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Synthetic peptides, nucleic acids and molecular probes to study ADP-Ribosylation

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Chapter 2

Synthesis of α - and
 β -Ser-ADP-Ribosylated Peptides

Introduction

Post-translational modifications (PTMs) of proteins are involved in many biological processes and entail covalent and reversible modification of specific amino acid side chains.^[1] Adenosine diphosphate ribosylation (ADP-ribosylation) is a PTM that has been associated with DNA damage response pathways, transcriptional regulation, ageing and apoptosis.^[2] ADP-ribosylation is catalyzed by a series of enzymes known as adenosine ribosyltransferases (ARTs).^[3] In this process, ARTs consume β -NAD⁺ and transfer ADP-ribose (ADPr) onto nucleophilic side chains of various amino acids, including arginine (Arg),^[4] glutamate/aspartate (Glu, Asp),^[5,6] and serine (Ser).^[7,8] This mono-ADP-ribosylated (MARylated) site can then be modified further by some ARTs forming long and also branched poly-ADPr (PAR) chains.

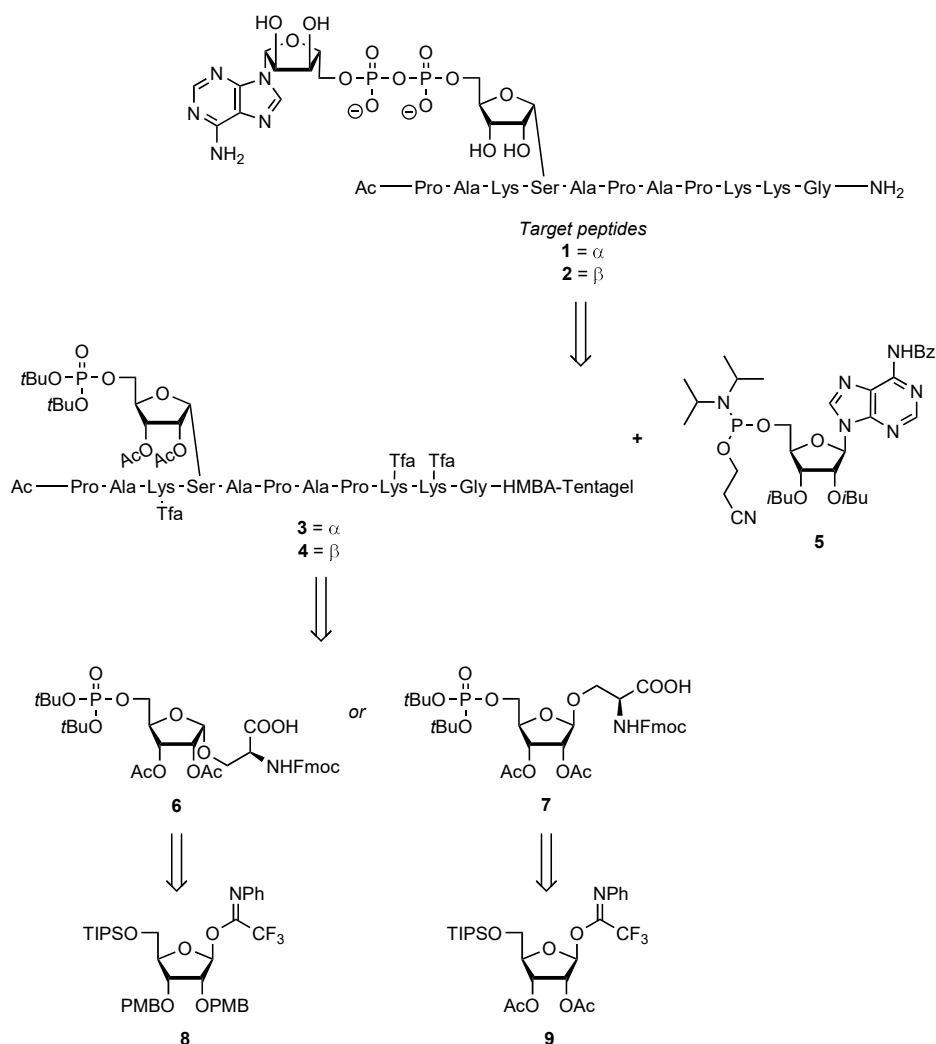
Recently, Ser residues were shown to be both acceptors of ADPr^[8] and the primary target of ADP-ribosylation upon DNA damage.^[9] Many of the proteins identified as Ser-ADP-ribosylated are critical for the maintenance of genome stability.^[10,11] Moreover, amino acid selectivity in this pathway appears to be dependent on a switch of the catalytic preference of the ARTs PARP1 and PARP2 from Glu/Asp toward Ser residues upon the formation of a newly identified PARP1:HPF1 complex.^[10,12] Transfer of ADPr by PARP1 onto Glu and Asp residues involves stereochemical inversion at the anomeric carbon and results in the formation of α -glycosidic linkages as the sole products of the reaction.^[13] However, in case of Ser-ADPr and the previously unknown PARP1:HPF1 complex, this α -selectivity is not yet confirmed. Studies of this complex as well as other ADP-ribosylation systems has been restrained by the unavailability of sufficient quantities of well-defined fragments of Ser-ADPr containing peptides. This chapter describes the preparation of such Ser-ADPr peptides from phosphoribosylated Fmoc-Ser building blocks designed specifically for the incorporation in peptide chains via a modified Fmoc SPPS. These building blocks were applied in a solid phase approach similar to the one previously described for the synthesis of ADPr-peptides that contained Gln-, Asn-, or Cit-ADPr,^[14] which are close isosteres of the native Glu-, Asp- and Arg-ADPr linkages. In this way, an *N*-terminal H2B peptide was prepared that contains Ser10, which is a confirmed *in vivo* ADPr acceptor site.^[8,11] Preparation of the Ser-ADPr H2B peptide allowed for unambiguously determining the anomeric configuration of the ribose residue and thus to gain early mechanistic insights into the Ser-ADPr biochemistry.

Results and discussion

As shown in Scheme 1, retrosynthetic analysis of the target mono-ADP-ribosylated peptides **1** and **2** relies on the $P^{III} - P^V$ method as described in Chapter 1. The intermediary phosphoribosylated peptides **3** and **4** (α - and β -configured respectively) can be coupled with adenosine 5'-phosphoramidite **5** as phosphorylating agent.^[15] Adenosine amidite **5** as well as intermediates **3** and **4** bear base-labile protecting groups (e.g. acetyl/isobutyryl, Ac and *i*Bu, on the 2' and 3' hydroxyls and trifluoroacetyl, Tfa, on the side chain of lysines (Lys)) which are to be removed in the final stage of the synthesis. To furnish **3** and **4**, key phosphoribosylated Ser building blocks **6** (α) and **7** (β) were chosen to allow for the orthogonal cleavage of the benzyl ester prior to SPPS. Furthermore, the phosphates in **6** and **7** are protected with *tert*-butyl (tBu) groups to enable the on-resin orthogonal deprotection and ensuing pyrophosphate formation via a procedure developed by Kistemaker *et al.*^[14] In order to obtain the α -configured phosphoribosylated **6** in a stereoselective manner, donor **8** was synthesized for its known α -selectivity.^[16] For a β -selective glycosylation to furnish **7**, it was expected that participation of the 2-O-acetyl group will steer the nucleophilic attack of the acceptor to the β -face leading to the design of donor **9**.

Building block synthesis

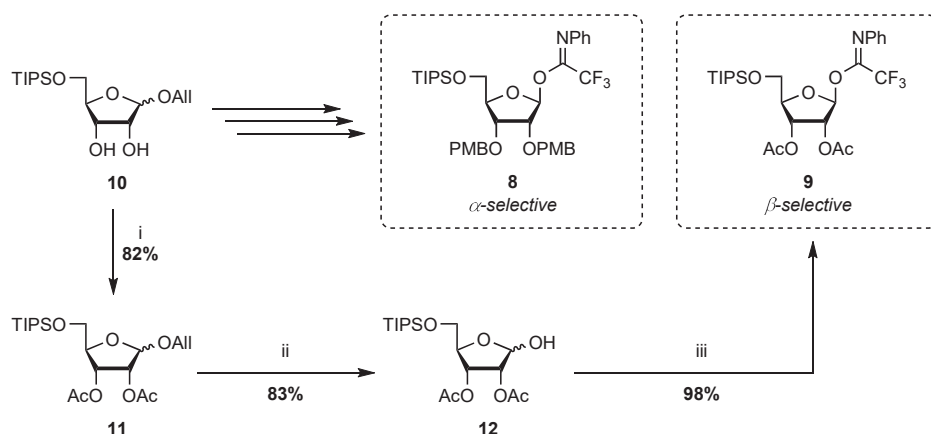
Known ribosyl donor **8** was prepared according to a reported procedure.^[16] Synthesis of β -steering donor **9** could be accomplished via a three-step sequence from allyl 5-O-triisopropylsilyl- α,β -D-ribofuranoside **10** (Scheme 2), an intermediate towards donor **8**. Acetylation of the 2- and 3-OH groups followed by Pd mediated cleavage of the anomeric allyl group furnished alcohol **12** which was then converted to *N*-phenyltrifluoroacetimidate donor **9**.



