_2]PF_6$ and I_2 in $NaHCO_3$ and subsequent sodium borohydride mediated reduction of **20** resulted in linear diol **21**. Mesylation of both hydroxyls using mesyl chloride and triethylamine gave diester **22**, the starting compound for two ensuing key S_N2 substitutions. Replacement of both mesyl esters by bromines required a high concentration

of lithium bromide in 2-butanone, a temperature of 80 °C and a reaction time of 50 hrs. The obtained dibromide **23** was then treated with sodium sulfide in DCM inducing the ring closure to give thioether **24**. Finally, the target donors **26** and **28** were obtained by oxidation of **24** with m-CPBA in DCM at -40 °C, followed by a Pummerer rearrangement of sulfoxide **25** using acetic anhydride at 100 °C yielding donor **26** in 93% yield over two steps. Donor **28** was prepared by anomeric deacetylation of **26** using sodium methoxide in methanol, followed by treatment with 2,2,2-trifluoro-N-phenylacetimidoyl chloride and cesium carbonate.

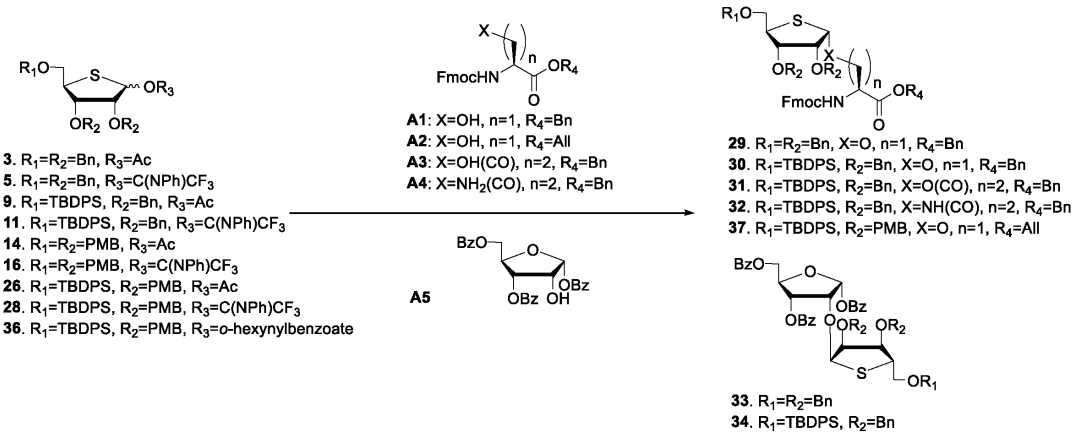


Scheme 4. Reagents and conditions: a) AllOH, AcCl; b) TBDPSCl, imidazole, DCM; c) DMF, PMBCl, NaH (93% over 3 steps); d) i) $[Ir(cod)(PPh_2Me)_2]PF_6$, THF; ii) I_2, sat. $NaHCO_3$ (aq.) (68% (quant. brsm) over 2 steps); e) MeOH, $NaBH_4$, 0 °C; f) DCM, Et_3N, MsCl, -20 °C (81% over 2 steps); g) 2-butanone, LiBr, reflux; h) DMF, Na_2S, 100 °C (73% over 2 steps); i) DCM, m-CPBA, -40 °C; j) Ac_2O, 100 °C (93% over 2 steps); k) NaOMe, MeOH, 0 °C (88%); l) $ClC(NPh)CF_3$, $CsCO_3$, acetone, H_2O (52% over 2 steps)

Having the donors (Table 1) available the coupling with protected serine was investigated (Table 1, entries 5-12). Unfortunately, none of the coupling reactions resulted in the isolation of the desired 4-thioribosylated serine. The byproduct, originating from the migration of PMB protecting groups from the donor (**14**, **16**, **26**, **28**) to the serine acceptor hydroxyl, was isolated in yields up to 70% (Scheme 5). Varying the conditions, the type and concentration of activator did not prevent the formation of this type of side product. Potentially one of the PMB groups in

the donor is cleaved by reaction of the PMB-ether oxygen with the (Lewis) acid and subsequent (intramolecular) trapping by the ring sulfur providing a transient sulfonium ion that transfers the PMB group to the acceptor.

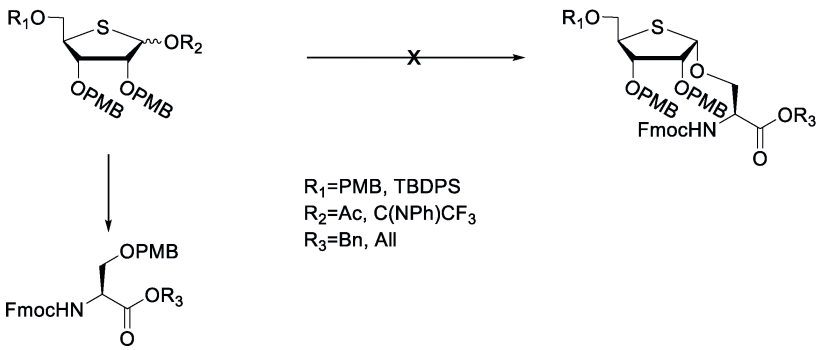
Table 1. Explorative 4-thioribosylation reactions.



No.	Donor	Acceptor	Product	Activator	Solvent	Temp (°C)	RT (hr)	Yield (%)	Ratio (α/β)
1	**3**	**A1**	**29**	0.2 eq. TMSOTf	DCM	-20	3	50	60/40
2	**3**	**A5**	**-**	0.1 eq. TMSOTf	DCM	-50	4	-	-
3	**5**	**A1**	**29**	0.3 eq. TMSOTf	DCM	-50	1.5	27	64/36
4	**5**	**A5**	**33**	0.2 eq. TMSOTf	DCM	-50	2.5	24	68/32
5	**14**	**A1**	**-**	0.2 eq. TMSOTf	DCM	-50	2	-	-
6	**16**	**A1**	**-**	0.1 eq. TBDMSOTf	DCM	-40	4	-	-
7	**26**	**A1**	**-**	0.1 eq. TMSOTf	DCM	-30	2	-	-
8	**26**	**A1**	**-**	0.1 eq. TBDMSOTf	DCM	-30	4	-	-
9	**26**	**A1**	**-**	0.1 eq. TMSOTf	DCM	-50	1	-	-
10	**28**	**A1**	**-**	0.1 eq. $HClO_4$-SiO_2	DCM	-50	1	-	-

Table 1. Continued

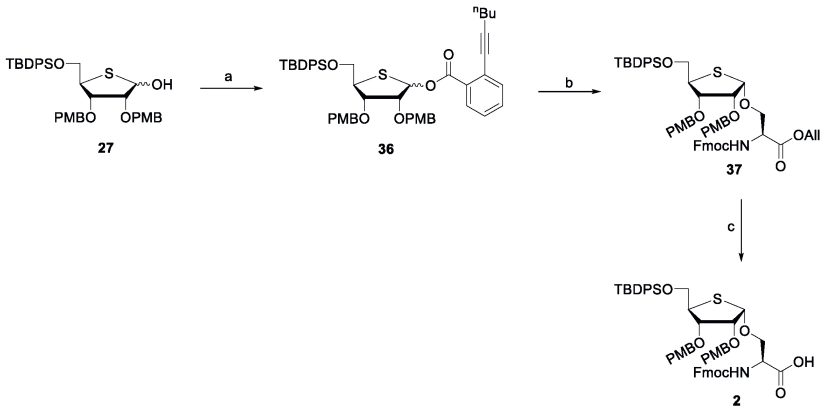
No.	Donor	Acceptor	Product	Activator	Solvent	Temp (°C)	RT (hr)	Yield (%)	Ratio (α/β)
11	**28**	**A5**	**-**	0.1 eq. TMSOTf	DCM	-80	3	-	-
12	**28**	**A5**	**-**	0.3 eq. $HClO_4$-SiO_2	DCM	-80	2	-	-
13	**9**	**A1**	**30**	0.3 eq. TMSOTf	DCM	-50	2.5	30	65/35
14	**11**	**A1**	**30**	0.1 eq. TfOH	DCM	-50	1.5	64	63/73
15	**11**	**A1**	**30**	0.3 eq. TMSOTf	DCM	-50	2	49	79/21
16	**11**	**A3**	**31**	0.2 eq. TMSOTf	DCM	-50	2	63	37/64
17	**11**	**A4**	**32**	0.2 eq. TMSOTf	Diox. / DCM	-15	1.5	81	86/14
18	**11**	**A5**	**34**	0.2 eq. TMSOTf	DCM	-80	1.5	66	91/9
19	**36**	**A2**	**37**	0.1 eq. PPh_3AuNTf_2	DCM	-30	O.N.	73	91/9



Scheme 5. The side reaction in which activating the donor results in migration of a PMB group to the acceptors nucleophilic sidechain.

Next, it was decided to seek a milder glycosylation procedure and this was found in the gold(I) catalyzed glycosylation of ortho-alkynylbenzoate donors, as introduced by the group of Yu (Scheme 6).[25] The donor (**36**) can be easily made from 4-thioribofuranose having a free anomeric hydroxyl (**27**) and ortho-hexynylbenzoic acid, that was prepared according to literature.[26,27] Compound **27**

was dissolved in DCM and DiPEA and DMAP were added. The ensuing sequential addition of EDCI and ortho-hexynylbenzoic acid in DCM resulted in the formation of donor **36** in a quantitative yield. This newly acquired donor was then used in a glycosylation using a catalytic amount of PPh_3AuNTf_2 and serine acceptor **A2** yielding thioribosylated serine (**36**) in 73% yield and with an 11:1 α/β-ratio. The allyl group was then removed using tetrakis(triphenylphosphine)palladium and DMBA, followed by the addition of THT (tetrahydrothiophene) as a scavenger, liberating the carboxylic acid and yielding the essential solid phase peptide synthesis building block **2** in 68% yield.

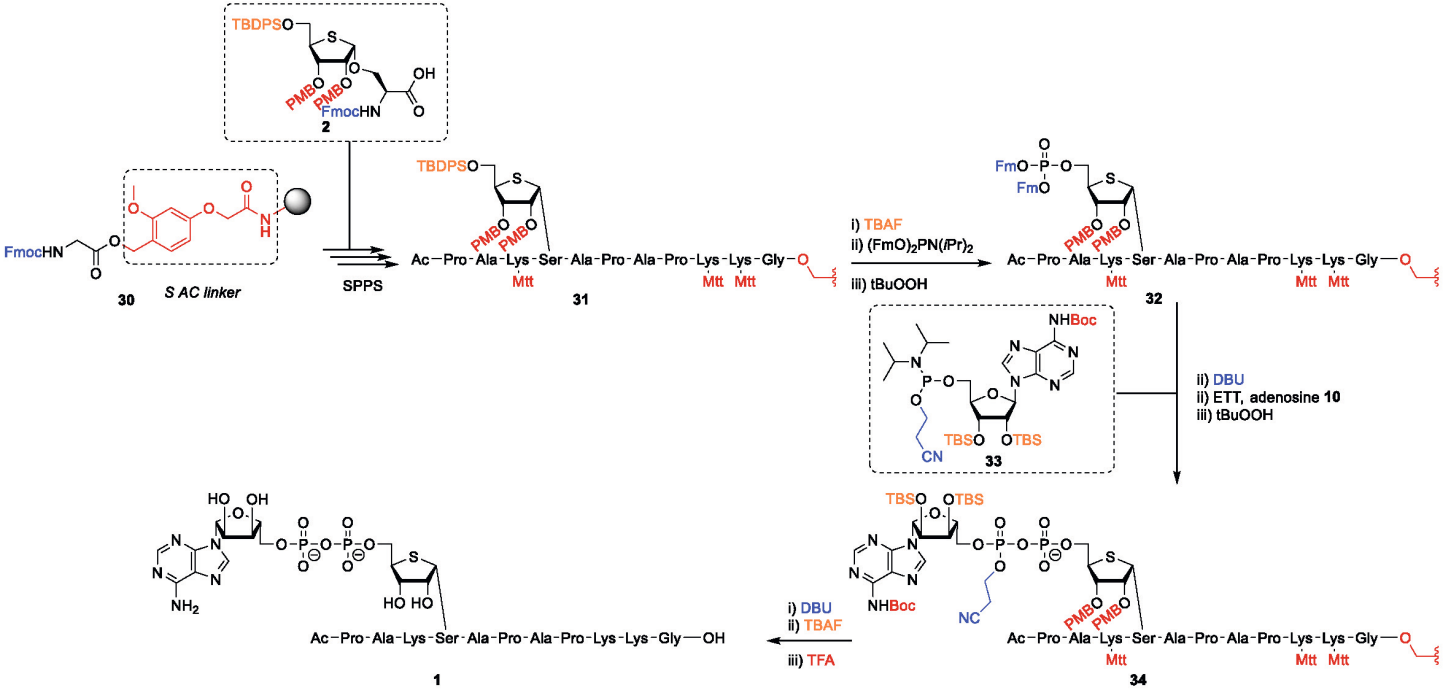


Scheme 6. Synthesis of alkynylbenzoate donor (**36**). a) ortho-hexynylbenzoic acid, DiPEA, DMAP, EDCI·HCl, DCM (quant.); b) **A2**, PPh_3AuNTf_2, DCM (73%); c) $Pd(PPh_3)_4$, DMBA, DCM (68%)