Scheme 1. Retrosynthetic analysis for the synthesis of mono-ADP-ribosylated peptides **1** and **2**.

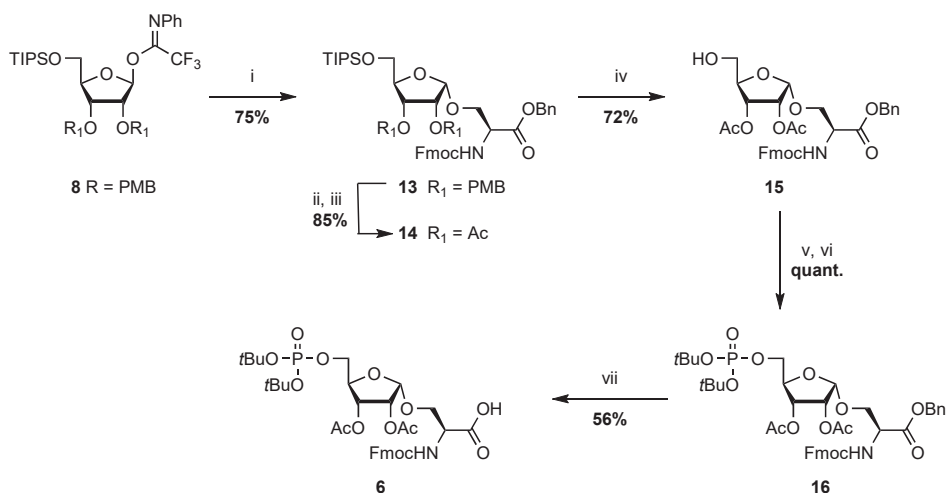
Having prepared the required ribofuranosyl donors, an α -selective glycosylation of Fmoc-Ser-OBn was performed with donor **8** (Scheme 3). When 0.2 eq of TMSOTf was employed at $-50\text{ }^{\circ}\text{C}$, α -configured ribosylated Ser **13** was isolated in 75% yield, while the corresponding β -product could not be detected. Subsequent cleavage of the PMB protecting groups in **13** using a previously reported HCl/HFIP cocktail^[17] (0.1 M) gave unsatisfactory yields due to acid-mediated hydrolysis of the Ser moiety. Although lowering the concentration of HCl (down to 0.01 M) in the mixture reduces the cleavage of the *O*-glycosidic bond, scaling up of the reaction resulted again in moderate yields. Fortunately, treatment of **13** with

TFA in DCM showed no sign of acidolysis of the *O*-glycosidic bond and upscaling of the reaction combined with the subsequent acetylation of the crude intermediate gave **14** in reproducible yields that exceeded 80% over two steps. Removal of the silyl ether in **14** using TEA·3HF proceeded uneventfully, while treatment with pyr·HF was accompanied by Fmoc cleavage. Phosphitylation of the primary hydroxyl in **15** using di-*tert*-butyl *N,N*-di-*iso*-propylphosphoramidite, and subsequent oxidation of the phosphite intermediate yielded phosphotriester **16**. Finally, hydrogenolysis of the benzyl ester gave orthogonally protected, α -phosphoribosylated Ser building block **6**.



Scheme 2. Synthesis of the α - and β -selective ribosyl donors **8** and **9**. Reagents and conditions: i) Ac₂O, pyr., rt. ii) PdCl₂, O₂, CHCl₃, H₂O, 45 °C. iii) Cl(C=NPh)CF₃, Cs₂CO₃, acetone, H₂O, rt.

For the β -selective glycosylation of Fmoc-Ser-OBn with *N*-phenyltrifluoroacetimidate donor **9**, different reaction conditions proved to be necessary. The conditions that were used for α -selective glycosylation (−50 °C, 0.2 eq. of activator, entry 1 Table 1) gave a low yield and a significant amount of acetyl migration to the Ser aglycon giving **19** as a significant side product.^[18] Therefore, an optimization of the β -selective glycosylation reaction with respect to the donor/activator ratio and the reaction temperature was performed (Table 1). Increasing the temperature to 0 °C (entry 2) not only improved the yield of **17** from 5% to 21% but also led to the formation of desilylated product **18** in 16% yield, giving a combined yield of 37%. Noteworthy, this acid-catalyzed desilylation was not observed in the α -directed glycosylation with donor **8**. Upon further increase of the temperature to room temperature (entry 3) **17** was not isolated but only the desilylated product **18** was obtained in a low yield. A reduced amount of activator (0.1 eq.) applied either at 0 °C or at room temperature (entries 4 and 5) increased the yields of both products significantly. As a result, the conditions in entry 4 (0 °C with 0.1 eq. TMSOTf) providing a cumulative yield of 55% were selected as the optimum conditions for β -selective glycosylation of Fmoc-Ser-OBn with donor **9**.



Scheme 3. Synthesis of orthogonally protected α -phosphoribosylated Ser building block **6**. Reagents and conditions: i) Fmoc-Ser(OH)-OBn, TMSOTf, DCM, -50 °C. ii) TFA, DCM, rt. iii) Ac₂O, pyr., rt. iv) TEA·3HF, THF, 0 °C to rt. v) (tBuO)₂PN(iPr)₂, 1-methyl imidazole, 1-methylimidazolium chloride, DMF, rt. vi) tBuOOH, nonane, rt. vii) H₂, 10 wt% Pd/C, tBuOH, 1,4-dioxane, H₂O.

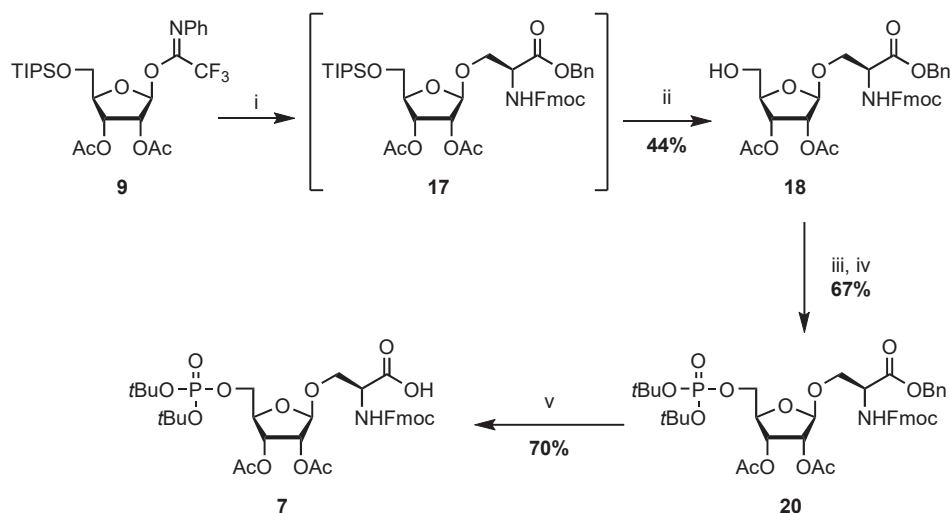
Table 1. optimization of the glycosylation of β -selective donor **6** with Fmoc-Ser(OH)-OBn.

Entry	T (°C)	Activator (eq.)	Yield 17 (%)	Yield 18 (%)	Cumulative yield (%)
1	-50	0.2	5	n.d.	5
2	0	0.2	21	16	37
3	rt.	0.2	0	16	16
4	0	0.1	32	23	55
5	rt.	0.1	13	21	34

Thus, the route to β -phosphoribosylated Ser building block **7**, depicted in Scheme 4, started by condensation of donor **9** with Fmoc-Ser-OBn using the optimized conditions described above. Due to the partial desilylation observed, the crude mixture was immediately treated with TEA·3HF to produce **18** in 44% yield over two steps after silica gel chromatography. Analogous to the synthesis of α -phosphoribosylated Ser, standard phosphitylation of building block **18** ensuing oxidation gave **20**. Finally, cleavage of the benzyl ester furnished β -phosphoribosylated building block **7**.