4.2.2 Solid phase peptide synthesis

With all building blocks in hand the solid phase assembly of mono-4-S-ADPribosylated peptide **1**, derived from the *N*-terminus of histone H2B, was undertaken. The standard Fmoc-based methodology was combined with amino acid building blocks carrying highly acid sensitive protecting groups on the side chains (Mtt for lysine, Trt for serine and threonine). As depicted in Scheme 7, the acid labile TentaGel® S AC resin, preloaded with glycine was elongated using the selected protected amino acid building blocks. The ensuing cleavage of the TBDPS-protecting group was tested using three different fluoride sources: TEA·3HF, HF·pyridine and TBAF. Both TEA·3HF and HF·pyridine needed reaction times of up to 16 hours to fully remove the silyl protecting group whereas a 1 M TBAF solution in THF ensured full deprotection in 30 minutes. The TBAF treatment was not only superior with regard to reaction rate but also in the quality of the product according to LC-MS analysis of the peptides after desilylation.[20] The released primary hydroxyl of the ribose moiety was then phosphitylated with di(9-fluorenylmethyl)-*N*,*N*-di-*iso*-propylphosphoramidite

using ethylthiotetrazole (ETT) as activator to give the corresponding phosphite triester. Care must be exercised in the oxidation of the phosphite to the phosphotriester, as it has been observed in the synthesis of ADP-ribosylated peptides linked to a biotin tag, that the biotin sulfur was oxidized when using CSO. Test reactions using protected thioribose substrates also showed quick and efficient oxidation of the thioether using CSO, whereas the use of tBuOOH led to minor or no oxidation of the thioether moiety. Gratifyingly, oxidation of the immobilized phosphite using tBuOOH resulted in fully protected phosphoribosyl peptide **40**, as shown by LC-MS analysis after deprotection and cleavage from the solid support. Based on this favorable result the Fm protecting groups in the immobilized phosphotriester **40** were removed by treatment of the resin with 10% DBU in DMF. Monitoring the reaction progress by LC-MS showed that both Fm-protecting groups were completely eliminated in 20 minutes to give the corresponding phosphomonoester. The assembly of the 4-thio ADP-ribosylated peptide was continued with the installation of the pyrophosphate by coupling of the phosphomonoester with adenosine phosphoramidite **41** and the tBuOOH mediated oxidation of the P^{III} – P^{V} intermediate to give immobilized peptide **42**, containing a partially protected pyrophosphate moiety. Now the terminal stage of the SPPS was reached. The cyanoethyl group was eliminated from the pyrophosphate in **42** by treatment of the resin with 10% DBU in DMF and the silyl ethers were cleaved with TBAF. Finally, the remaining protecting groups were removed with concomitant cleavage of the target 4-thio ADP-ribosylated peptide from the resin by treatment with a 10% TFA solution in DCM containing 2.5% TIS as a scavenger for trityl and *para*-methoxybenzyl carbocations. Monitoring of the deprotection by LC-MS analysis revealed that the Mtt and PMB-protecting groups were split off instantly while the more stable Boc-group on the exocyclic amine of adenosine needed at least 4 hours for its removal. Purification with RP-HPLC of the obtained crude product led to the isolation of MARylated peptide **1**, derived from the *N*-terminus histone H2B in a 3.6% overall yield.



Scheme 7. Assembly of thio-ADP-ribosylated H2B peptide (**1**). a) SPPS; b) i) TBAF; ii) $(FmO)_2PN(iPr)_2$, ETT; iii) ^tBuOOH; c) i) DBU; ii) ETT, **41**; iii) tBuOOH; d) i) DBU; ii) TBAF; iii) TFA.

4.3 Enzymatic cleavage assay

Having synthesized the Ser-thio-ADPr peptide **1**, we evaluated the Ser-ADPr isostere with respect to its sensitivity to enzymatic cleavage. ARH3 is known to hydrolyze the *O*-glycosidic linkage of Ser-ADPr and indeed was able to convert the Ser-ADPr control peptide efficiently (Figure 3). In contrast, our preliminary screen using a panel of human hydrolases utilizing an established luminescence reporter assay,[21,28] showed only marginal activity of ARH3 against Ser-thio-ADPr. While a complete hydrolysis of native Ser-ADPr was achieved in 45 min, only approximately 3% of the Ser-thio-ADPr counterpart was removed in the same time. Note, the ability of NudT16, which directly cleaves AMP from the modified peptide and acts as internal control, is also severely reduced (to ~9%) by the ribose to thio-ribose exchange. While we cannot fully exclude that the thio-ribose influences the enzyme-substrate interaction, our results suggest a strong stabilizing effect of the thio-sugar and the adjacent *O*-glycosidic bond.

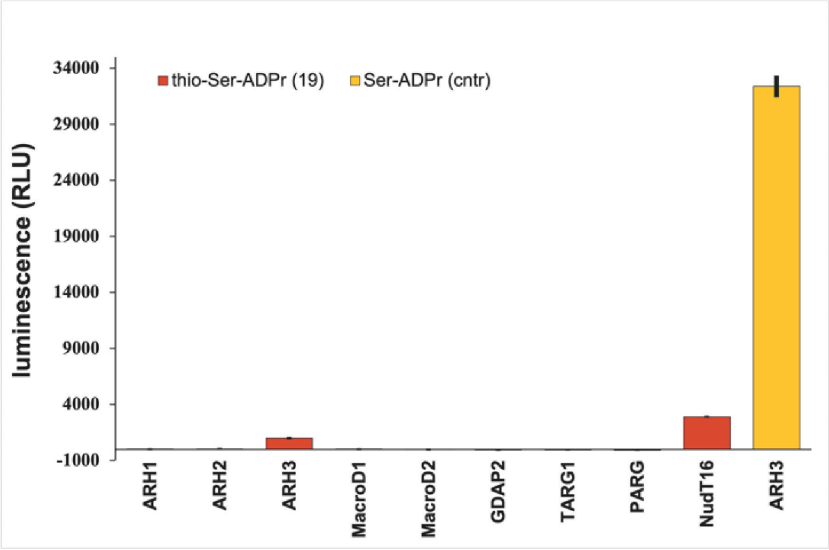


Figure 3. Enzymatic hydrolysis of the glycosidic linkage in thio-ADP-ribosylated peptide **19** and *O*-ribose control. Enzymatic turnover of the peptide was assessed by measuring the AMP release directly (NudT16) or converting released thio-ADPr via NudT5 to AMP. AMP was measured using the AMP-Glo assay (Promega). Reactions were measured in triplicates ± SD.

4.4 Conclusion

Various differently protected 4-thioribofuranose donors were tested to construct an α-glycosidic bond with different *O*-nucleophiles, particularly the serine side chain. Glycosylation procedures based on imidate and acetate donors proved to be inefficient in terms of yield and stereoselectivity while the gold(I)-catalyzed glycosylation of a newly developed 4-thioribose ortho-alkynylbenzoate donor was not only compatible with the applied PMB-protective groups but also produced the required orthogonally protected 4-thioribosylated serine building block in good yield and with excellent stereoselectivity. Subsequent deblocking of the allyl ester yielded a building block suitable for application in SPPS of mono-4-thio-ADP-ribosylated peptides. In this procedure the pyrophosphate function was introduced by the application of the P^{III}-P^{V} methodology leading to the first synthesis of a mono-4-thio-ADP-ribosylated peptide, the sequence of which was derived from H2B histone. This methodology might be extended to different thiosugar donors and amino acid acceptors opening the possibility of developing other bioisosteres, particularly of ADPr peptides, and probes for activity and structural biology studies on ARH3 and related hydrolases. The discovered glycosylation strategy may find wider application in the glycosylation with other thiosugar donors.

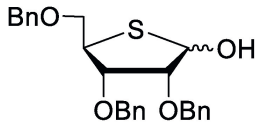
4.5 Experimental

General procedures

All solvents used under anhydrous conditions were stored over 4Å molecular sieves, except for methanol which was stored over 3Å molecular sieves. Solvents used for workup and column chromatography were of technical grade from Sigma Aldrich and used directly. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40 °C. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F254 with detection by spraying with 20% H_2SO_4 in EtOH, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), followed by charring at approx. 150 °C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm) or by automation using a Biotage® Isolera™ Spektra Four machine. For LC-MS analysis a JASCO HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C18 column (4.6 mmD × 50 mmL, 3µ particle size) in combination with buffers A: H_2O, B: MeCN and C: 0.5% aq. TFA and coupled to a PE/SCIEX API 165 single quadruple mass spectrometer (Perkin-Elmer) was used, unless stated otherwise. Alternatively, a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer with an electrospray ion source coupled to

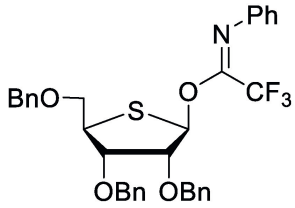
Surveyor HPLC system (Thermo Finnegan) was used with the same analytical column. High resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray 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 = 150-2000) and dioctylpthalate (m/z = 391.2842) as a "lock mass". The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). 1H- and 13C-NMR spectra were measured on a Bruker AV-400 (400 MHz) or a Bruker DMX-600 (600 MHz) and all individual signals were assigned using 2D-NMR spectroscopy. Chemical shifts are given in ppm (δ) relative to TMS (0 ppm) and coupling constants are given in Hz. Optical rotations were measured in $CHCl_3$ or methanol at a concentration of 10 mg/mL at 25 °C. Stereoisomers were, when possible, separated by silica gel column chromatography for analytical purposes.

2,3,5-tri-O-benzyl-4-thio-α,β-D-ribofuranoside (4)



Compound **3** (1.08 mmol, 0.52 g) was dissolved in methanol (6 ml), sodium methoxide (0.54 mmol, 0.03 g) was added at 0 °C and the reaction was stirred for 1 hour. The reaction was quenched upon addition of acetic acid and concentrated *in vacuo*. The residue was dissolved in DCM and washed with water. The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and used in the next step without purification.

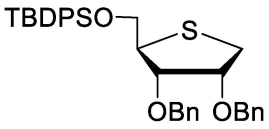
1-((N-Phenyl)-2,2,2-trifluoroacetimido)-2,3,5-tri-O-benzyl-4-thio-β-D-ribofuranoside (5)



Crude **4** (1.08 mmol) and cesium carbonate (1.62 mmol, 0.57 g) were dissolved in acetone (6 ml) and water (0.1 ml). 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (1.62 mmol, 0.15 ml) was added and the reaction mixture was stirred at room

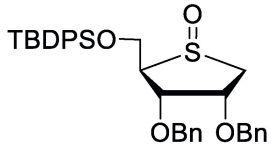
temperature for 16 hours. The reaction mixture was filtered over a pad of celite and concentrated *in vacuo*. Column chromatography, after neutralizing the silica with a triethylamine solution (pentane:triethylamine, 90/10), (pentane: EtOAc, 99/1 – 90/10) yielded the title compound (0.75 mmol, 0.46 g, 70% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.39 – 7.24 (m, 17H, arom.), 7.10 (t, *J* = 7.4 Hz, 1H, arom.), 6.84 – 6.77 (m, 2H, arom.), 6.03 (s, 1H, H1'), 4.75 – 4.59 (m, 2H, CH_2 Bn), 4.53 (d, *J* = 3.4 Hz, 4H, CH_2 Bn), 4.21 (d, *J* = 3.3 Hz, 1H, H2'), 4.09 – 4.01 (m, 1H, H3'), 3.90 – 3.82 (m, 1H, H4'), 3.80 (AB, *J* = 9.8, 4.5 Hz, 1H, H5'), 3.63 – 3.54 (m, 1H, H5'). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 143.69, 138.16, 137.64, 128.80, 128.56, 128.54, 128.44, 128.14, 128.06, 128.03, 127.95, 127.69, 127.61, 124.36, 119.60 (arom.), 83.94 (C1'), 81.19 (C3'), 80.84 (C2'), 73.18 (CH_2 Bn), 73.09 (CH_2 Bn), 72.45 (CH_2 Bn), 71.73 (C5'), 47.75 (C4'). IR: 3088, 3067, 3031, 2864, 1704, 1597, 1489, 1453, 1338, 1204, 1132, 1102, 1027. HRMS: $[M+Na]^+$ calcd for $C_{34}H_{32}F_3NO_4SNa$ 630.1896, found 630.1893.

1,4-Anhydro-2,3-bis-O-benzyl-5-O-tert-butyldiphenylsilyl-4-thio-D-ribitol (7)



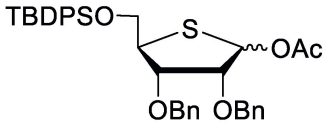
Compound **6** (0.39 mmol) was dissolved in DMF. The solution was cooled to 0 °C and sodium hydride (1.48 mmol, 0.067 g, 60% dispersion in mineral oil) was slowly added. After hydrogen gas formation stopped benzyl bromide (1.48 mmol, 0.176 ml) was slowly added and the reaction mixture was stirred at room temperature for 1 hour. The reaction was quenched by the addition of ice. The reaction mixture was diluted with EtOAc and washed with water, brine, dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 98/2 – 80/20) yielded the title compound (0.20 mmol, 0.115 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 – 7.57 (m, 4H, arom.), 7.45 – 7.21 (m, 16H, arom.), 4.61 (d, *J* = 10.4 Hz, 2H, CH_2 Bn), 4.56 – 4.45 (m, 2H, CH_2 Bn), 4.06 (d, *J* = 2.9 Hz, 1H, H3'), 3.95 (qd, *J* = 6.1, 5.5, 3.3 Hz, 1H, H2'), 3.74 – 3.52 (m, 3H, H4'; H5'), 3.08 – 2.97 (m, 1H, H1'), 2.92 – 2.79 (m, 1H, H1'), 1.04 (s, 9H, tBu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.35 (Cq. arom.), 138.16 (Cq. arom.), 135.70 (arom.), 135.65 (arom.), 133.28 (Cq. arom.), 129.85, 128.48, 128.39, 127.82, 127.81, 127.78, 127.73, 127.67, 127.63 (arom.), 80.52 (C3'), 80.24 (C2'), 72.07 (CH_2 Bn), 71.75 (CH_2 Bn), 65.88 (C5'), 49.78 (C4'), 30.55 (C1'), 26.94 (tBu), 19.34 (tBu). IR: 3069, 3031, 2930, 2857, 1427, 1360, 1111, 1105, 1067, 1028, 1007. HRMS: $[M+H]^+$ calcd for $C_{35}H_{40}O_3SSiH$ 569.2540, found 569.2543.

1,4-Anhydro-2,3-bis-O-benzyl-5-O-tert-butyldiphenylsilyl-4-sulfoxide-D-ribitol (8)



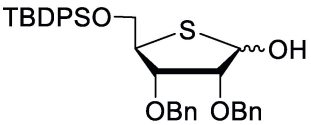
Compound **7** (6.3 mmol, 3.58 g) was dissolved in DCM (32 ml) and m-CPBA (6.9 mmol, 1.20 g) was added at -40 °C. The reaction mixture was stirred for 1 hour. The reaction mixture was quenched by the addition of aq. $Na_2S_2O_3$ (sat.) and the organic layer was washed with aq. $NaHCO_3$ (sat.). The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and used in the next step without purification.

1-O-Acetyl-5-O-tert-butyldiphenylsilyl-2,3-bis-O-benzyl-4-thio-α,β-D-ribofuranoside (9)



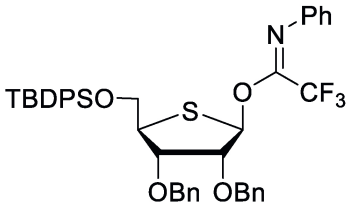
Crude **8** (6.3 mmol) was dissolved in acetic anhydride (32 ml) and stirred at 100 °C for 3 hours. The reaction mixture was concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 95/5 – 80/20) yielded the title compound (4.86 mmol, 3.05 g, 77% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (dt, *J* = 8.0, 1.8 Hz, 2H, arom.), 7.58 (ddd, *J* = 8.1, 3.0, 1.5 Hz, 2H, arom.), 7.46 – 7.20 (m, 16H, arom.), 6.20 (d, *J* = 4.7 Hz, 1H, H1''), 5.99 (d, *J* = 2.0 Hz, 1H, H1'), 4.79 – 4.44 (m, 4H, CH_2 Bn), 4.20 (dd, *J* = 4.1, 1.8 Hz, 1H, H3''), 4.16 (dd, *J* = 8.3, 3.5 Hz, 1H, H3'), 4.09 (dd, *J* = 3.5, 2.1 Hz, 1H, H2'), 3.99 (t, *J* = 4.4 Hz, 1H, H2''), 3.87 (d, *J* = 4.5 Hz, 2H, H5'), 3.77 (dt, *J* = 8.8, 4.5 Hz, 1H, H4'), 3.69 (ddd, *J* = 7.4, 5.3, 1.7 Hz, 1H, H4''), 3.57 (AB, *J* = 10.7, 5.4 Hz, 1H, H5''), 3.45 (AB, *J* = 10.7, 7.8 Hz, 1H, H5''), 2.13 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.05 (s, 9H, tBu), 1.01 (s, 9H, tBu). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.06, 170.03, 138.53, 137.83, 137.74, 137.70 (Cq. arom.), 135.85, 135.71, 135.64 (arom.), 133.48, 133.08, 133.02 (Cq. arom.), 132.97, 130.00, 129.87, 129.81, 128.59, 128.57, 128.50, 128.37, 128.03, 128.01, 127.92, 127.83, 127.77, 127.70, 127.64, 127.58 (arom.), 81.66 (C2'), 80.78 (C2''), 80.58 (C3'), 80.27 (C3''), 80.02 (C1''), 76.91 (C1'), 72.95, 72.22, 72.10, 65.56 (C5'), 63.30 (C5''), 50.79 (C4'), 50.27 (C4''), 26.92 (tBu), 26.88 (tBu), 21.56 (OAc), 21.27 (OAc), 19.49 (tBu), 19.33 (tBu). IR: 3070, 3030, 2931, 2893, 2858, 1733, 1454, 1427, 1370, 1363, 1226, 1111, 1072. HRMS: $[M+Na]^+$ calcd for $C_{37}H_{42}O_5SSiNa$ 649.2414, 649.2411 found.

5-O-tert-butyldiphenylsilyl-2,3-bis-O-benzyl-4-thio-α,β-D-ribofuranoside (10)



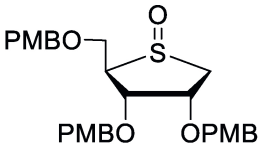
Compound **9** (4.71 mmol, 2.95 g) was dissolved in methanol (30 ml), sodium methoxide (2.36 mmol, 0.13 g) was added at 0 ˚C and the reaction was stirred for 1 hour. The reaction was quenched upon addition of acetic acid and concentrated *in vacuo*. The residue was dissolved in DCM and washed with water. The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and used in the next step without purification.

1-((N-Phenyl)-2,2,2-trifluoroacetimido)-5-O-tert-butyldiphenylsilyl-2,3-bis-paramethoxybenzyl-4-thio-β-D-ribofuranoside (11)



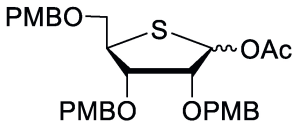
Compound **10** (4.71 mmol) and cesium carbonate (7.1 mmol, 2.51 g) were dissolved in acetone (30 ml) and water (0.5 ml). 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (7.1 mmol, 1.15 ml) was added and the reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was filtered over a pad of celite and concentrated *in vacuo*. Column chromatography, after neutralizing the silica with a triethylamine solution (pentane:triethylamine, 90/10), (pentane: EtOAc, 99/1 – 90/10) yielded the title compound (4.51 mmol, 3.41 g, 96% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.71 – 7.65 (m, 4H, arom.), 7.47 – 7.20 (m, 15H, arom.), 7.19 – 7.04 (m, 4H, arom.), 6.81 (d, *J* = 7.8 Hz, 2H, arom.), 6.03 (s, 1H, H1'), 4.75 – 4.60 (AB, 2H, CH_2 Bn), 4.58 – 4.48 (AB, 2H, CH_2 Bn), 4.26 – 4.17 (m, 2H, H2'; H3'), 3.94 – 3.82 (m, 2H, H5'), 3.78 (dt, *J* = 7.4, 4.7 Hz, 1H, H4'), 1.06 (s, 9H, tBu). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 143.85, 137.83, 137.78 (Cq. arom.), 135.88, 135.74 (arom.), 133.55, 133.19 (Cq. arom.), 129.89, 129.84, 129.28, 128.85, 128.59, 128.55, 128.03, 128.00, 127.97, 127.85, 127.84, 127.54, 124.34, 120.77, 119.61 (arom.), 84.10 (C1'), 81.05 (C3'), 80.36 (C2'), 73.18 (CH_2 Bn), 72.52 (CH_2 Bn), 63.63 (C5'), 50.18 (C4'), 26.85 (tBu), 19.45 (tBu). IR: 3053, 2932, 1076, 1598, 1428, 1265, 1205, 1162, 1111, 1027. HRMS: $[M+H]^+$ calcd for $C_{43}H_{44}F_3NO_4SSiH$ 756.2785, found 756.2787.

1,4-Anhydro-2,3,5-tri-O-paramethoxybenzyl-4-sulfoxide-D-ribitol (13)



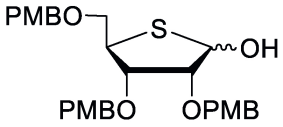
Compound **12** (1.98 mmol, 1.01 g) was dissolved in DCM (10 ml) and m-CPBA (2.18 mmol, 0.38 g) was added at -40 °C. The reaction mixture was stirred for 1 hour. The reaction mixture was quenched by the addition of aq. $Na_2S_2O_3$ (sat.) and the organic layer was washed with aq. $NaHCO_3$ (sat.). The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and used in the next step without purification.

1-O-Acetyl -2,3,5-tri-O-paramethoxybenzyl-4-thio-α,β-D-ribofuranoside (14)



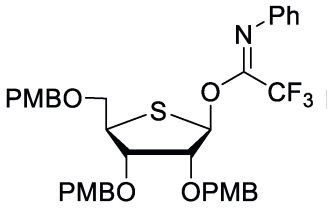
Crude **13** (1.98 mmol) was dissolved in acetic anhydride (10 ml) and stirred at 100 °C for 3 hours. The reaction mixture was concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 95/5 – 80/20) yielded the title compound (0.79 mmol, 0.45 g, 40% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.31 – 7.25 (m, 2H, arom.), 7.25 – 7.19 (m, 2H, arom.), 7.19 – 7.13 (m, 2H, arom.), 6.90 – 6.79 (m, 6H, arom.), 6.16 (d, *J* = 4.7 Hz, 1H, H1'α), 5.95 (d, *J* = 1.9 Hz, 1H, H1'β), 4.69 – 4.31 (m, 6H, CH_2 Bn), 4.03 (td, *J* = 4.4, 3.7, 2.1 Hz, 1H, H2'β), 3.98 (t, *J* = 4.5 Hz, 1H, H2'α), 3.91 (dd, *J* = 8.3, 3.5 Hz, 1H, H3'β), 3.86 (dd, *J* = 7.0, 3.7 Hz, 1H, H3'α), 3.79 (d, *J* = 1.9 Hz, 10H, OMe; H4'β), 3.77 – 3.67 (m, 1H, H5'β; H4'α), 3.52 (AB, *J* = 9.7, 7.0 Hz, 1H, H5'β), 3.34 (AB, *J* = 10.0, 5.5 Hz, 1H, H5'α), 3.26 (dd, *J* = 9.9, 7.7 Hz, 1H, H5'α), 2.13 (s, 3H, OAc'α), 2.03 (s, 3H, OAc'β). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 169.93, 159.46, 159.24, 130.34, 129.94, 129.89, 129.78 (Cq. arom.), 129.65, 129.59, 129.52, 129.47, 129.35, 129.27, 113.90, 113.84, 113.81, 113.71 (arom.), 80.83 (C3'α), 80.72 (C3'β), 80.36 (C2'α), 80.05 (C2'β), 80.00 (C1'β), 77.19 (C1'α), 72.85 (CH_2 Bn), 72.50 (CH_2 Bn), 72.42 (CH_2 Bn), 71.78 (CH_2 Bn), 71.69 (CH_2 Bn), 71.41 (C5'α), 71.36 (C5'β), 55.37 (OMe), 48.47 (C4'α), 47.89 (C4'β), 21.56 (OAc'α), 21.28 (OAc'β). IR: 3005, 2936, 2911, 2865, 2836, 1733, 1611, 1586, 1511, 1244, 1226, 1173, 1099, 1030. HRMS: $[M+Na]^+$ calcd for $C_{31}H_{36}O_8SNa$ 591.2023, found 591.2020.

2,3,5-tri-O-paramethoxybenzyl-4-thio-α,β-D-ribofuranoside (15)



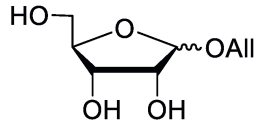
Compound **14** (0.274 mmol, 0.156 g) was dissolved in methanol (1.5 ml), sodium methoxide (0.137 mmol, 0.007 g) was added at 0 °C and the reaction was stirred for 1 hour. The reaction was quenched upon addition of acetic acid and concentrated *in vacuo*. The residue was dissolved in DCM and washed with water. The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and used in the next step without purification.