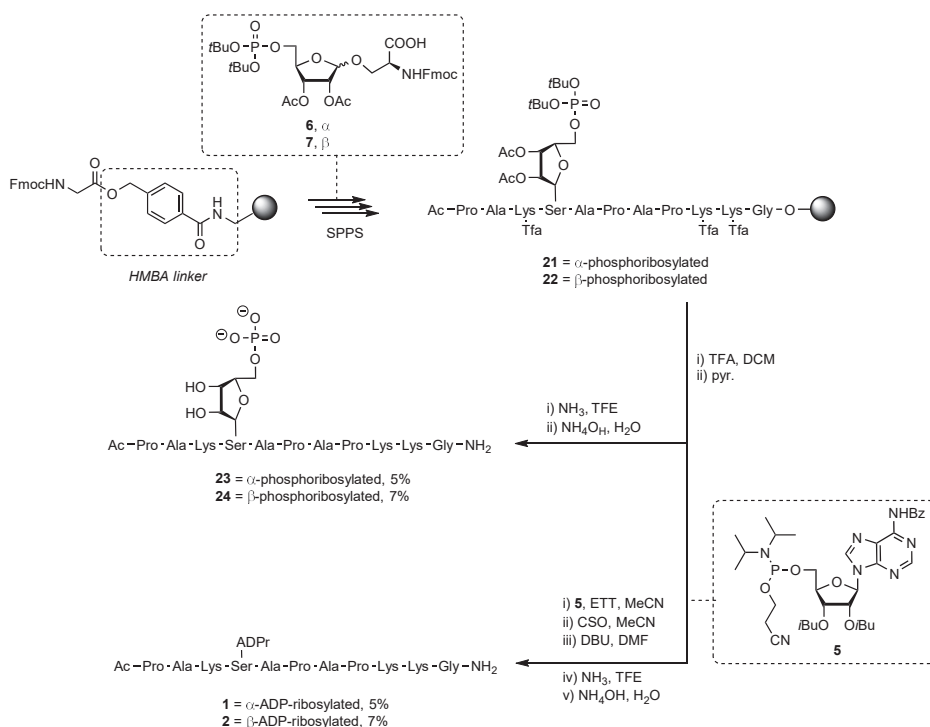
Peptide synthesis

Solid phase peptide synthesis (SPPS) of hendecapeptides **1** and **2** was performed using TentaGel® resin equipped with HMBA-linker as solid support (Scheme 5). Immobilized peptides **21** and **22** were assembled with standard Fmoc SPPS, utilizing trifluoroacetyl protected Lys (Fmoc-Lys(Tfa)-OH), Fmoc-Pro-OH, Fmoc-Ala-OH, Fmoc-Gly-OH and building blocks **6** or **7**. The *t*Bu groups of the phosphotriester were then removed by treatment of the immobilized peptides **21** and **22** with TFA in DCM. The conversion of the phosphotriester to the phosphate monoester could be monitored on-resin by ³¹P-NMR (a shift of the phosphorus resonance from approximately -9 ppm for the phosphotriester to -4 ppm for the phosphodiester to 0 ppm for the unprotected phosphomonoester). After complete removal of the *t*Bu groups, the resin was washed with pyridine to obtain the more stable pyridinium salt of the phosphomonoester. To obtain phosphoribosylated peptides **23** and **24**, the protected phosphoribosylated intermediates were cleaved from the resin by treatment with a saturated NH₃ solution in 2,2,2-trifluoroethanol (TFE). The use of TFE ensures the formation of only carboxamide at the C-terminus of the target peptide chain and excludes formation of a carboxylic acid.^[19] Finally, to effect complete removal of all protecting groups (i.e. the (trifluoroacetyl groups), NH₄OH was added, furnishing α- and β-phosphoribosylated peptides **23** and **24**.



Scheme 4. Synthesis of the β-phosphoribosylated Ser building block **20**. Reagents and conditions: i) Fmoc-Ser(OH)-OBn, TMSOTf, DCM, 0 °C. ii) TEA·3HF, THF, 0 °C to rt. iii) *t*BuO)₂PN(*i*Pr)₂, 1-methylimidazole, 1-methylimidazolium chloride, DMF, rt. iv) *t*BuOOH, nonane, rt. v) H₂, 10 wt% Pd/C, *t*BuOH, 1,4-dioxane, H₂O.

Next the synthesis of ADP-ribosylated peptides **1** and **2** was undertaken. Removal of the *t*Bu groups in the immobilized fully protected peptides **21** and **22** to the corresponding phosphomonoesters was followed by the installation of the pyrophosphate via a three step procedure developed by Kistemaker *et al.*^[14] On-resin treatment of the obtained phosphate monoesters with activator ETT and phosphoramidite **5**, subsequent immediate oxidation of the $P^{III} - P^V$ intermediate species with CSO and, finally, removal of the cyanoethyl group with DBU gave the immobilized and unprotected pyrophosphate. Similar conditions as described for phosphoribosyl peptides **23** and **24** were then used for the cleavage from the resin and deprotection of the ADP-ribosylated peptides to furnish, after purification, homogeneous α - and β -ADP-ribosylated peptides **1** and **2**, respectively.



Scheme 5. Solid phase peptide synthesis of the α - and β -ADP-ribosylated peptides **1** and **2** and their phosphoribosylated derivatives **23** and **24**. Tfa = trifluoroacetyl.

Chemical and enzymatic degradation of peptides **1** and **2**

Having obtained Ser-ADPr peptides **1** and **2**, the native stereochemistry at the anomeric centre of the ribose residue attached to the side chain of Ser was investigated. Human ARH3 is the only enzyme known to hydrolyze the *O*-glycosidic Ser-ADPr linkage.^[20] A homologue of ARH3, termed ARH1, is a stereoselective ADP-ribosylarginine hydrolase.^[21,22]

It was hypothesized that ARH3 is likewise only capable of hydrolyzing glycosidic linkages with the native stereochemistry, leaving the non-natural epimer intact. Accordingly, a de-MARylation assay by treating the homogeneous α - and β -configured H2B peptides **1** and **2** with hARH3 was performed. In addition, the reaction mixture contained human NUDT5 (hNUDT5), which converts the released ADPr into AMP, and thus allows detection of the peptide turnover by a commercial assay. As expected, no turnover was observed either by hNUDT5 alone^[23] or in the presence of the catalytically inactive hARH3 double mutant (D77N D78N) (Figure 1). On the other hand, wild-type hARH3 exclusively hydrolyzes the α anomeric Ser-ADPr linkage. Since no spontaneous epimerization in solution was observed previously, this data strongly suggest that the α -linked form is the endogenous epimer.

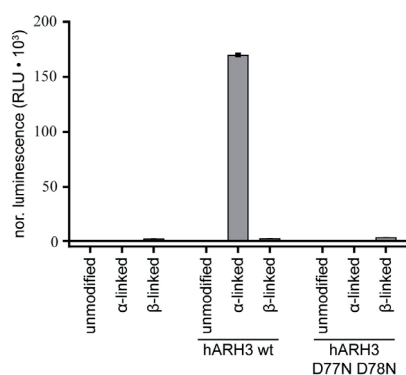


Figure 1. Analysis of the stereospecificity of hARH3. ADPr released in the ARH3 reaction was converted into AMP using hNUDT5 and subsequently measured using the AMP-Glo assay (Promega). Control reactions were carried out both in absence of hARH3 as well as using a catalytically inactive hARH3 mutant (D77N D78N). The data were normalized to reactions containing only hNUDT5 and represent triplicate measurement $\pm$ SD.

After confirming the stereochemistry of the naturally occurring Ser-ADPr modification, the chemical stability of the native α -Ser-ADPr was tested. Such knowledge about the stability of ADP-ribosylation sites is relevant for the future synthetic and proteomics studies and for a retrospective evaluation of already documented observations. A set of chemically divergent conditions were chosen (aqueous acid, diluted NaOH and 0.5 M NH_2OH) as these are broadly encountered in proteomic studies for identifying PAR- and MARylation sites (NH_2OH , NaOH)^[22,24] or in the course of sample preparation (aqueous acid).^[7] Peptide **1** was dissolved in aqueous solutions of either 0.1 M TFA, 0.1 M NaOH, or 0.5 M NH_2OH , and after various time points (15 min, 60 min, 2.5 h, 5 h, and 24 h), a sample was taken for LC-MS analysis of the quality of the peptide. For TFA and NH_2OH , no detectable degradation was observed after 24 hours. This leads to the conclusion that the isolation and purification of Ser-ADPr-peptides can safely be performed under acidic conditions and that the treatment

of ADPr-proteins with NH_2OH is indeed selective toward Asp and Glu residues since Ser-ADPr is unreactive toward this nucleophile.^[24] However, treatment of our MArYlated peptide **1** with 0.1 M aqueous NaOH (pH 13) showed a measurable elimination of ADP-ribose after 15 minutes (7%, Table 2). After 2.5 hours, the eliminated ADPr was partially hydrolyzed, giving phosphoribose and adenosine monophosphate (AMP). In a time span of 5 hours, more than half of the starting material was converted into a mixture of ADPr or AMP and complete degradation ensued after 24 hours. This elimination reaction leads to transformation of Ser-ADPr into dehydroalanine at the modification site, a reaction not possible with amino acids such as Asp, Glu, or Arg. This result is in line with the known behavior of Ser O-glycopeptides, in which the glycosyl linkage is also cleaved by β -elimination upon treatment with base.^[25] The formation of dehydroalanine residue has been exploited in studies on phosphoproteomics^[26,27] and glycomics,^[28] and the present observation that Ser-ADPr sites behave similarly points to a possibility of extending such “foot printing” to the ADP-ribosylome as well.

Table 2. Elimination of ADPr from Ser and its subsequent hydrolysis of to phosphoribose and AMP.

The diagram illustrates the chemical reaction of ADPr elimination from a serine residue. In the first step, a hydroxide ion (OH^-) acts as a base, abstracting an α -proton from the serine residue of a peptide. This results in the formation of a dehydroalanine residue and the release of ADP-ribose (ADPr). In the second step, the released ADPr undergoes further hydrolysis in the presence of OH^- , breaking down into phosphoribose and adenosine monophosphate (AMP).

t (h)	Intact ADPr peptide (%)	Free ADPr (%)	Free AMP (%)
0	100	0	0
0.25	93	7	0
1	85	15	0
2.5	66	26	8
5	45	32	23
24	0	50	50

Conclusion

In conclusion, suitably protected phosphoribosylated Ser building blocks have been synthesized and used for the SPPS-mediated assembly of H2B-derived peptides, MARYlated on their Ser residues both in α - and β -glycosidic form, via an SPPS based strategy. The work presented in this chapter showed the synthesis of the first Ser-ADPr-peptides via a modified literature procedure.^[14] The thus prepared H2B-derived ADPr-peptides were then used to investigate fundamental, but as yet unknown, properties of the Ser-ADPr modification. The striking specificity of ARH3 for Ser-ADPr substrates was utilized to gain insight into its substrate preference and by extension the stereoselectivity by which the PARP1-HPF1 complex catalyzes MARYlation of Ser residues. ARH3 catalysis resulted only in the hydrolysis of the α -linked peptide, thus strongly implying that this is the natural epimer. Also, since the Ser O-ribosidic bond is chemically distinct from the formerly studied Asp, Glu, and Arg modifications, the stability of this linkage was tested under conditions typically encountered in biochemical and proteomic studies. Ser-ADPr proved to be stable for a minimum of 24 hours when subjected to TFA (0.1 M) and NH_2OH (0.5 M) with no detectable degradation. In contrast, when Ser-ADPr was treated with NaOH (0.1 M) β -elimination occurred, in which the glycosidic bond was cleaved to give dehydroalanine residue and free ADPr.

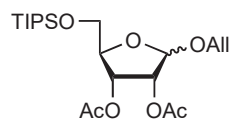
Experimental section

General synthetic procedures

All reagents were used as received unless stated otherwise. Solvents used in synthesis were dried and stored over 4 Å molecular sieves, except for MeOH and MeCN which were stored over 3 Å molecular sieves. Triethylamine (TEA) and diisopropylethylamine (DIPEA) were stored over KOH pellets. Column chromatography was performed on silica gel 60 Å (40–63 μ m, Macherey-Nagel). TLC analysis was performed on Macherey-Nagel aluminium sheets (silica gel 60 F₂₅₄). TLC was used to visualize compounds by UV at wavelength 254 nm and by spraying with either cerium molybdate spray (25 g/L (NH₄)₆Mo₇O₂₄, 10 g/L (NH₄)₄Ce(SO₄)₂·H₂O in 10% H₂SO₄ water solution) or KMnO₄ spray (20 g/L KMnO₄ and 10 g/L K₂CO₃ in water) followed by charring at c.a. 250 °C. LC-MS analysis was performed on a Finnigan Surveyor HPLC system with a Nucleodur C18 Gravity 3 μ m 50 x 4.60 mm column (detection at 200–600 nm) coupled to a Finnigan LCQ Advantage Max mass spectrometer with ESI or coupled to a Thermo LCQ Fleet Ion mass spectrometer with ESI. The method used was 10→90% 13.5 min (0→0.5 min: 10% MeCN; 0.5→8.5 min: 10% to 90% MeCN; 8.5→11 min: 90% MeCN; 11→13.5 min: 10% MeCN) or 0→50% 13.5 min. High resolution mass spectra were recorded by direct injection (2 μ L of a 2 μ M 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 (mass range m/z = 150–2,000) and dioctylphthalate (m/z = 391.2843) as a “lock mass”. The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). HPLC purification was performed on an Agilent 1200 series using a 6130 quadrupole MS equipped with a Develosil C30 column, size 10.0/250. NMR spectra were recorded on a Bruker AV-400 or AV-600 NMR. Chemical shifts (δ) are given in ppm relative to tetramethyl silane as internal standard. Coupling constants (J) are given in Hz. For the peptides, a small amount of EDTA was added to the NMR sample to sharpen the peaks for ³¹P-NMR. All given ¹³C-APT spectra are proton decoupled. Optical rotation measurements were performed on an Anton Paar MCP100 modular circular polarimeter. FT-IR spectra were recorded on a Shimadzu IRAffinity-1 FT-IR spectrometer.

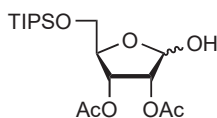
2,3-*O*-Acetyl-1-*O*-allyl-5-*O*-triisopropylsilyl- α,β -D-ribofuranoside (11)

Compound **10**^[14] (1.04 g, 3.0 mmol) was dissolved in pyridine (6.0 mL, 0.5 M). Ac₂O (1.13 mL, 12.0 mmol, 4 eq.) was added and the reaction was stirred overnight. The reaction was diluted with EtOAc and transferred into a separatory funnel. The reaction was subsequently washed with 1 M HCl, sat. aq. NaHCO₃ and brine. The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. Flash column chromatography (10% EtOAc in pentane) yielded the title compound as a clear oil (α and β combined 1.06 g, 2.47 mmol, 82%). *α -anomer* ¹H NMR (400 MHz, CDCl₃) δ 5.90 (ddt, J = 16.4, 10.8, 5.4 Hz, 1H, OCH₂CHCH₂), 5.39–5.30 (m, 2H, H-3 + OCH₂CHCH₂), 5.28 (d, J = 4.1 Hz, 1H, H-1), 5.18 (dd, J = 10.4, 1.8 Hz, 1H, OCH₂CHCH₂), 5.03 (dd, J = 7.0, 4.8 Hz, 1H, H-2), 4.25 (dd, J = 13.5, 3.0 Hz, 1H, OCH₂CHCH₂), 4.17 (d, J = 3.2 Hz, 1H, H-4), 4.10 (dd, J = 13.7, 6.2 Hz, 1H, OCH₂CHCH₂), 3.95 (dd, J = 10.9, 2.8 Hz, 1H, H-5_a), 3.84 (dd, J = 11.0, 2.9 Hz, 1H, H-5_b), 2.18–2.02 (m, 6H, 2x Ac), 1.18–0.98 (m, 21H, TIPS). ¹³C NMR (101 MHz, CDCl₃) δ 170.53, 169.85 (C=O Ac), 134.21 (OCH₂CHCH₂), 116.90 (OCH₂CHCH₂), 99.46 (C-1), 82.88 (C-4), 71.36 (C-2), 70.53 (C-3), 68.41 (OCH₂CHCH₂), 63.37 (C-5), 20.88, 20.57 (CH₃ Ac), 17.88 (CH TIPS), 11.87 (CH₃ TIPS). *β -anomer* [α]_D²⁰: -22.2° (c = 1.0, CHCl₃). IR cm⁻¹: 2934, 2866, 1750, 1464, 1368, 1239, 1215, 1128, 1067, 881, 756. ¹H NMR (400 MHz, CDCl₃) δ 5.87 (ddt, J = 15.8, 10.8, 5.5 Hz, 1H, OCH₂CHCH₂), 5.41 (dt, J = 6.7, 3.6 Hz, 1H, H-3), 5.34–5.22 (m, 2H, H-2 + OCH₂CHCH₂), 5.18 (dt, J = 10.7, 2.0 Hz, 1H, OCH₂CHCH₂), 5.05 (s, 1H, H-1), 4.27–4.14 (m,



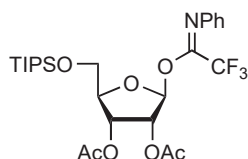
2H, H-4 + OCH₂CHCH₂), 4.04 – 3.91 (m, 1H, OCH₂CHCH₂), 3.91 – 3.73 (m, 2H, H-5), 2.10 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.14 – 1.00 (m, 21H, TIPS). **¹³C NMR** (101 MHz, CDCl₃) δ 169.63 (C=O Ac), 133.68 (OCH₂CHCH₂), 117.29 (OCH₂CHCH₂), 104.09 (C-1), 81.32 (C-4), 75.00 (C-2), 72.30 (C-3), 68.35 (OCH₂CHCH₂), 64.72 (C-5), 20.61 (CH₃ Ac), 20.54 (CH₃ Ac), 17.90 (CH TIPS), 11.88 (CH₃ TIPS). **HRMS**: [C₂₁H₃₈O₇Si + Na]⁺ found: 453.2277, calculated: 453.2279

2,3-O-Acetyl-1-5-O-triisopropylsilyl-α,β-D-ribofuranoside (12)