1-((N-Phenyl)-2,2,2-trifluoroacetimido)-2,3,5-tri-O-paramethoxybenzyl-4-thio-β-D-ribofuranoside (16)



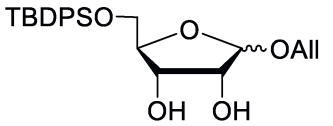
Crude **15** (0.274 mmol) and cesium carbonate (0.411 mmol, 0.145 g) were dissolved in acetone (3 ml) and water (0.1 ml). 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (0.411 mmol, 58 µl) was added and the reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was filtered over a pad of celite and concentrated *in vacuo*. Column chromatography, after neutralizing the silica with a triethylamine solution (pentane:triethylamine, 90/10), (pentane: EtOAc, 99/1 – 90/10) yielded the title compound (0.19 mmol, 0.100 g, 52% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 – 7.16 (m, 9H, arom.), 6.91 – 6.73 (m, 8H, arom.), 5.97 (s, 1H, H1'), 4.64 – 4.54 (m, 2H, CH_2 Bn), 4.51 – 4.41 (m, 4H, CH_2 Bn), 4.14 (dd, *J* = 3.5, 2.2 Hz, 1H, H2'), 3.98 (dd, *J* = 7.9, 3.5 Hz, 1H, H3'), 3.89 – 3.70 (m, 11H, OMe; H4'; H5'), 3.53 (AB, *J* = 9.6, 7.0 Hz, 1H, H5'). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 159.52, 159.25, 143.73, 130.29 (Cq. arom.), 129.79 (arom.), 129.74 (Cq. arom.), 129.62, 129.22, 128.78, 124.31, 119.59, 113.92, 113.89, 113.81 (arom.), 84.08 (C1'), 80.77 (C3'), 80.44 (C2'), 72.82 (CH_2 Bn), 72.67 (CH_2 Bn), 72.12 (CH_2 Bn), 71.54 (C5'), 55.34 (OMe), 47.80 (C4'). IR: 2959, 2937, 2864, 2843, 1733, 1717, 1612, 1513, 1455, 1303, 1286, 1247, 1173, 1154, 1103, 1033. HRMS: $[M+Na]^+$ calcd for $C_{37}H_{38}F_3NO_7SNa$ 720.2213, found 720.2216.

Allyl-α,β-D-ribofuranoside (17)



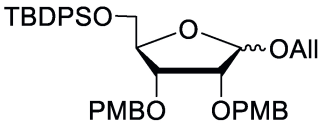
D-ribose (31.48 g, 210 mmol) was suspended in allylalcohol (500 ml, 0.4 M). The suspension was cooled to 0 °C and acetyl chloride (3.0 ml, 42 mmol) was slowly added. The reaction mixture stirred over night at 4 °C. The reaction was quenched by adding pyridine. The reaction was quenched by adding Na_2CO_3. The reaction mixture filtrated, concentrated *in vacuo* and used in the next step without purification.

Allyl-5-O-tertbutyldiphenylsilyl-α,β-D-ribofuranoside (18)



Crude **17** (210 mmol) was used without further purification and after coevaporation with toluene was dissolved in dry DCM (550 ml). Imidazole (21.10 g, 310 mmol), dried after coevaporation with toluene, was added and TBDPSCl (58.5 ml, 225 mmol) was slowly added. The reaction was then stirred for 1 hour, quenched by adding water and then concentrated *in vacuo*. The residue was dissolved with diethyl ether and washed with water, dried ($MgSO_4$), concentrated *in vacuo* and used in the next step without purification.

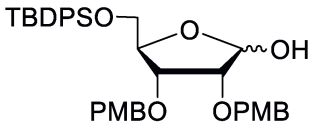
Allyl-5-O-tertbutyldiphenylsilyl-2,3-bis-O-paramethoxybenzyl-α,β-D-ribofuranoside (19)



Crude **18** (210 mmol) was used without further purification and was dissolved in dry DMF (700 ml). The reaction was cooled to 0 °C and sodium hydride (472 mmol, 18.9 g, 60% dispersion in mineral oil) was added in small portions. After all sodium hydride has reacted PMBCl (62.8 ml, 461 mmol) was slowly added and the reaction was allowed to warm up to room temperature. The reaction was stirred for 3 hours and then quenched by addition of water. The reaction mixture was diluted with EtOAc and then partitioned between EtOAc and water.

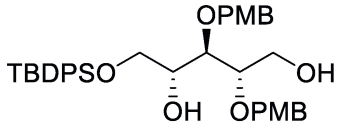
The organic layer was washed with water, brine, dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 95/5 – 80/20) yielded the title compound (124.67 g, 186.55 mmol, 89% over three steps).

5-O-tertbutyldiphenylsilyl-2,3-O-paramethoxybenzyl-D-ribose (20)



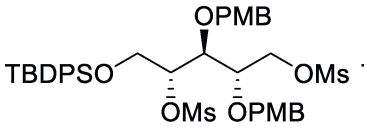
$[Ir(COD)(PPh_2Me)_2]PF_6$ (0.44 g, 0.52 mmol) was dissolved in freshly distilled THF (500 ml) and stirred under argon atmosphere. Hydrogen gas was bubbled through the solution until solution turns from red to yellow. The atmosphere was then replaced with argon while making sure the solutions colour does not change back to red. A dropping funnel was charged with compound **19** (114.94 g, 172.0 mmol) dissolved in freshly distilled THF (900 ml), and slowly added to the catalyst in solution. The reaction was stirred over night on room temperature. Aqueous $NaHCO_3$ (sat.) solution (1 liter) and iodine (52.4 g, 206.4 mmol) was added and the reaction was stirred for 1.5 hrs. The reaction mixture was quenched by the addition of aq. $Na_2S_2O_3$ (sat.) and extracted with chloroform. The organic layer was dried ($MgSO_4$), concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 90/10 – 70/30) yielded the title compound (72.96 g, 116.1 mmol, 68% over two steps (quantitative based on recovered starting material).

(2S,3R,4R)-5-O-tert-butyldiphenylsilyl-2,3-bis-paramethoyxybenzyl-pentane-1,4-diol (21)



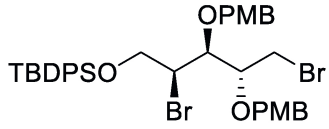
Compound **20** (73 g, 116 mmol) was dissolved in methanol (460 ml) and sodium borohydride (8.8 g, 232 mmol) was added in small portions over a period of 30 minutes at 0 °C. The reaction was then stirred for 1,5 hour at room temperature. After these 1,5 hours the solvent was removed *in vacuo* and the residue was dissolved in EtOAc and washed with water and brine. The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and used in the next step without purification.

(2S,3R,4R)-5-O-tert-butyldiphenylsilyl-2,3-bis-paramethoyxybenzyl-pentane-1,4-dimesylate (22)



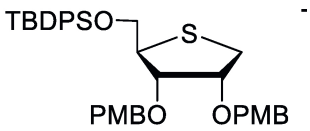
Crude **21** (116 mmol) was used without further purification and dissolved in DCM (580 ml) and triethylamine (65 ml, 464 mmol) was added. Mesyl chloride (27.2 ml, 348 mmol) was slowly added at -15 °C and the reaction was stirred for 45 minutes after which it was quenched upon addition of ice. The organic layer was washed with water, aq. $NaHCO_3$ (sat.), brine, dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 90/10 – 70/30) yielded the title compound (69.23 g, 88.0 mmol, 76% over two steps). ^{1}H NMR (300 MHz, DMSO) δ 7.70 – 7.58 (m, 4H), 7.51 – 7.29 (m, 6H), 7.23 – 7.09 (m, 4H), 6.89 – 6.74 (m, 4H), 5.06 (dd, *J* = 6.7, 3.3 Hz, 1H), 4.66 – 4.38 (m, 4H), 4.42 – 4.22 (m, 2H), 4.04 – 3.91 (m, 1H), 3.87 – 3.66 (m, 9H), 2.98 (s, 3H), 2.91 (s, 3H), 1.06 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.78, 135.70, 133.96, 130.30, 130.13, 130.09, 129.98, 129.08, 127.99, 114.01, 83.35, 76.40, 75.87, 73.29, 72.21, 67.88, 63.12, 55.38, 38.82, 37.73, 26.97, 19.26.

(2S,3R,4S)-5-O-tert-butyldiphenylsilyl-2,3-bis-paramethoyxybenzyl-1,4-di-bromopentane (23)



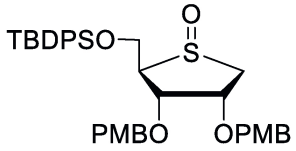
Compound **22** (66.58 g, 88.0 mmol), co-evaporated with toluene to remove traces of water, was dissolved in dry 2-butanone (440 ml). Lithium bromide (53 g, 616 mmol) was added and the reaction was refluxed for 3 nights. The reaction mixture was diluted with EtOAc and washed with water, aq. $NaHCO_3$ (sat.), brine, dried ($MgSO_4$) and concentrated *in vacuo*.

5-O-tert-butyldiphenylsilyl-1,4-anhydro-2,3-bis-paramethoxybenzyl-4-thio-D-ribitol (24)



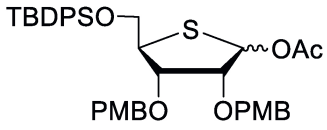
Crude **23** (88.0 mmol) and sodium sulfide nonahydrate (25.36 g, 105 mmol) were dissolved in DMF (300 ml). The reaction mixture was stirred at 100° C for 1 hour. Then the reaction mixture was diluted with EtOAc and washed with water, brine, dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 95/5 – 80/20) yielded the title compound (40.37 g, 64.2 mmol, 73% over 2 steps). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.63 (ddd, *J* = 8.1, 2.7, 1.4 Hz, 4H, arom.), 7.42 (dd, *J* = 7.5, 2.0 Hz, 2H, arom.), 7.37 (tt, *J* = 8.0, 1.0 Hz, 4H, arom.), 7.27 – 7.20 (m, 4H, arom.), 6.90 – 6.84 (m, 2H, arom.), 6.84 – 6.76 (m, 2H, arom.), 4.55 (d, *J* = 1.8 Hz, 2H, CH_2 PMB), 4.44 (q, *J* = 9.1 Hz, 2H, CH_2 PMB), 4.02 (t, *J* = 3.3 Hz, 1H, H3'), 3.93 (ddd, *J* = 7.6, 5.6, 3.4 Hz, 1H, H2'), 3.80 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.67 – 3.53 (m, 3H, H4'; H5'), 2.99 (AB, *J* = 7.7 Hz, 1H, H1'), 2.83 (AB, *J* = 5.6 Hz, 1H, H1'), 1.04 (s, 9H, tBu). ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.33, 159.22 (Cq. arom.), 135.75, 135.70, 133.37, 133.34, 130.43, 130.24, 129.43, 129.36, 127.85, 127.83, 113.89, 113.81 (arom.), 80.00 (C3'), 79.76 (C2'), 71.71 (CH_2 PMB), 71.39 (CH_2 PMB), 65.95 (C5'), 55.38 (OMe), 55.38 (OMe), 49.82 (C4'), 30.64 (C1'), 26.95 (tBu), 19.39 (Cq. tBu). IR: 3071, 2930, 2858, 1612, 1512, 1246, 1105, 1069, 1034. HRMS: $[M+Na]^+$ calcd for $C_{37}H_{44}O_5SSiNa$ 651.2571, found 651.2565

5-O-tert-butyldiphenylsilyl-1,4-anhydro-2,3-bis-paramethoxybenzyl-4-sulfoxide-D-ribitol (25)



Compound **24** (7.68 mmol, 4.8 g) was dissolved in DCM (52 ml) and m-CPBA (8.06 mmol, 1.39 g) was added at -20 °C. The reaction mixture was stirred for 2 hours. The reaction mixture was quenched by the addition of aq. $Na_2S_2O_3$ (sat.) and the organic layer was washed with aq. $NaHCO_3$ (sat.). The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and used in the next step without purification.

1-O-Acetyl-5-O-tert-butyldiphenylsilyl-2,3-bis-paramethoxybenzyl-4-thio-α,β-D-ribofuranoside (26)

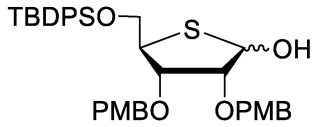


Crude **25** (7.68 mmol) was dissolved in acetic anhydride (6 ml) and stirred at 100° for 3 hours. The reaction mixture was concentrated *in vacuo*. Column

chromatography (pentane: EtOAc, 95/5 – 80/20) yielded the title compound (7.12 mmol, 4.89 g, 93% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$, **α**-anomer) δ: 7.59 (ddd, *J* = 7.9, 3.5, 1.5 Hz, 4H), 7.48 – 7.31 (m, 6H), 7.30 – 7.19 (m, 4H), 6.90 – 6.76 (m, 4H), 6.17 (d, *J* = 4.7 Hz, 1H, H1'), 4.70 – 4.41 (m, 4H, CH_2 Bn), 4.14 (dd, *J* = 4.1, 2.0 Hz, 1H, H3'), 3.96 (t, *J* = 4.4 Hz, 1H, H2'), 3.83 – 3.73 (m, 6H, OMe), 3.67 (ddd, *J* = 7.6, 5.5, 1.9 Hz, 1H, H4'), 3.55 (AB, *J* = 10.7, 5.5 Hz, 1H, H5'), 3.45 (AB, *J* = 10.7, 7.6 Hz, 1H, H5'), 2.13 (s, 3H, OAc), 1.02 (s, 9H, OAc).