Compound **11** (995 mg, 2.31 mmol) was dissolved in a 3:1 (v/v) mixture of CHCl₃:H₂O (total volume 12 mL, 0.2 M) and PdCl₂ (62 mg, 0.35 mmol, 0.15 eq.) was added. Oxygen gas was bubbled through the solution for 15 minutes while the temperature was raised to 45°C. The reaction was stirred vigorously for 2 days under oxygen atmosphere. The reaction mixture was carefully concentrated *in vacuo* and the residue was dissolved in 12 mL EtOAc and 12 mL sat. aq. NaHCO₃. Iodine (1 eq.) was added and the reaction was transferred into a separating funnel and shaken. The organic layer was separated and washed with sat. aq. Na₂S₂O₃. The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. Flash column chromatography yielded the title compound as a clear oil (750 mg, 1.92 mmol, 83%, α:β ratio: 0.6:1). **¹H NMR** (400 MHz, CDCl₃) δ 5.56 (td, *J* = 5.3, 2.1 Hz, 1H, β H-3), 5.50 (d, *J* = 4.6 Hz, 1H, α H-1), 5.44 (dt, *J* = 6.0, 1.9 Hz, 1H, α H-3), 5.27 (s, 1H, β H-1), 5.22 (d, *J* = 5.1 Hz, 1H, β H-2), 5.20 – 5.14 (m, 1H, α H-2), 4.27 (t, *J* = 2.3 Hz, 1H, α H-4), 4.21 (dt, *J* = 5.2, 2.6 Hz, 1H, β H-4), 3.99 – 3.77 (m, 4H, α + β H-5), 2.15 – 2.04 (m, 12H, α + β Ac), 1.13 – 1.01 (m, 42H, α + β TIPS). **¹³C NMR** (101 MHz, CDCl₃) δ 170.02, 169.98, 169.88, 169.68 (α + β C=O Ac), 100.27 (β C-1), 95.61 (α C-1), 82.95 (α C-4), 82.61 (β C-4), 76.77 (β C-2), 71.86 (α C-3), 71.55 (α C-2), 71.22 (β C-3), 63.57, 63.37 (α + β C-5), 20.89, 20.65, 20.61, 20.59 (α + β CH₃ Ac), 17.95, 17.91, 17.88 (α + β CH TIPS), 11.89 (α + β CH₃ TIPS). **IR cm⁻¹**: 3456, 29492, 2866, 1746, 1464, 1369, 1220, 1090, 1066, 1013, 881, 754, 680. **HRMS**: [C₁₈H₃₄O₇Si + Na]⁺ found: 413.1968, calculated: 413.1966

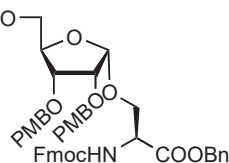
2,3-O-Acetyl-1-((N-phenyl)-2,2,2-trifluoroacetimido)-5-O-triisopropylsilyl-α,β-D-ribofuranoside (9)



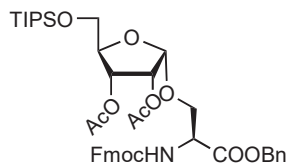
Compound **12** (623 mg, 1.60 mmol) was dissolved in acetone (8 mL, 0.2 M) and H₂O (0.16 mL) was added. Cs₂CO₃ (782 mg, 2.4 mmol, 1.5 eq.) and 2,2,2-trifluoro-N-phenylacetimidoyl chloride (0.36 mL, 2.4 mmol, 1.5 eq.) were added and the reaction was stirred overnight. The reaction was filtered and concentrated *in vacuo*. Flash column chromatography (5 → 10% EtOAc in pentane) yielded the title compound as a pale oil (α and β combined 880 mg, 1.57 mmol, 98%). **¹H NMR** (400 MHz, CDCl₃) δ 7.32 – 7.27 (m, 3H Ph arom.), 7.10 (t, *J* = 7.5 Hz, 1H, Ph arom.), 6.82 (d, *J* = 7.1 Hz, 2H, Ph arom.), 6.32 (bs, 1H, H-1), 5.57 (d, *J* = 3.4 Hz, 1H, H-2), 5.50 (t, *J* = 5.5 Hz, 1H, H-3), 4.33 (dt, *J* = 6.2, 4.6 Hz, 1H, H-4), 3.90 (d, *J* = 4.6 Hz, 2H, H-5), 2.12 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.10 – 1.06 (m, 21H, TIPS). **¹³C NMR** (101 MHz, CDCl₃) δ 169.77, 169.53 (C=O Ac), 143.59 (Cq Ph), 128.82, 124.41, 119.54 (CH arom. Ph), 101.54 (C-1), 83.36 (C-2), 74.50 (C-3), 71.18 (C-4), 63.79 (C-5), 20.63 (CH₃ Ac), 17.97 (CH TIPS), 11.93 (CH₃ TIPS). **IR cm⁻¹**: 2943, 2867, 1753, 1713, 1599, 1451, 1322, 1237, 1205, 1153, 1088, 881, 776, 690.

O-(2,3-O-di-(4-methoxybenzyl)-5-O-triisopropylsilyl- α -D-ribose)-N-fluorenylmethoxycarbonyl serine benzyl ester (13)

Fmoc-Ser(OH)-OBn (877 mg, 2.10 mmol, 1.0 eq.) together with donor **8** (1.65 g, 2.30 mmol, 1.1 eq.) was co-evaporated twice with 1,4-dioxane and once with DCE before the mixture was dissolved in DCM (23 mL, 0.09 M). The solution was cooled to -50°C before TMSOTf (42 μL , 0.23 mmol, 0.1 eq. relative to the donor **5**) was added. The reaction was stirred for 90 minutes at -50°C before TLC showed full conversion of the acceptor and a higher running product. The reaction was quenched by the addition of TEA and concentrated *in vacuo*. Flash column chromatography (0.5 $\rightarrow$ 3% acetone in DCM) yielded the title compound as a colorless oil (1.64 g, 1.73 mmol, 75%). **Rf**: 0.6 in 3% acetone in DCM. $[\alpha]_{\text{D}}^{20} = +21^{\circ}$ ($c = 1.0$, CHCl_3). **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 7.72 (dd, $J = 7.6, 3.9$ Hz, 2H, Fmoc arom.), 7.56 (d, $J = 7.4$ Hz, 2H, Fmoc arom.), 7.41 – 7.16 (m, 9H, Fmoc arom. + Bn arom. + CHCl_3), 6.90 – 6.70 (m, 4H, PMB arom.), 6.60 (d, $J = 9.0$ Hz, 1H, NH), 5.23 – 5.12 (m, 2H, CH_2 Bn.), 4.91 (d, $J = 4.1$ Hz, 1H, H-1), 4.69 – 4.56 (m, 2H, CH Ser + CH_2 PMB), 4.53 – 4.45 (m, 3H, 2 CH_2 PMB), 4.39 (dd, $J = 10.3, 6.8$ Hz, 1H, CH_2 Fmoc), 4.18 (m, 4H, CH_2 Fmoc + CH Fmoc + CH_2 Ser + H-4), 4.02 – 3.92 (m, 2H, CH_2 Ser + H-3), 3.76 (m, 4H, CH_3 PMB + H-2), 3.69 (s, 3H, CH_3 PMB), 3.66 – 3.55 (m, 2H, H-5), 0.98 (m, 21H, TIPS). **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 170.56 (C=O COOBn), 159.40, 159.21 (C-OMe PMB), 156.57 (C=O Fmoc), 144.27, 143.87, 141.32, 141.23 (Cq arom. Fmoc), 135.69 (Cq arom. Bn), 130.43, 129.97 (Cq arom. PMB), 129.84, 129.52, 128.58, 128.22, 128.09, 127.68, 127.62, 127.12, 127.06 (CH arom. Fmoc + Bn + PMB), 125.48, 125.22, 119.96, 119.93 (CH Fmoc arom.), 113.84–113.72 (CH PMB arom.), 101.13 (C-1), 84.44 (C-4), 78.44 (C-2), 75.16 (C-3), 72.33, 72.03 (CH_2 PMB), 67.52 (CH_2 Ser), 67.19 (CH_2 Fmoc), 67.15 (CH_2 Bn), 63.74 (C-5), 55.31, 55.24 (CH_3 PMB), 54.70 (CH Ser), 47.17 (CH Fmoc), 18.03, 17.99 (CH TIPS), 11.94 (CH_3 TIPS). **IR cm^{-1}** : 2941, 2864, 1722, 1610, 1512, 1246, 1029, 908, 729. **HRMS** [$\text{C}_{55}\text{H}_{67}\text{NO}_{11}\text{Si} + \text{Na}$]: 968.4398 found, 968.4375 calculated.

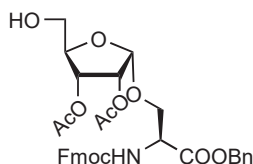
**O-(2,3-O-diacetyl-5-O-triisopropylsilyl- α -D-ribose)-N-fluorenylmethoxycarbonyl serine benzyl ester (14).**

Compound **13** (1.64 g, 1.73 mmol) was dissolved in DCM (35 mL, 0.05 M) and TFA (1.0 mL, 13.5 mmol, 7.8 eq.) was added. Next, the reaction was stirred for 30 minutes. TLC showed full conversion of starting material and a product on the baseline (3:97 acetone:DCM). The reaction was diluted with toluene (30 mL) and the reaction was concentrated *in vacuo*. The diol was co-evaporated thrice with toluene to remove traces of TFA. Then residue was dissolved in pyridine (17.5 mL, 0.1 M) and Ac_2O (3.3 mL, 34.6 mmol, 20 eq.) was added. The reaction was stirred overnight after which TLC showed full conversion of the diol. The reaction was diluted with DCM and transferred into a separating funnel. The organic layer was washed with 1 M HCl, sat. aq. NaHCO_3 , and brine. The organic layer was collected, dried over MgSO_4 , and concentrated *in vacuo*. Flash column chromatography (1 $\rightarrow$ 3% acetone in DCM) yielded the title compound as a white foam (1163 mg, 1.47 mmol, 85%). **Rf**: 0.58 in 2% acetone in DCM. $[\alpha]_{\text{D}}^{20} = +47.5^{\circ}$ ($c = 1.0$, CHCl_3). **$^1\text{H NMR}$** (400 MHz, CDCl_3) δ 7.81 – 7.72 (m, 2H, Fmoc arom.), 7.58 (t, $J = 6.9$ Hz, 2H, Fmoc arom.), 7.44 – 7.23 (m, 9H, Fmoc arom. + Bn arom. + CHCl_3), 5.98 (d, $J = 9.2$ Hz, 1H, NH), 5.40 (dd, $J = 6.4, 2.0$ Hz, 1H, H-3), 5.21 (d, $J = 4.4$ Hz, 1H, H-1), 5.18 (m, 2H, CH_2 Bn), 4.98 (dd, $J = 6.4, 4.4$ Hz, 1H, H-2), 4.57 (dt, $J = 9.3, 2.8$ Hz, 1H, CH Ser), 4.50 (dd, $J = 10.7, 6.6$ Hz, 1H, CH_{2a} Fmoc), 4.41 (dd, $J = 10.6, 7.0$ Hz, 1H, CH_{2b} Fmoc), 4.23 (t, $J = 6.8$ Hz, 1H, CH Fmoc), 4.08 (q, $J = 2.5$ Hz, 1H, H-4), 3.99 (qd, $J = 10.1, 2.9$ Hz, 2H, CH_2 Ser), 3.82 (ddd, $J = 30.3, 11.0, 2.5$ Hz, 2H, H-5), 2.00 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.08 – 1.03 (m, 21H, TIPS). **$^{13}\text{C NMR}$** (101 MHz, CDCl_3) δ 170.40, 170.20, 169.96 (C=O COOBn/Ac), 156.20 (C=O Fmoc), 144.0, 143.79, 141.45, 141.40 (Cq arom.



Fmoc), 135.43 (Cq arom. Bn), 128.75, 128.54, 128.12, 127.85, 127.82, 127.20, 127.13 (CH arom. Fmoc + Bn), 125.09, 124.97, 120.14, 120.10 (CH arom. Fmoc), 100.14 (C-1), 83.28 (C-4), 71.83 (C-2), 70.80 (C-3), 67.50 (CH₂ Ser), 67.32 (CH₂ Bn), 67.01 (CH₂ Fmoc), 63.47 (C-5), 54.34 (CH Ser), 47.25 (CH Fmoc), 20.68, 20.55 (CH₃ Ac), 18.05 (CH TIPS), 18.02 (CH 11.99 (CH₃ TIPS). **IR** cm⁻¹: 2943, 2866, 1732, 1506, 1221, 1057, 1030, 908, 729. **HRMS**: [C₄₃H₅₅NO₁₁Si + Na]⁺: 812.3454 found, 812.3436 calculated.

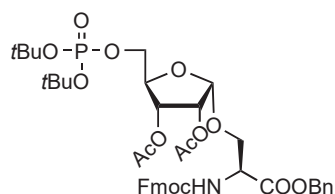
O-(2,3-O-diacetyl- α -D-ribose)-N-fluorenylmethoxycarbonyl serine benzyl ester (15)



Compound **14** (1.16 g, 1.47 mmol, 1.0 eq.) was dissolved in THF (15 mL, 0.1 M) and cooled to 0 °C. TEA·3HF (4.8 mL, 29.4 mmol, 20 eq.) was added slowly and the reaction was stirred three days while slowly allowed to warm to room temperature. TLC showed full conversion of the starting material into a lower running product. The reaction was cooled to 0 °C and carefully quenched by the addition of sat. aq. NaHCO₃. The reaction

was transferred into a separating funnel and extracted with EtOAc. The organic layers were combined, dried over MgSO₄, filtered and concentrated *in vacuo*. Flash column chromatography (3 → 15% acetone in DCM) yielded the title compound as a white foam (673 mg, 1.06 mmol, 72%). **Rf**: 0.32 in 10% acetone in DCM. **[α]_D²⁰** = +69.9° (c = 1.0, CHCl₃). **¹H NMR** (400 MHz, CDCl₃) δ 7.79 (d, J = 7.5 Hz, 2H, Fmoc arom.), 7.65 – 7.59 (m, 2H, Fmoc arom.), 7.47 – 7.30 (m, 9H, Fmoc arom. + Bn arom.), 5.95 (d, J = 9.1 Hz, 1H, NH), 5.29 (dd, J = 6.9, 3.1 Hz, 1H, H-3), 5.24 (d, J = 4.3 Hz, 1H, H-1), 5.23 – 5.19 (m, 2H, CH₂ Bn), 4.93 (dd, J = 6.9, 4.3 Hz, 1H, H-2), 4.60 (dt, J = 9.3, 2.8 Hz, 1H, CH Ser), 4.54 (dd, J = 10.7, 6.6 Hz, 1H, CH₂ Fmoc), 4.46 (dd, J = 10.8, 6.9 Hz, 1H, CH₂ Fmoc), 4.26 (t, J = 6.7 Hz, 1H, CH Fmoc), 4.11 (q, J = 3.2 Hz, 1H, H-4), 4.08 – 3.95 (m, 2H, CH₂ Ser), 3.85 – 3.68 (m, 3H, H-5 + OH), 2.05 (s, 3H, Ac), 2.02 (s, 3H, Ac). **¹³C NMR** (101 MHz, CDCl₃) δ 170.47, 170.08, 170.01 (C=O COOBn/Ac), 156.14 (C=O Fmoc), 143.98, 143.75, 141.47, 141.42 (Cq arom. Fmoc), 135.34 (Cq arom. Bn), 128.77, 128.60, 128.20, 127.88, 127.86, 127.16 (CH arom. Fmoc + Bn), 125.07, 124.98, 120.15, 120.12 (CH arom. Fmoc), 100.39 (C-1), 82.45 (C-4), 71.49 (C-2), 70.20 (C-3), 67.99 (CH₂ Ser), 67.43 (CH₂ Bn), 66.99 (CH₂ Fmoc), 62.27 (C-5), 54.33 (CH Ser), 47.26 (CH Fmoc), 20.65, 20.52 (CH₃ Ac). **IR** cm⁻¹: 3431, 2939, 1738, 1511, 1451, 1369, 1219, 1055, 1027, 974, 739, 698. **HRMS**: [C₃₄H₃₅NO₁₁ + Na]⁺: 656.2118 found, 656.2102 calculated.

O-(2,3-O-diacetyl-5-O-(di-*tert*-butyl)-phosphoryl- α -D-ribose)-N-fluorenylmethoxycarbonyl serine benzyl ester (16)



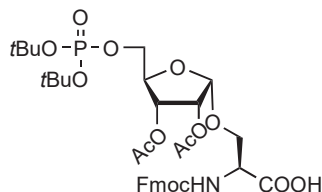
Compound **15** (668 mg, 1.05 mmol, 1.0 eq.) was co-evaporated thrice with 1,4-dioxane and dissolved in a 0.2 M 1-methyl-imidazole and 0.3 M 1-methyl-imidazole-HCl solution in DMF (14.2 mL, 0.1 M). Di-*tert*-butyl-*N,N*-diisopropylphosphoramidite (167 μL, 2.12 mmol, 1.5 eq.) was added and the reaction was stirred for 20 minutes. TLC indicated full conversion (*R_f* = 1.0, 1:9 acetone:DCM) after which *t*BuOOH (5.5 M in nonane, 2.58 mL, 14.2 mmol, 10 eq.)

was added. After 120 minutes, ³¹P-NMR indicated full conversion of the amidite into the protected phosphate. The reaction was quenched by the addition of sat. aq. NaHCO₃ and transferred into a separating funnel. The water layer was extracted with Et₂O and the combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. Flash column chromatography (30 → 50% EtOAc in pentane) yielded the title compound as a white foam in quantitative yield. **[α]_D²⁰** = +53.6° (c = 1.0, CHCl₃). **¹H NMR** (400 MHz, CDCl₃) δ 7.76 (d, J = 7.6 Hz, 2H, Fmoc arom.), 7.59 (t, J = 6.6 Hz, 2H, Fmoc arom.), 7.45 – 7.29 (m, 9H, Fmoc arom. + Bn arom.), 5.87 (d, J = 9.2 Hz, 1H, NH), 5.34 (dd, J = 6.7, 2.5 Hz, 1H, H-3), 5.23 (d, J = 4.3 Hz, 1H, H-1), 5.17 (s, 2H, CH₂ Bn), 4.92 (dd, J = 6.7, 4.3 Hz, 1H, H-2), 4.57 (dt, J = 9.3, 2.9 Hz, 1H, CH Ser), 4.51 (dd, J = 10.7, 6.5 Hz, 1H, CH₂ Fmoc), 4.42 (dd, J = 10.7, 6.9 Hz, 1H, CH₂ Fmoc), 4.23 (t, J = 6.7 Hz, 1H,