^{13}C NMR (101 MHz, $CDCl_3$, **α**-anomer) δ: 171.00, 159.47, 159.18 (Cq. arom.), 135.70 (arom.), 135.64 (arom.), 133.09 (Cq. arom.), 133.06 (Cq. arom.), 130.57 (Cq. arom.), 129.96 (arom.), 129.77 (Cq. arom.), 129.36, 129.27, 127.88, 113.96, 113.75 (arom.), 81.22 (C2'), 80.11 (C3'), 77.02 (C1'), 72.57 (CH_2 Bn), 71.89 (CH_2 Bn), 65.61 (C5'), 55.35 (OMe), 50.91 (C4'), 26.92 (tBu), 21.55 (OAc), 19.33 (Cq. tBu).^{1}H NMR (400 MHz, $CDCl_3$, β-anomer) δ: 7.66 (ddd, *J* = 5.4, 3.9, 1.8 Hz, 4H, arom.), 7.38 (dddd, *J* = 16.2, 8.4, 6.1, 1.6 Hz, 6H, arom.), 7.29 (d, *J* = 8.6 Hz, 2H, arom.), 7.14 (d, *J* = 8.6 Hz, 2H, arom.), 6.86 (d, *J* = 8.5 Hz, 2H, arom.), 6.80 (d, *J* = 8.6 Hz, 2H, arom.), 5.97 (d, *J* = 1.9 Hz, 1H, H1'), 4.67 (AB, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.54 (AB, *J* = 12.0 Hz, 1H, CH_2Bn), 4.42 – 4.33 (m, 2H, CH_2 Bn), 4.12 (dd, *J* = 8.3, 3.5 Hz, 1H, H3'), 4.07 (dd, *J* = 3.5, 2.1 Hz, 1H, H2'), 3.84 (t, *J* = 4.6 Hz, 2H, H5'), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.74 (dt, *J* = 8.7, 4.4 Hz, 1H, H4'), 2.02 (s, 3H, OAc), 1.05 (s, 9H, tBu).^{13}C NMR (101 MHz, $CDCl_3$, β-anomer) δ: 169.96, 159.48, 159.39 (Cq. arom.), 135.84 (arom.), 135.71 (arom.), 133.55 (Cq. arom.), 133.16 (Cq. arom.), 129.98 (Cq. arom.), 129.83, 129.78, 129.67, 129.38, 127.79, 113.93, 113.86 (arom.), 80.36 (C2'), 80.11 (C1'), 79.91 (C3'), 72.54 (CH_2 Bn), 71.71 (CH_2 Bn), 63.46 (C5'), 55.38 (OMe), 50.35 (C4'), 26.88 (tBu), 21.25 (OAc), 19.48 (Cq. tBu). IR: 3072, 3006, 2952, 2933, 2858, 1735, 1612, 1587, 1513, 1246, 1224, 1173, 1104, 1032, 1016. HRMS: $[M+Na]^+$ calcd for $C_{39}H_{46}O_7SSiNa$ 709.2626, found 709.2626.

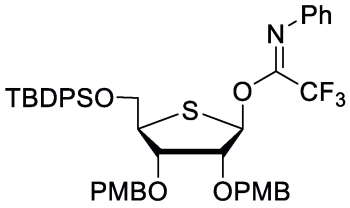
5-O-tert-butyldiphenylsilyl -2,3-bis-paramethoxybenzyl-4-thio-α,β-D-ribofuranoside (27)



Compound **26** (7.12 mmol, 4.89 g) was dissolved in methanol (35 ml), sodium methoxide (3.56 mmol, 0.19 g) was added at 0 °C and the reaction was stirred for 1 hour. The reaction was quenched upon addition of acetic acid, diluted with toluene, and concentrated *in vacuo*. The residue was dissolved in DCM and washed with water. The organic layer was dried ($MgSO_4$), concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 95/5 – 80/20) yielded the title

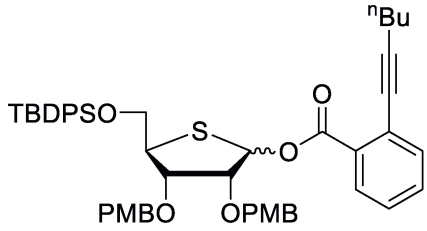
compound (4.05 g, 6.30 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 – 7.51 (m, 4H), 7.51 – 7.33 (m, 6H), 7.33 – 7.22 (m, 3H), 7.22 – 7.08 (m, 2H), 6.93 – 6.69 (m, 4H), 5.20 (dd, *J* = 5.8, 2.2 Hz, 1H), 4.60 (q, *J* = 12.0 Hz, 2H), 4.42 (d, *J* = 11.5 Hz, 1H), 4.33 (d, *J* = 11.5 Hz, 1H), 4.18 (dd, *J* = 7.6, 3.5 Hz, 1H), 4.08 – 3.97 (m, 1H), 3.90 – 3.64 (m, 9H), 2.16 (d, *J* = 5.8 Hz, 1H), 1.04 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.46, 159.39, 135.79, 135.70, 135.66, 133.26, 133.20, 130.15, 130.01, 129.92, 129.91, 129.71, 129.55, 129.51, 129.46, 127.96, 127.93, 127.87, 113.97, 113.94, 113.90, 82.81, 80.25, 80.13, 72.46, 72.02, 64.34, 55.40, 55.38, 50.95, 26.96, 19.43. HRMS: $[M+Na]^+$ calcd for $C_{37}H_{44}O_6SSiNa$ 667.2526, found 667.2520 found.

1-((N-Phenyl)-2,2,2-trifluoroacetimido)-5-O-tert-butyldiphenylsilyl-2,3-bis-paramethoxybenzyl-4-thio-β-D-ribofuranoside (28)



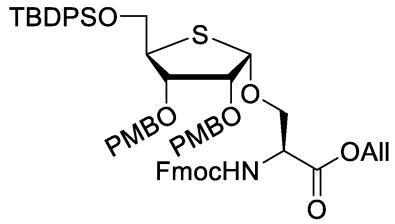
Crude **27** (0.32 mmol) and cesiumcarbonate (0.48 mmol, 0.010 g) were dissolved in acetone (3.5 ml) and water (0.1 ml). 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (0.48 mmol, 0.067 ml) was added and the reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was filtered over a pad of celite and concentrated *in vacuo*. Column chromatography, after neutralizing the silica with a triethylamine solution (pentane:triethylamine, 90/10), (pentane: EtOAc, 99/1 – 90/10) yielded the title compound (0.13 mmol, 0.106 g, 41% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.72 – 7.63 (m, 4H, arom.), 7.47 – 7.32 (m, 6H, arom.), 7.29 (td, *J* = 8.2, 3.4 Hz, 4H, arom.), 7.19 (dd, *J* = 8.0, 6.1 Hz, 2H, arom.), 7.14 – 7.06 (m, 1H, arom.), 6.91 – 6.76 (m, 6H, arom.), 5.98 (s, 1H, H1'), 4.67 – 4.55 (m, 2H, CH_2 Bn), 4.52 – 4.40 (m, 2H, CH_2 Bn), 4.25 – 4.08 (m, 2H, H2'; H3'), 3.91 – 3.81 (m, 2H, H5'), 3.81 – 3.72 (m, 7H, OMe; H4'), 1.06 (s, 9H, tBu). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 159.48 (Cq. arom.), 143.84 (Cq. arom.), 135.85 (arom.), 135.71 (arom.), 133.56 (Cq. arom.), 133.18 (Cq. arom.), 129.84, 129.80, 129.67, 129.64, 128.81, 127.79, 124.28, 120.61, 119.56, 113.94 (arom.), 84.14 (C1'), 80.52 (C2'), 79.85 (C3'), 72.78 (CH_2 Bn), 72.14 (CH_2 Bn), 63.56 (C5'), 55.33 (OMe), 50.20 (C4'), 26.81 (tBu), 19.42 (tBu). IR: 3072, 2955, 2933, 2857, 1706, 1612, 1513, 1248, 1206, 1162, 1135, 1111, 1073, 1035 HRMS: $[M+H]^+$ calcd for $C_{45}H_{48}F_3NO_6SSiH$ 816.2996, found 816.2999.

(3R,4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3,4-bis((4-methoxybenzyl)oxy)tetrahydrothiophen-2-yl 2-(hex-1-yn-1-yl) benzoate (36)



Compound **27** (1.44 mmol, 0.93 g) was dissolved in DCM (8 ml) and DiPEA (12.96 mmol, 1.68 g, 2.26 ml), DMAP (1.44 mmol, 0.18 g) and EDCI·HCl (4.32 mmol, 0.83 g). Next, a solution of ortho-hexynylbenzoic acid (4.32 mmol, 0.87 g) in DCM (7 ml) was added to the reaction mixture and it was stirred overnight on room temperature. The reaction mixture was washed with with aq. $NaHCO_3$ (sat.), dried (Na_2SO_4) and concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 95/5 – 85/15) yielded the title compound (1.44 mmol, 1.19 g, quant.). ^{1}H NMR (300 MHz, DMSO) δ 7.78 – 7.63 (m, 1H), 7.69 – 7.58 (m, 5H), 7.56 – 7.07 (m, 16H), 6.94 – 6.73 (m, 5H), 6.25 (d, *J* = 1.7 Hz, 1H), 4.76 (d, *J* = 12.1 Hz, 1H), 4.69 – 4.53 (m, 1H), 4.51 – 4.35 (m, 1H), 4.41 – 4.29 (m, 1H), 4.27 – 4.15 (m, 1H), 4.20 – 4.02 (m, 2H), 3.95 – 3.82 (m, 2H), 3.79 (s, 4H), 3.77 (s, 4H), 2.41 (t, *J* = 7.0 Hz, 2H), 1.58 (s, 2H), 1.68 – 1.39 (m, 4H), 1.04 (s, 2H), 0.99 – 0.88 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.86, 135.75, 134.61, 131.98, 130.55, 129.91, 129.79, 129.49, 127.81, 127.79, 127.13, 113.96, 113.89, 80.50, 80.36, 79.96, 77.58, 77.16, 76.74, 72.50, 71.66, 63.75, 55.40, 50.44, 30.88, 26.82, 22.21, 19.70, 19.40. HRMS: $[M+Na]^+$ calcd for $C_{50}H_{56}O_7SSiNa$ 851.3408, found 851.3400.

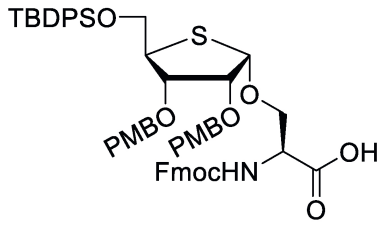
(E)-prop-1-en-1-yl N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2S,3R,4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3,4-bis((4-methoxybenzyl)oxy)tetrahydrothiophen-2-yl)-L-serinate (37)



Donor **36** (0.09 mmol, 0.083 g) and acceptor **A2** (0.135 mmol, 0.050 g), dried by co-evaporating with dry toluene thrice, were dissolved in DCM (1.8 ml). Freshly dried molecular sieves (rods) were added. After stirring for 30 mins the

reaction was cooled to -30 °C, next freshly prepared PPh_3AuNTf_2 (0.1M in DCM) solution was added (0.1 ml). The reaction mixture was stirred overnight at -30 °C. The reaction mixture was filtered over a pad of celite and concentrated *in vacuo*. Column chromatography (pentane: EtOAc, 95/5 – 75/25) yielded the title compound (0.066 mmol, 0.065 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (ddd, *J* = 15.7, 7.5, 3.1 Hz, 3H), 7.72 – 7.46 (m, 7H), 7.46 – 7.36 (m, 3H), 7.40 – 7.29 (m, 8H), 7.31 – 7.20 (m, 4H), 6.89 – 6.83 (m, 2H), 6.75 (dd, *J* = 15.7, 8.5 Hz, 2H), 6.39 (d, *J* = 8.8 Hz, 1H), 5.87 (ddt, *J* = 17.3, 10.7, 5.5 Hz, 1H), 5.34 – 5.18 (m, 1H), 5.17 – 5.08 (m, 2H), 4.64 (d, *J* = 2.2 Hz, 1H), 4.66 – 4.50 (m, 5H), 4.50 – 4.36 (m, 1H), 4.40 – 4.29 (m, 1H), 4.29 – 4.12 (m, 3H), 4.04 (dd, *J* = 9.1, 3.5 Hz, 1H), 4.01 – 3.90 (m, 1H), 3.86 (t, *J* = 4.2 Hz, 1H), 3.78 (s, 3H), 3.82 – 3.71 (m, 3H), 3.67 (s, 3H), 3.68 – 3.51 (m, 2H), 3.46 (dd, *J* = 10.4, 7.6 Hz, 1H), 1.03 (s, 11H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.42, 159.36, 159.12, 156.52, 144.30, 143.91, 141.36, 141.27, 135.77, 135.74, 135.69, 133.14, 133.09, 131.85, 130.52, 130.10, 129.97, 129.83, 129.62, 129.53, 129.27, 127.90, 127.88, 127.83, 127.70, 127.66, 127.16, 127.14, 125.57, 125.32, 120.10, 119.98, 119.94, 118.43, 113.88, 113.84, 113.74, 85.00, 82.56, 80.09, 72.24, 71.95, 67.86, 67.34, 67.13, 66.09, 65.79, 55.36, 55.33, 55.24, 54.28, 50.92, 47.19, 26.93, 26.87, 19.36. HRMS: $[M+Na]^+$ calcd for $C_{58}H_{63}NO_{10}SSiNa$ 1016.3834, found 1016.3837.

N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2S,3R,4S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3,4-bis((4-methoxybenzyl)oxy) tetrahydrothiophen-2-yl)-L-serine (2)



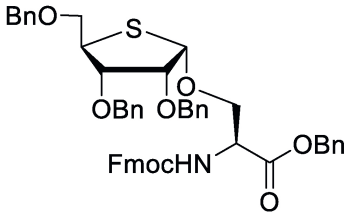
Compound **29** (0.24 mmol, 0.24 g) and DMBA (0.36 mmol, 0.056 g) were dissolved in DCM (2.4 ml). $Pd(PPh_3)_4$ (0.024 mmol, 0.028 g) was added and the reaction mixture was stirred on room temperature for 1 hour. After completion of the reaction THT (2.4 mmol, 0.21 g, 0.21 ml) was added, the reaction mixture was diluted with DCM and washed with a 1M HCl (aq.) solution. The reaction mixture was filtered over a pad of celite and concentrated *in vacuo*. Column chromatography (DCM: MeOH, 99/1 – 90/10) followed by size exclusion column chromatography yielded the title compound (0.16 mmol, 0.157 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 7.5 Hz, 2H), 7.53 (d, *J* = 7.4 Hz, 6H), 7.41 (dd, *J* = 9.6, 5.6 Hz, 2H), 7.33 (t, *J* = 7.8 Hz, 6H), 7.26 – 7.10 (m, 7H), 6.79 – 6.68 (m, 4H), 6.24 (s, 1H), 4.90 (s, 1H), 4.61 – 4.56 (m, 1H), 4.49 (d, *J* = 11.9 Hz, 2H), 4.39

(s, 1H), 4.32 – 4.21 (m, 2H), 4.13 (t, *J* = 7.1 Hz, 1H), 4.04 (s, 1H), 3.93 (s, 1H), 3.82 – 3.73 (m, 2H), 3.73 – 3.60 (m, 6H), 3.53 (t, *J* = 6.1 Hz, 1H), 3.43 (s, 2H), 3.35 – 3.26 (m, 1H), 0.97 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 141.40, 135.73, 135.66, 133.27, 130.17, 130.07, 129.87, 128.33, 127.96, 127.94, 127.74, 127.17, 125.35, 120.02, 114.11, 114.03, 87.00, 81.00, 79.00, 72.00, 71.50, 69.00, 67.02, 65.20, 55.28, 55.26, 54.00, 50.00, 47.29, 26.96, 19.36. HRMS: $[M+Na]^+$ calcd for $C_{55}H_{59}NO_{10}SSiH$ 954.3702, found 954.3702

General procedure A:

The donor and acceptor were co-evaporated with 1,4-dioxane (2x) and DCE (1x), dissolved in dry DCM and stirred with freshly activated 4 Å molecular sieves at room temperature for 1 h under argon to remove traces of water. The solution was then cooled and TMSOTf (~0.2 eq.) was added to the reaction mixture. The reaction was stirred for 1.5-2 hours and quenched by the addition of triethylamine. The reaction mixture was filtered through a pad of celite, concentrated *in vacuo* and purified.

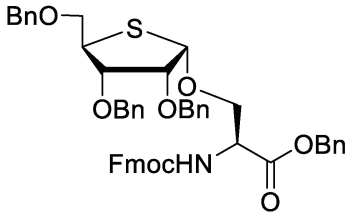
O^{β}-(2,3,5-tri-*O*-benzyl-4-thio-α,β-D-ribosyl)-N^{α}-fluorenylmethoxycarbonyl serine benzyl ester (29)



General procedure A using compound **3** (0.34 mmol, 0.163 g), Fmoc-Ser-OBn (0.40 mmol, 0.170 g), TMSOTf (34 µmol, 6 µl) and DCM (2 ml) was performed at -40 °C. Column chromatography (pentane: EtOAc, 90/10 – 70/30) yielded the title compound (0.06 mmol, 0.054 g, 54%) as an anomeric mixture (**α**:β, 60:40). ^{1}H NMR (400 MHz, $CDCl_3$, **α**-anomer) δ: 7.71 (dd, *J* = 7.6, 4.9 Hz, 2H, arom.), 7.56 (dd, *J* = 7.4, 5.6 Hz, 2H, arom.), 7.40 – 7.14 (m, 24H, arom.), 6.36 (d, *J* = 8.8 Hz, 1H, NH), 5.23 – 5.06 (m, 3H, CH_2 Bn; H1'), 4.65 – 4.54 (m, 4H, CH_2 Bn; CH **α**-Ser), 4.49 – 4.41 (m, 3H, CH_2 Bn), 4.35 (dd, *J* = 10.5, 7.1 Hz, 1H, CH_2 Fmoc), 4.26 (dd, *J* = 10.5, 7.6 Hz, 1H, CH_2 Fmoc), 4.14 (t, *J* = 7.3 Hz, 1H, CH Fmoc), 4.10 – 4.00 (m, 2H, CH_2 β –Ser; H3'), 3.98 – 3.86 (m, 2H, CH_2 β –Ser; H2'), 3.73 (ddd, *J* = 8.1, 5.7, 2.7 Hz, 1H, H4'), 3.47 – 3.30 (m, 2H, H5'). ^{13}C NMR (101 MHz, $CDCl_3$, **α**-anomer) δ: 170.51, 156.52, 144.23, 143.93, 141.36, 141.28, 138.22, 138.01, 137.91, 135.63 (Cq. arom.), 128.64, 128.55, 128.53, 128.38, 128.33, 128.20, 128.18, 127.89, 127.76, 127.73, 127.71, 127.67, 127.17, 125.47, 125.31, 120.00, 119.97 (arom.), 85.47 (C1'), 82.15 (C2'), 80.38 (C3'), 73.25 (CH_2 Bn), 72.45 (CH_2 Bn), 72.36 (CH_2 Bn), 71.69

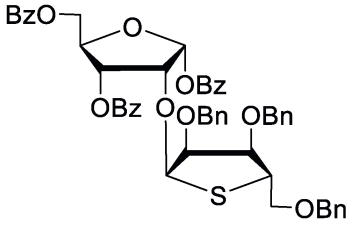
(C5'), 68.28 (CH_2 β –Ser), 67.27 (CH_2 Bn), 67.20 (CH_2 Fmoc), 54.36 (CH **α**-Ser), 48.63 (C4'), 47.22 (CH Fmoc). ^{1}H NMR (400 MHz, $CDCl_3$, β-anomer) δ: 7.75 (d, *J* = 7.5 Hz, 2H, arom.), 7.60 (d, *J* = 7.5 Hz, 2H, arom.), 7.42 – 7.17 (m, 24H, arom.), 5.61 (d, *J* = 8.5 Hz, 1H, NH), 5.19 – 5.00 (AB, 2H, CH_2 Bn), 4.98 (d, *J* = 2.1 Hz, 1H, H1'), 4.62 (d, *J* = 2.0 Hz, 2H, CH_2 Bn), 4.56 (dt, *J* = 8.5, 3.5 Hz, 1H, CH **α**-Ser), 4.51 – 4.40 (m, 5H, CH_2 Bn; CH Fmoc), 4.37 (AB, *J* = 7.1 Hz, 1H, CH_2 Fmoc), 4.22 (AB, *J* = 7.0 Hz, 1H, CH_2 Fmoc), 4.09 – 3.96 (m, 2H, CH_2 β –Ser; H2'), 3.91 – 3.78 (m, 2H, H3'; H4'), 3.71 (AB, *J* = 9.7, 4.4 Hz, 1H, H5'), 3.55 (AB, *J* = 9.6, 3.2 Hz, 1H, CH_2 β –Ser), 3.41 (AB, *J* = 9.7, 7.1 Hz, 1H, H5'). ^{13}C NMR (101 MHz, $CDCl_3$, β-anomer) δ: 169.78, 156.00, 143.92, 143.75, 141.37, 141.35, 138.07, 137.90, 137.75, 135.14 (Cq. arom.), 128.67, 128.53, 128.49, 128.46, 128.40, 128.05, 127.93, 127.89, 127.81, 127.65, 127.17, 125.17, 125.10, 120.09 (arom.), 88.24 (C1'), 81.92 (C2'; C3'), 73.00 (CH_2 Bn), 72.89 (CH_2 Bn), 72.70 (C5'), 72.42 (CH_2 Bn), 68.63 (CH_2 β –Ser), 67.58 (CH_2 Bn), 67.21 (CH_2 Fmoc), 54.29 (CH **α**-Ser), 47.31 (C4') , 47.21 (CH Fmoc). IR: 3065, 3033, 2939, 2870, 1723, 1498, 1452, 1265, 1198, 1101, 1073, 1027, 1002. HRMS: $[M+Na]^+$ calcd for $C_{51}H_{49}NO_8SNa$ 858.3071, found 858.3070.

N$^{\beta}$-(2,3,5-tri-*O*-benzyl-4-thio-α,β-D-ribosyl)-*N*$^{\alpha}$-fluorenylmethoxycarbonyl serine benzyl ester (29)



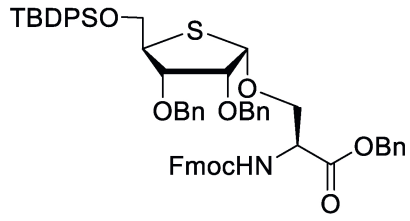
General procedure A using compound **21** (0.145 mmol, 0.088 g), Fmoc-Ser-OBn (0.096 mmol, 0.040 g), TMSOTf (43 µmol, 8 µl) and DCM (1 ml) was performed at -40 °C. Column chromatography (pentane: EtOAc, 90/10 – 70/30) yielded the title compound (0.017 mmol, 0.022 g, 27%) as an anomeric mixture (**α**:β, 64:36).

1-O-(1,3,5-tri-O-benzoyl-α-D-ribofuranosyl)-2,3,5-tri-O-benzyl-4-thio-α,β-D-ribofuranoside (34)



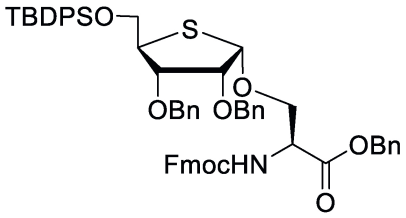
General procedure A using compound **21** (0.38 mmol, 0.231 g), 1,3,5-tri-O-benzoyl-benzoyl-α-D-ribofuranoside (0.25 mmol, 0.116 g), TMSOTf (72 µmol, 14 µl) and DCM (2 ml) was performed at -50 °C. Column chromatography (pentane: EtOAc, 90/10 – 70/30) yielded the title compound (0.06 mmol, 0.054 g, 24%) as an anomeric mixture (**α**:β, 68:32). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18 – 8.04 (m, 6H), 7.68 – 7.62 (m, 1H), 7.62 – 7.40 (m, 6H), 7.39 – 7.01 (m, 21H), 6.99 – 6.92 (m, 2H), 6.78 (d, *J* = 4.2 Hz, 1H), 6.64 (d, *J* = 4.6 Hz, 1H), 5.71 – 5.62 (m, 1H), 5.54 (d, *J* = 4.3 Hz, 1H), 5.29 (d, *J* = 1.5 Hz, 1H), 4.90 (d, *J* = 2.1 Hz, 1H), 4.81 (dd, *J* = 6.4, 4.2 Hz, 1H), 4.74 (dt, *J* = 4.9, 2.2 Hz, 1H), 4.71 – 4.30 (m, 9H), 4.06 (d, *J* = 11.7 Hz, 1H), 4.03 – 3.97 (m, 1H), 3.92 (t, *J* = 4.4 Hz, 1H), 3.90 – 3.79 (m, 1H), 3.80 – 3.72 (m, 1H), 3.67 (dd, *J* = 3.4, 1.5 Hz, 1H), 3.55 – 3.40 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.31, 166.18, 165.97, 165.71, 165.30, 138.24, 138.09, 138.02, 138.00, 137.95, 137.69, 133.68, 133.61, 133.45, 133.36, 133.26, 130.22, 130.14, 130.06, 130.02, 129.96, 129.94, 129.91, 129.85, 129.63, 129.54, 129.34, 128.83, 128.68, 128.66, 128.56, 128.50, 128.38, 128.36, 128.32, 128.28, 128.23, 128.12, 127.92, 127.84, 127.81, 127.76, 127.72, 127.64, 127.62, 127.60, 127.44, 127.31, 95.89, 95.11, 87.72, 85.58, 83.30, 82.59, 81.62, 81.14, 80.42, 75.98, 75.55, 73.20, 73.12, 73.03, 72.75, 72.65, 72.41, 72.10, 71.89, 71.34, 70.39, 64.42, 64.37, 60.52, 49.13, 47.93, 46.89. IR: 3095, 3060, 3035, 2925, 2860, 1715, 1601, 1451, 1363, 1264, 1107, 1092, 1068, 1025, 1001. HRMS: $[M+Na]^+$ calcd for $C_{52}H_{48}O_{11}SNa$ 903.2810, found 903.2813.

O$^{\beta}$-(2,3-bis-*O*-benzyl-5-tert-butyldiphenylsilyl-4-thio-α,β-D-ribosyl)-*N*$^{\alpha}$-fluorenylmethoxycarbonyl serine benzyl ester (30)



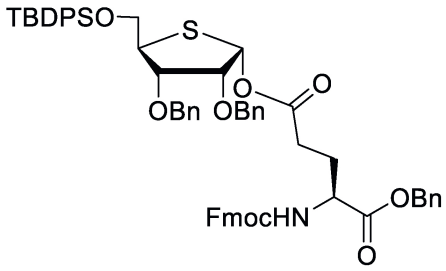
General procedure A using compound **26** (0.20 mmol, 0.124 g), Fmoc-Ser-OBn (0.16 mmol, 0.069 g), TMSOTf (40 µmol, 7 µl) and DCM (1.5 ml) was performed at -50 °C. Column chromatography (pentane: EtOAc, 95/5 – 80/20) yielded the title compound (0.047 mmol, 0.046 g, 30%) as an anomeric mixture (**α**:β, 65:35).