CH Fmoc), 4.15 (p, $J = 4.0, 3.5$ Hz, 1H, H-4), 4.14 – 4.03 (m, 2H, H-5), 3.98 (d, $J = 2.9$ Hz, 2H, CH₂ Ser), 2.00 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.52 – 1.47 (s, 18H, tBu). **¹³C NMR** (101 MHz, CDCl₃) δ 170.09, 170.01, 169.72 (C=O COOBn/Ac), 156.04 (C=O Fmoc), 143.88, 143.65, 141.35, 141.30 (Cq arom. Fmoc), 135.26 (Cq arom. Bn), 128.67, 128.49, 128.08, 127.78, 127.76, 127.08 (CH arom. Fmoc + Bn), 124.97, 124.86, 120.06, 120.01 (CH arom. Fmoc), 100.02 (C-1), 82.93, 82.90, 82.86, 82.83 (Cq tBu), 80.79, 80.71 (C-4), 71.31 (C-2), 70.21 (C-3), 67.49 (CH₂ Ser), 67.29 (CH₂ Bn), 66.89 (CH₂ Fmoc), 65.79, 65.73 (C-5), 54.18 (CH Ser), 47.14 (CH Fmoc), 29.87, 29.86, 29.83, 29.81 (CH₃ tBu), 20.48, 20.38 (CH₃ Ac). **³¹P NMR** (162 MHz, CDCl₃) δ -9.35. **IR cm⁻¹**: 2942, 1744, 1512, 1450, 1371, 1242, 1082, 1037, 996, 741. **HRMS**: [C₄₂H₅₂NO₁₄P + Na]⁺: 848.3043 found, 848.3017 calculated.

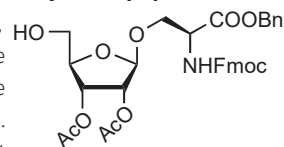
O-(2,3-O-diacetyl-5-O-(di-tert-butyl)-phosphoryl- α -D-ribose)-N-fluorenylmethoxycarbonyl serine (6)

Compound **16** (1.15 g, 1.39 mmol) was dissolved in a 4:4:1 v/v mixture of tBuOH:1,4-dioxane:H₂O (total volume 27.8 mL, 0.05 M). The reaction was purged with nitrogen and Pd/C (10 wt.% 172 mg) was added. Hydrogen gas was then bubbled through the reaction mixture for 1 hour. TLC indicated full conversion of the starting material. The reaction was purged with nitrogen gas and filtered through a pad of Celite. The reaction was concentrated *in vacuo* and flash column chromatography (2 → 10% MeOH in DCM, 1 v/v% AcOH) yielded the title compound as a white foam (568 mg 0.77 mmol, 56%). **Rf**: 0.47 in 10% MeOH in DCM + AcOH. **[α]_D²⁰** = +66.0° ($c = 1.0$, CHCl₃). **¹H NMR** (400 MHz, CDCl₃) δ 10.55 (s, 1H COOH), 7.75 (d, $J = 7.6$ Hz, 2H, Fmoc. arom.), 7.59 (t, $J = 7.6$ Hz, 2H, Fmoc. arom.), 7.39 (t, $J = 7.4$ Hz, 2H, Fmoc. arom.), 7.33 – 7.25 (m, 2H, Fmoc. arom.), 5.86 (d, $J = 8.9$ Hz, 1H, NH), 5.31 (dd, $J = 6.7, 2.6$ Hz, 1H, H-3), 5.26 (d, $J = 4.3$ Hz, 1H, H-1), 4.93 (dd, $J = 6.7, 4.3$ Hz, 1H, H-2), 4.54 (dt, $J = 9.2, 3.2$ Hz, 1H, CH Ser), 4.48 (dd, $J = 10.6, 6.7$ Hz, 1H, CH₂ Fmoc), 4.40 (dd, $J = 10.6, 7.0$ Hz, 1H, CH₂ Fmoc), 4.27 – 4.15 (m, 2H, CH Fmoc + H-4), 4.15 – 4.03 (m, 2H, H-5), 4.03 – 3.90 (m, 2H, CH₂ Ser), 2.02 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.48 (s, 18H, tBu). **³¹P NMR** (162 MHz, CDCl₃) δ -10.17. **¹³C NMR** (101 MHz, CDCl₃) δ 176.37 (C=O COOH), 170.33, 170.08 (C=O Ac), 156.16 (C=O Fmoc), 143.92, 143.73, 141.35 (Cq arom. Fmoc), 127.82, 127.13, 125.08, 124.98, 120.09, 120.06 (CH arom. Fmoc), 100.33 (C-1), 83.67, 83.64, 83.60, 83.57 (Cq tBu), 80.82, 80.73 (C-4), 71.28 (C-2), 70.22 (C-3), 67.73 (CH₂ Ser), 67.02 (CH₂ Fmoc), 66.08, 66.02 (C-5), 54.01 (CH Ser), 47.18 (CH Fmoc), 29.89, 29.84 (CH₃ tBu), 20.58 (CH₃ Ac), 20.36 (CH₃ Ac). **IR cm⁻¹**: 3421, 2981, 1739, 1512, 1373, 1238, 1041, 1002, 744. **HRMS**: [C₃₅H₄₆NO₁₄P + Na]⁺: 758.2567 found, 758.2548 calculated.



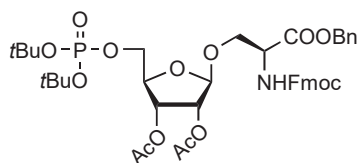
O-(2,3-O-diacetyl- β -D-ribose)-N-fluorenylmethoxycarbonyl serine benzyl ester (18)

Fmoc-Ser-OBn (584 mg, 1.40 mmol, 1.0 eq.) together with donor **9** (851 mg, 1.52 mmol, 1.1 eq.) was co-evaporated twice with 1,4-dioxane and once with DCE before the mixture was dissolved in DCM (15 mL, 0.1 M). The solution was cooled to 0 °C before TMSOTf (27 μ L, 0.15 mmol, 0.1 eq. relative to donor **6**) was added. The reaction was stirred for 1 hour at 0 °C before TLC (3% acetone in DCM) showed full conversion of the starting material into both the 5'-silylidene protected product ($R_f = 0.58$) and its deprotected equivalent. The reaction was quenched by adding three drops of TEA and concentrated *in vacuo*. Flash column chromatography (0 → 10% acetone in DCM) obtained 87 mg of the title compound and 669 mg of a 1 : 2 mixture of Fmoc-Ser(Ac)-OBn and protected product (calculated by ¹H-NMR). This mixture was dissolved in THF (8.5 mL, 0.1 M) and the solution was cooled to 0 °C. TEA·3HF (2.77 mL, 17 mmol, 20 eq.) was added and the reaction was stirred for three days while the reaction temperature was slowly allowed to warm to room temperature. The reaction was cooled to 0 °C and carefully quenched by the addition of sat. aq.



NaHCO₃. The mixture was transferred into a separating funnel and extracted with EtOAc. The organic layers were combined, were dried over MgSO₄, filtered and concentrated. Flash column chromatography (5 → 10% acetone in DCM) yielded the title compound as a white foam (combined yield of 391 mg, 0.62 mmol, 44%). **Rf**: 0.16 in 3% acetone in DCM. $[\alpha]_D^{20} = -1.40^\circ$ ($c = 1.0$, CHCl₃). **¹H NMR** (400 MHz, CDCl₃) δ 7.77 (d, $J = 7.5$ Hz, 2H, Fmoc arom.), 7.62 (dd, $J = 7.5, 3.6$ Hz, 2H, Fmoc arom.), 7.44 – 7.28 (m, 9H Fmoc arom. + Bn arom.), 6.00 (d, $J = 8.6$ Hz, 1H, NH), 5.34 (dd, $J = 6.9, 4.9$ Hz, 1H, H-3), 5.27 (d, $J = 5.0$ Hz, 1H, H-2), 5.27 – 5.15 (m, 2H, CH₂ Bn), 4.98 (s, 1H, H-1), 4.58 (dt, $J = 8.6, 3.3$ Hz, 1H, CH Ser), 4.45 (dd, $J = 10.5, 7.0$ Hz, 1H, CH₂ Fmoc), 4.36 (dd, $J = 10.5, 7.1$ Hz, 1H, CH₂ Fmoc), 4.28 – 4.17 (m, 2H, CH Fmoc + CH₂ Ser), 4.14 (ddd, $J = 6.7, 3.6, 2.7$ Hz, 1H, H-4), 3.82 (dd, $J = 10.4, 3.7$ Hz, 1H, CH₂ Ser), 3.73 (dd, $J = 12.6, 2.8$ Hz, 1H, H-5_a), 3.51 (dd, $J = 12.6, 3.7$ Hz, 1H, H-5_b), 2.12 (s, 3H, Ac), 2.07 (s, 3H, Ac). **¹³C NMR** (101 MHz, CDCl₃) δ 170.47, 170.11, 169.74 (C=O COOBn/Ac), 156.30 (C=O Fmoc), 144.04, 143.87, 141.38 (Cq arom. Fmoc), 135.15 (Cq arom. Bn), 128.76, 128.64, 128.51, 127.83, 127.81, 127.26, 127.24 (CH arom. Fmoc + Bn), 125.33, 125.29, 120.07 (CH arom. Fmoc), 105.90 (C-1), 82.16 (C-4), 75.45 (C-2), 70.21 (C-3), 68.85 (CH₂ Ser), 67.88 (CH₂ Bn), 67.36 (CH₂ Fmoc), 61.68 (C-5), 54.56 (CH Ser), 47.22 (CH Fmoc), 20.77, 20.73 (CH₃ Ac). **IR cm⁻¹**: 3368, 2921, 1745, 1722, 1519, 1450, 1370, 1240, 1215, 1045, 739. **HRMS**: [C₃₄H₃₅NO₁₁ + Na]⁺ found: 656.2108, calculated: 656.2102.