O$^{\beta}$-(2,3-bis-*O*-benzyl-5-tert-butyldiphenylsilyl-4-thio-α,β-D-ribosyl)-*N*$^{\alpha}$-fluorenylmethoxycarbonyl serine benzyl ester (30)



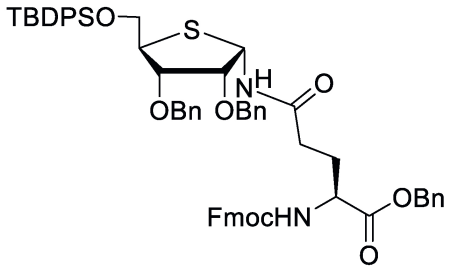
General procedure A compound **27** (0.08 mmol, 0.061 g), Fmoc-Ser-OBn (0.05 mmol, 0.021 g), TMSOTf (24 µmol, 4 µl) and DCM (0.5 ml) was performed at -50 °C. Column chromatography (pentane: EtOAc, 95/5 – 80/20) yielded the title compound (0.024 mmol, 0.024 g, 49%) as an anomeric mixture (**α**:β, 79:21). ^{1}H NMR (500 MHz, $CDCl_3$, **α**-anomer) δ: 7.71 (dd, *J* = 7.5, 4.8 Hz, 2H, arom.), 7.64 – 7.57 (m, 4H, arom), 7.54 (t, *J* = 7.5 Hz, 2H, arom.), 7.41 (t, *J* = 7.4 Hz, 2H, arom.), 7.38 – 7.14 (m, 23H, arom.), 6.33 (d, *J* = 9.0 Hz, 1H, NH), 5.18 – 5.08 (m, 3H, H1'; CH_2 Bn), 4.66 (s, 2H, CH_2 Bn), 4.62 – 4.52 (m, 2H, CH **α**-Ser; CH_2 β –Ser), 4.42 (AB, *J* = 11.8 Hz, 1H, CH_2 β –Ser), 4.34 (AB, *J* = 10.6, 7.0 Hz, 1H, CH_2 Fmoc), 4.23 (AB, *J* = 10.3, 7.9 Hz, 1H, CH_2 Fmoc), 4.20 – 4.15 (m, 1H, H3'), 4.12 (t, *J* = 7.4 Hz, 1H, CH Fmoc), 4.04 (AB, *J* = 9.1, 3.3 Hz, 1H, CH_2 Bn), 3.93 (AB, *J* = 9.3, 2.3 Hz, 1H, CH_2 Bn), 3.86 (t, *J* = 4.2 Hz, 1H, H2'), 3.67 (td, *J* = 5.6, 2.7 Hz, 1H, H4'), 3.61 (AB, *J* = 10.7 Hz, 1H, H5'), 3.47 (AB, *J* = 10.5 Hz, 1H, H5'), 1.02 (s, 9H, tBu). ^{13}C NMR (126 MHz, $CDCl_3$, **α**-anomer) δ: 170.52, 156.51, 144.29, 143.93, 141.37, 141.27, 138.43, 138.07 (Cq. arom.), 135.74 (arom.), 135.69 (arom.), 135.66 (Cq. arom.), 133.14 (Cq. arom.), 133.12 (Cq. arom.), 129.97, 128.62, 128.54, 128.38, 128.30, 128.15, 127.92, 127.91, 127.89, 127.87, 127.71, 127.66, 127.54, 127.16, 125.51, 125.31, 119.98, 119.94 (arom.), 85.10 (C1'), 82.70 (C2'), 80.56 (C3'), 72.60 (CH_2 Bn), 72.35 (CH_2β-Ser), 68.08 (CH_2 Bn), 67.24 (CH_2 Bn), 67.21 (CH_2 Fmoc), 65.73 (C5'), 54.39 (CH **α**-Ser), 50.94 (C4'), 47.24 (CH Fmoc), 26.97 (tBu), 19.38 (tBu). IR: 3435, 3068, 3039, 2931, 2857, 1723, 1506, 1452, 1338, 1265, 1196, 1103, 1081, 1028, 1007. HRMS: $[M+Na]^+$ calcd for $C_{60}H_{61}NO_8SSiNa$ 1006.3779, found 1006.3783.

O$^{\delta}$-(2,3-bis-*O*-benzyl-5-tert-butyldiphenylsilyl-4-thio-α,β-D-ribosyl)-*N*$^{\alpha}$-fluorenylmethoxycarbonyl glutamic acid benzyl ester (31)



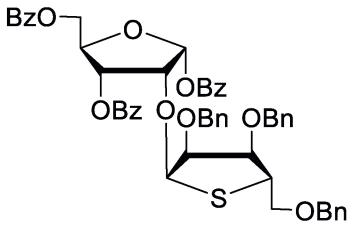
General procedure A using compound **27** (0.30 mmol, 0.227 g), Fmoc-Glu-OBn (0.20 mmol, 0.092 g), TMSOTf (60 µmol, 10 µl) and DCM (2 ml) was performed at -50 °C. Column chromatography (pentane: EtOAc, 90/10 – 70/30) yielded the title compound (0.125 mmol, 0.128 g, 63%) as an anomeric mixture (**α**:β, 37:63). ^{1}H NMR (400 MHz, MeOD) δ: 7.76 – 7.68 (m, 4H, arom.), 7.67 – 7.61 (m, 5H, arom.), 7.61 – 7.51 (m, 6H, arom.), 7.46 – 7.38 (m, 4H, arom.), 7.38 – 7.20 (m, 41H), 7.19 – 7.11 (m, 3H), 6.08 (d, *J* = 4.6 Hz, 1H, H1'), 5.96 (d, *J* = 2.0 Hz, 1H, H1''), 5.21 – 5.03 (m, 4H), 4.69 – 4.38 (m, 6H), 4.38 – 4.24 (m, 7H), 4.21 – 4.12 (m, 3H, H2'), 4.08 (dd, *J* = 8.2, 3.6 Hz, 2H), 3.94 (t, *J* = 4.4 Hz, 1H, H2''), 3.86 (dd, *J* = 10.7, 3.9 Hz, 1H), 3.79 (dd, *J* = 10.7, 5.7 Hz, 1H), 3.70 (ddd, *J* = 9.0, 5.6, 3.8 Hz, 1H), 3.62 – 3.49 (m, 1H), 3.39 (dd, *J* = 10.5, 7.6 Hz, 1H), 2.55 – 2.30 (m, 3H), 2.30 – 2.13 (m, 2H), 2.13 – 1.98 (m, 2H), 1.98 – 1.83 (m, 2H), 1.01 (s, 9H, tBu), 0.98 (s, 9H, tBu). ^{13}C NMR (101 MHz, MeOD) δ 172.00, 171.55, 171.49, 171.41, 156.72, 156.64, 143.40, 143.29, 143.19, 140.78, 137.51, 137.05, 137.01, 135.11, 135.03, 135.02, 134.98, 132.64, 132.44, 132.28, 132.26, 129.38, 129.26, 129.24, 127.95, 127.89, 127.88, 127.83, 127.75, 127.66, 127.57, 127.52, 127.49, 127.39, 127.34, 127.26, 127.17, 127.05, 126.52, 124.58, 124.55, 124.52, 124.48, 119.29, 81.11, 80.41, 79.87 (C1'), 79.65, 79.46, 76.96 (C1''), 72.24, 72.16, 71.57, 71.07, 66.54, 66.48, 66.44, 66.29, 64.98, 63.35, 53.34, 52.73, 50.12, 49.87, 46.64, 46.58, 30.45, 29.74, 26.05, 25.97, 25.91, 25.46, 18.61, 18.48. IR: 3067, 3031, 2955, 2931, 2858, 1725, 1451, 1505, 1451, 1333, 1288, 1253, 1228, 1203, 1179, 1162, 1111, 1105, 1085, 1070, 1053, 1028. HRMS: $[M+Na]^{+}$ calcd for $C_{62}H_{63}NO_9SSiNa$ 1048.3885, found 1048.3894.

N$^{\delta}$-(2,3-bis-*O*-benzyl-5-tert-butyldiphenylsilyl-4-thio-α-D-ribosyl)-*N*$^{\alpha}$-fluorenylmethoxycarbonyl glutamine benzyl ester (32)



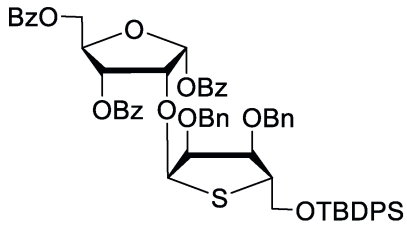
General procedure A using compound **27** (0.30 mmol, 0.227 g), Fmoc-Gln-OBn (0.20 mmol, 0.092 g), TMSOTf (60 µmol, 10 µl), DCM (2 ml) and dioxane (2 ml) was performed at -50 °C. Column chromatography (pentane: EtOAc, 90/10 – 70/30) yielded the title compound (0.154 mmol, 0.158 g, 77%) as an anomeric mixture (**α**:β, 86:14). ^{1}H NMR (500 MHz, MeOD, **α**-anomer) δ: 7.75 (d, *J* = 7.7 Hz, 2H, arom.), 7.65 – 7.56 (m, 7H, arom.), 7.43 (td, *J* = 7.5, 1.5 Hz, 2H, arom.), 7.39 – 7.13 (m, 21H, arom.), 5.53 (d, *J* = 5.7 Hz, 1H, H1'), 5.17 – 5.07 (m, 2H, CH_2 Bn), 4.68 – 4.54 (m, 3H), 4.54 – 4.39 (m, 3H), 4.39 – 4.27 (m, 3H), 4.27 – 4.11 (m, 4H, H3'; H4'; CH Fmoc), 3.97 (dd, *J* = 5.7, 3.4 Hz, 1H, H2'), 3.66 – 3.55 (m, 2H, H5'; CH **α**-Gln), 3.48 (td, *J* = 10.6, 4.8 Hz, 1H, H5'), 2.19 – 2.02 (m, 3H, CH_2 γ-Gln; CH β-Gln), 1.93 – 1.82 (m, 2H, CH β-Gln), 1.01 (s, 9H, tBu). ^{13}C NMR (126 MHz, MeOD, **α**-anomer) δ: 173.45, 173.02, 158.23, 145.04, 144.88, 142.36, 138.94, 138.75, 136.80 (Cq. arom.), 136.57 (arom.), 136.52 (arom.), 133.98 (Cq. arom.), 133.96 (Cq. arom.), 130.91, 130.90, 129.40, 129.39, 129.36, 129.17, 129.15, 129.03, 128.82, 128.76, 128.70, 128.62, 128.59, 128.01, 127.98, 126.10, 126.04, 120.74 (arom.), 83.10 (C3'), 81.35 (C2'), 73.44 (CH_2 Bn), 73.09 (CH_2 Bn), 67.86 (CH_2 Bn), 67.84 (CH_2 Fmoc), 66.66 (C5'), 55.24 (C1'), 54.75 (C4'), 50.46 (CH **α**-Gln), 48.22 (CH Fmoc), 33.40 (CH_2 γ-Gln), 28.02 (CH_2 β-Gln), 27.37 (tBu), 19.94 (tBu). IR: 3419, 3068, 3036, 2958, 2933, 2859, 1723, 1664, 1632, 1498, 1451, 1427, 1264, 1104, 1081, 1049, 1028, 1007. HRMS: $[M+Na]^+$ calcd for $C_{62}H_{64}N_2O_8SSiNa$ 1025.4225, found 1025.4240.

1-O-(1,3,5-tri-O-benzoyl-α-D-ribofuranosyl)-2,3,5-tri-O-benzyl-4-thio-α,β-D-ribofuranoside (33)



General procedure A using compound **20** (0.21 mmol, 0.099 g), 1,3,5-tri-O-benzoyl-benzoyl-α-D-ribofuranoside (0.21 mmol, 0.097 g), TMSOTf (21 µmol, 3.8 µl) and DCM (2 ml) was performed at -50 °C. Column chromatography (pentane: EtOAc, 90/10 – 70/30) yielded the title compound (0.06 mmol, 0.054 g, 29%) as an anomeric mixture (**α**:β, 4:96).

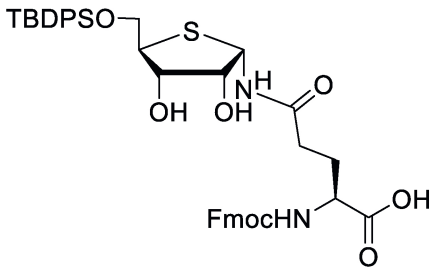
1-O-(1,3,5-tri-O-benzoyl-α-D-ribofuranosyl)-2,3-bis-O-benzyl-5-tert-butyldiphenylsilyl-4-thio-α,β-D-ribofuranoside (34)



General procedure A using compound **27** (0.08 mmol, 0.060 g), 1,3,5-tri-O-benzoyl-benzoyl-α-D-ribofuran-oside (0.05 mmol, 0.023 g), TMSOTf (16 µmol, 3 µl) and DCM (0.5 ml) was performed at -80 °C. Column chromatography (pentane: EtOAc, 95/5 – 80/20) yielded the title compound (0.06 mmol, 0.034 g, 66%) as an anomeric mixture (**α**:β, 91:9). ^{1}H NMR (500 MHz, $CDCl_3$, **α**-anomer) δ: 8.16 – 8.06 (m, 6H, arom.), 7.64 – 7.57 (m, 4H, arom.), 7.56 – 7.47 (m, 3H, arom.), 7.47 – 7.38 (m, 4H, arom.), 7.38 – 7.32 (m, 5H, arom.), 7.32 – 7.26 (m, 2H, arom.), 7.26 – 7.19 (m, 3H, arom.), 7.19 – 7.05 (m, 6H, arom.), 6.97 (d, *J* = 7.2 Hz, 2H, arom.), 6.81 (d, *J* = 4.1 Hz, 1H, H1' (O)), 5.69 (dd, *J* = 6.4, 1.7 Hz, 1H, H3' (O)), 5.53 (d, *J* = 4.4 Hz, 1H, H1' (S)), 4.84 (dd, *J* = 6.4, 4.1 Hz, 1H, H2' (O)), 4.77 – 4.71 (m, 1H, (H4' (O)), 4.64 (d, *J* = 3.2 Hz, 1H), 4.62 – 4.60 (m, 1H), 4.59 (s, 1H), 4.51 (dd, *J* = 12.0, 3.9 Hz, 1H), 4.46 – 4.37 (m, 2H), 4.33 (d, *J* = 12.0 Hz, 1H), 4.02 (t, *J* = 4.0 Hz, 1H, H3' (S)), 3.95 (t, *J* = 4.2 Hz, 1H, H2' (S)), 3.79 (tt, *J* = 6.5, 3.3 Hz, 1H, H4' (S)), 3.61 (dd, *J* = 6.2, 3.2 Hz, 2H, H5' (S)), 1.02 (s, 9H, tBu). ^{13}C NMR (126 MHz, $CDCl_3$, **α**-anomer) δ: 166.33, 166.15, 165.74, 138.23, 138.05, 135.70,

135.65, 133.41, 133.32, 133.22, 133.16, 133.11, 130.21, 130.15, 130.03, 129.98, 129.91, 129.69, 128.62, 128.48, 128.34, 128.24, 128.23, 127.87, 127.86, 127.60, 127.46, 127.39, 127.18, 95.04 (C1' (O)), 85.39 (C1' (S)), 83.35 (C4' (O)), 81.85 (C2' (S)), 80.40 (C3' (S)), 75.76 (C2' (O)), 72.53 (C3' (O)), 72.48, 72.17, 65.45 (C5' (S)), 64.36, 51.54 (C4' (S)), 26.92 (tBu), 19.35 (tBu). IR: 3063, 2959, 2932, 2859, 1722, 1452, 1428, 1312, 1264m 1177, 1111, 1068, 1025, 1001. HRMS: $[M+Na]^+$ calcd for $C_{61}H_{60}O_{11}SSiNa$ 1051.3518, found 1051.3523.

***N*$^{\delta}$-(5-tert-butyldiphenylsilyl-4-thio-α,β-D-ribosyl)-*N*$^{\alpha}$-fluorenylmethoxycarbonyl glutamine (35)**



Compound **34** (0.144 mmol, 0.148 g) was dissolved in DCM (2 ml) and a BCl_3 solution (1M in DCM) (0.6 ml, 0.6 mmol) was added at -80 °C. After the reaction was stirred for 1 hr it was quenched upon addition of triethylamine (0.25 ml) and methanol (1 ml). The reaction mixture was concentrated *in vacuo* and column chromatography (DCM: MeOH, 100/0 – 90/10) yielded the title compound (0.062 g, 0.082 mmol, 57%). ^{1}H NMR (400 MHz, MeOD) δ: 7.76 (d, *J* = 7.3 Hz, 2H, arom.), 7.72 – 7.53 (m, 8H, arom.), 7.50 – 7.08 (m, 10H, arom.), 5.53 – 5.41 (m, 1H, H1'α), 5.32 – 5.19 (m, 1H, H1'β), 4.51 – 4.29 (m, 2H), 4.20 (m, 4H), 4.09 – 3.86 (m, 2H), 3.74 (m, 1H), 3.67 (m, 3H), 3.15 (m, 1H), 2.40 – 2.09 (m, 3H, CH_2 γ-Gln; CH_2 β-Gln), 2.09 – 1.84 (m, 1H, CH_2 β-Gln), 1.04 (s, 9H, tBu). ^{13}C NMR (101 MHz, MeOD) δ: 173.70, 172.53, 156.72, 143.46, 143.28, 140.79, 140.04, 138.88, 134.98, 132.68, 132.61, 132.57, 129.27, 128.30, 128.25, 128.18, 128.04, 127.96, 127.92, 127.88, 127.82, 127.68, 127.48, 127.18, 126.88, 126.53, 126.34, 125.47, 124.59, 119.31, 77.88, 77.35, 75.55, 75.50, 74.07, 74.01, 73.83, 73.72, 73.68, 66.42 (C5'α), 66.20 (C5'β), 57.71 (C1'β), 55.10 (C1'α), 53.18, 53.11, 53.07, 51.77, 51.75, 51.62, 50.98, 46.63, 46.59, 46.57, 46.04, 41.42, 39.00, 32.03 (CH_2 γ-Gln), 31.68 (CH_2 γ-Gln), 29.10 (CH_2 β-Gln), 27.08 (CH_2 β-Gln), 25.96 (tBu), 18.56 (tBu), 7.88. IR: 3407, 3072, 2953, 2931, 2901, 2863, 1684, 1640, 1471, 1452, 1427, 1420, 1113, 1106. HRMS: $[M+H]^+$ calcd for $C_{41}H_{46}N_2O_8SSiH$ 755.2817, found 755.2817.

Solid phase synthesis

Peptide synthesis

The intermediate peptides were synthesized using standard, Fmoc-based solid phase peptide synthesis utilizing (pre-loaded) Tentagel® S AC purchased from Rapp Polymer Gmbh. Coupling cycles were as followed: Fmoc deprotection: 2x2 min, 1x5 min treatment with 20% piperidine in DMF. Coupling: treatment of 6 eq. amino acid, 6 eq. HCTU (0.25M in DMF) and 12 eq. DIPEA (1M in DMF) for 30 minutes. Capping: 2x2 min treatment of the resin with a 10% Ac_2O solution in DMF and catalytic DIPEA. Washing between the steps was done with DMF. Ribosylated amino acids **28**, **29** and **30** were incorporated in the sequence by adding a solution of 2 eq. building block in a 0.25M HCTU solution (2 eq.) in DMF and a 1M DIPEA solution (4 eq.) in DMF to the resin in a fritted syringe. The resin was shaken overnight and thoroughly washed.

On-resin phosphorylation

The resin was treated with a sufficient amount of 1M TBAF in THF for 30 minutes. The resin was thoroughly washed with DCM and DMF before the treatment was repeated once, furnishing the desilylated intermediate. The resin was then extensively washed with dry MeCN and flushed with nitrogen to remove traces of water before the resin was subjected to a solution of 5 eq. of $(FmO)_2PN(iPr)_2$ (0.25M in MeCN) with 10 eq. ETT solution (0.25M in MeCN). The resin was shaken for 30 minutes after which the resin was washed with MeCN. The resin was then treated with a sufficient amount of tBuOOH solution (0.5M in MeCN) for 30 minutes. The resin was then treated with a 10% DBU solution in DMF (2x 15 minutes) to furnish the crude, immobilized and deprotected phosphoribosylated peptide.

Construction of the pyrophosphates

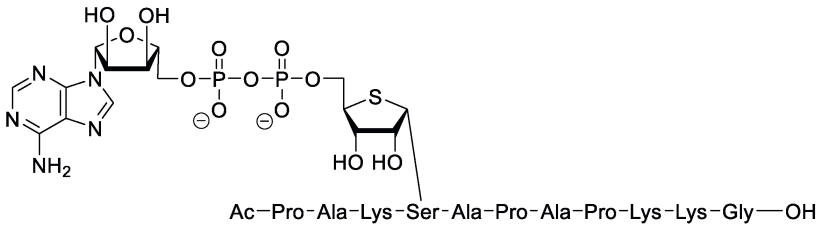
The resin was extensively washed with dry MeCN and flushed with nitrogen to remove traces of water. The resin was then treated with a solution of 5'-*O*-(N^6-*tert*-butyloxycarbonyl-2',3'-di-*O*-*tert*-butyldimethylsilyladenosine)-2-cyanoethyl-*N*-*N*-diisopropylphosphoramidite (3 eq., 0.3M in MeCN) and ETT (6 eq., 0.25M in MeCN) for 30 minutes. The resin was thoroughly washed with MeCN before a sufficient amount of tBuOOH (0.5M in MeCN) was added to the resin and shaken for 30 minutes.

Final deprotection and cleavage

The resin was treated with a 10% DBU solution in DMF (2x 10 minutes) to remove the cyano ethyl protecting group. The resin was then treated with a 1M TBAF solution in THF (2x 45 minutes) and washed with DMF followed by DCM. Final cleavage/deprotection occurred by treating the resin with a cleavage cocktail (2.5/10/87.5 TIS/TFA/DCM) for 4 hours. The crude products were collected by

filtration and the resin was washed with a solution of 1/1/1 water/*t*BuOH/MeCN. The solvents were evaporated *in vacuo* and co-evaporated with a 1/1/1 water/*t*BuOH/MeCN solution.

Ac-Pro-Ala-Lys-Ser(5-O-adenosine diphosphate-α-D-4-thio-ribosyl)-Ala-Pro-Ala-Pro-Lys-Lys-Gly-OH (1)



The general procedures described above were applied to 50 µmol Tentagel® S AC resin preloaded with glycine. The amino acids used were: Fmoc-Pro-OH, Fmoc-Ala-OH, Fmoc-Lys(Mtt)-OH and **2**. Oxidation steps were carried out with a 0.5M *t*BuOOH solution in MeCN. The crude peptide was purified by RP-HPLC in NH_4OAc buffer. The pure fractions were concentrated, co-evaporated extensively with a 1:1 mixture of MeCN:Milli-Q water, redissolved in Milli-Q water and lyophilized to obtain titled compound as a white solid (3.43 mg, 2.04 µmol, 4.1%). ^{1}H NMR (850 MHz, D_2O) δ 8.49 (s, 1H, H-2 adenine), 8.24 (s, 1H, H-8 adenine), 6.10 (d, J = 8.5 Hz, 1H, H-1' adenosine). ^{31}P NMR (202 MHz, D_2O) δ -10.72, -10.83, -11.04, -11.14. LC-MS (0 -> 50% B in A): Rt = 4.42. HRMS: $[M+2H]^{2+}$ calcd for $C_{64}H_{105}N_{19}O_{26}P_2S2H$ 825.8410; found 825.8408.

Expression plasmids and protein purification

The construction of the expression plasmids and the purification procedures were described earlier. Briefly, expression plasmids were transferred into Rossetta (DE3) cells and grown at 37 °C to an OD_{600} of 0.6 in LB medium supplemented with 1% (w/v) D-glucose and appropriate antibiotics. For (ADP-ribosyl)hydrolases (ARH1, ARH2, and ARH3) the medium was further enriched by addition of 2 mM $MgSO_4$. Expression was induced with 0.4 mM isopropyl-β-D-1-thioglactopyranoside (IPTG) and cultured were allowed to grow overnight at 17 °C. Cultures were harvested by centrifugation, pellets resuspended in lysis buffer (50 mM TrisHCl [pH 8], 500 mM NaCl and 25 mM imidazole) and stored at -20 °C until use. Proteins were purified by Ni^{2+}-NTA chromatography (Jena Bioscience) according to the manufacturer's protocol using the following buffers: all buffers contained 50 mM TrisHCl (pH 8) and 500 mM NaCl; additionally, the lysis buffer contained 25 mM, the washing buffer 40 mM, and the elution buffer 500 mM imidazole. Proteins were dialyzed overnight against 50 mM TrisHCl (pH 8), 200 mM NaCl, 1 mM dithiothreitol and 5% (v/v) glycerol and stored at -80 °C. For the

purification of ARH1, ARH2, and ARH3 all purification buffers were additionally supplemented with 10 mM $MgCl_2$.

(ADP-ribosyl)hydrolase activity assay

The peptide demodification assay was described earlier. Briefly, peptide concentrations for the assay were estimated using absorbance at l260nm using the molar extinction coefficient of ADP-ribose (13,400 M^{-1} cm^{-1}). 10 µM indicated peptide were demodified by incubation with 0.5 µM hydrolase for 60 min at 30 °C in assay buffer (50 mM TrisHCl [pH 8], 200 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol and 0.2 µM human NUDT5). Reactions were stopped and analysed by performing the AMP-Glo™ assay (Promega) according to the manufacturer's protocol. Luminescence was recorded on a SpectraMax M5 plate reader (Molecular Devices) and data analysed with GraphPad Prism 7. Control reactions were carried out in absence of peptide.

4.5 References

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