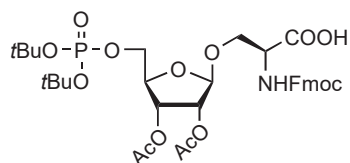
O-(2,3-O-diacetyl-5-O-(di-tert-butyl)-phosphoryl-β-D-ribose)-N-fluorenylmethoxycarbonyl serine benzyl ester (20)



Compound **18** (343 mg, 0.54 mmol, 1.0 eq.) was co-evaporated thrice with 1,4-dioxane before dissolved in a 0.2 M 1-methylimidazole and 0.3 M 1-methylimidazole-HCl solution in DMF (5.4 mL, 0.1 M). Di-tert-butyl-*N,N*-diisopropylphosphoramidite (256 μ L, 0.81 mmol, 1.5 eq.) was added and the reaction was stirred for 20 minutes. TLC indicated full conversion ($R_f = 1.0$, 1:9 acetone:DCM) and tBuOOH (5.5 M in nonane, 0.64 mL, 3.5 mmol, 10 eq.) was added. After 135 minutes, ³¹P-NMR indicated full conversion of the amidite into the protected phosphate. The reaction was quenched by the addition of sat. aq. NaHCO₃ and transferred into a separating funnel. The water layer was extracted with Et₂O and the organic layers were combined, dried over MgSO₄, filtered and concentrated in vacuo. Flash column chromatography (30 → 50% EtOAc in pentane) yielded the title compound as a white foam (298 mg, 0.36 mmol, 67%). **Rf**: 0.45 in 50% EtOAc in pentane. $[\alpha]_D^{20} = -9.30^\circ$ ($c = 1.0$, CHCl₃). **¹H NMR** (400 MHz, CDCl₃) δ 7.75 (d, $J = 7.5$ Hz, 2H, Fmoc arom.), 7.66 (t, $J = 7.2$ Hz, 2H, Fmoc arom.), 7.43 – 7.27 (m, 9H, Fmoc + Bn arom.), 6.51 (d, $J = 8.7$ Hz, 1H, NH), 5.28 (d, $J = 5.0$ Hz, 1H, H-2), 5.26 – 5.16 (m, 3H, CH₂ Bn + H-3), 5.05 (s, 1H, H-1), 4.65 (ddd, $J = 8.7, 5.4, 3.3$ Hz, 1H, CH Ser), 4.40 (dd, $J = 10.4, 7.4$ Hz, 1H, CH_{2a} Fmoc), 4.36 – 4.27 (m, 2H, CH_{2b} Fmoc + H-4), 4.27 – 4.19 (m, 2H, CH Fmoc + CH_{2a} Ser), 4.08 – 3.98 (m, 2H, H-5), 3.75 (dd, $J = 10.0, 3.4$ Hz, 1H, CH_{2b} Ser), 2.10 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.47 (m, $J = 5.6$ Hz, 18H, tBu). **¹³C NMR** (101 MHz, CDCl₃) δ 169.71, 169.59, 169.55 (C=O COOBn/Ac), 156.40 (C=O Fmoc), 143.97, 143.88, 141.20 (Cq Fmoc), 135.29 (Cq Bn), 128.58, 128.35, 128.23, 127.62, 127.07 (CH arom. Fmoc + Bn), 125.35, 119.87 (CH Fmoc arom.), 105.51 (C-1), 82.87, 82.79, 82.76, 82.69 (Cq tBu), 79.97, 79.89 (C-4), 74.63 (C-2), 71.58 (C-3), 67.47 (CH₂ Ser), 67.32, 67.30, 67.23, 67.17 (CH₂ Fmoc, CH₂ Bn, C-5), 54.47 (CH Ser), 47.06 (CH Fmoc), 29.83, 29.78 (CH₃ tBu), 20.58, 20.51 (CH₃ Ac). **³¹P NMR** (162 MHz, CDCl₃) δ -9.18. **IR cm⁻¹**: 2978, 1747, 1716, 1517, 1370, 1239, 1214, 1038, 989, 759, 739. **HRMS**: [C₄₂H₅₂NO₁₄P + Na]⁺ found: 848.3040, calculated: 848.3017.

O-(2,3-O-diacetyl-5-O-(di-tert-butyl)-phosphoryl- β -D-ribose)-N-fluorenylmethoxycarbonyl serine (7)

Compound **20** (298 mg, 0.36 mmol) was dissolved in a 4:4:1 v/v mixture of *t*BuOH:1,4-dioxane:H₂O (total volume 7.2 mL, 0.05 M). The reaction was purged with nitrogen and Pd/C (10 wt.%, 54 mg) was added. Hydrogen gas was then bubbled through the reaction mixture for 2 hours. TLC indicated full conversion of the starting material. The reaction was purged with nitrogen



gas and filtered through a pad of Celite. The reaction was concentrated *in vacuo* and flash column chromatography (1 $\rightarrow$ 5% MeOH in DCM, 1 v/v% AcOH) yielded the title compound as a white foam (185 mg 0.25 mmol, 70%). **RF:** 0.45 in 5% MeOH in DCM + AcOH. **[α]^D** = +10.40° (*c* = 1.0, CHCl₃). **¹H NMR** (400 MHz, CDCl₃) δ 7.76 (dt, *J* = 7.6, 1.3 Hz, 2H, Fmoc arom.), 7.69 – 7.62 (m, 2H, Fmoc arom.), 7.44 – 7.37 (m, 2H, Fmoc arom.), 7.33 (tdd, *J* = 7.4, 2.7, 1.3 Hz, 2H, Fmoc arom.), 5.81 (d, *J* = 8.1 Hz, 1H, NH), 5.33 (d, *J* = 4.7 Hz, 1H, H-2), 5.18 (dd, *J* = 7.9, 4.7 Hz, 1H, H-3), 4.98 (s, 1H, H-1), 4.52 (dt, *J* = 8.2, 2.4 Hz, 1H, CH Ser), 4.40 – 4.34 (m, 2H, CH₂ Fmoc), 4.34 – 4.18 (m, 3H, H-4 + CH Fmoc + CH_{2a} Ser), 4.15 – 3.98 (m, 2H, H-5), 3.89 (dd, *J* = 9.0, 2.6 Hz, 1H, CH_{2b} Ser), 2.12 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.51 (s, 18H, *t*Bu). **¹³C NMR** (101 MHz, CDCl₃) δ 170.93 (COOH), 169.68, 169.63 (C=O Ac), 155.94 (C=O Fmoc), 144.10, 144.03, 141.38 (Cq Fmoc), 129.17, 128.36, 127.79, 127.26, 125.49, 125.46, 125.43, 120.04 (CH Fmoc arom.), 105.09 (C-1), 79.30, 79.19 (C-4), 74.59 (C-2), 71.03 (C-3), 68.73 (CH₂ Ser), 67.48, 67.42, 67.37 (C-5 + CH₂ Fmoc), 54.20 (CH Ser), 47.24 (CH Fmoc), 29.97, 29.93, 29.89, 29.85 (CH₃ *t*Bu), 20.75, 20.63 (CH₃ Ac). **³¹P NMR** (162 MHz, CDCl₃) δ -8.66. **IR cm⁻¹:** 2981, 2935, 1750, 1717, 1506, 1451, 1371, 1238, 1214, 1039, 999, 758, 738. **HRMS:** [C₃₅H₄₆NO₁₄P + Na]⁺ found: 758.2565, calculated: 758.2548.

Solid phase synthesis*General procedure A, synthesis of protected 5-phosphate-ribose intermediate peptides 21 and 22*

The first intermediate peptide was synthesized using standard automated SPPS protocols starting from preloaded TentaGel® HMBA resin. The following amino acids were used: Fmoc-Pro-OH, Fmoc-Lys(Tfa)-OH, Fmoc-Ala-OH, Fmoc-Gly-OH. For the manual couplings, the resin was transferred into a fritted syringe. From the α/β -phosphoribosylated serine building block 12 or 14 (2 eq.), was dissolved in 0.25 M HCTU solution in NMP (2 eq.). This solution was added to the resin together with a 1 M DIPEA solution in NMP (4 eq.). The resin was shaken overnight before washed with DCM and NMP. Standard Fmoc-based SPPS protocols were used to couple the last three amino acids. Final Fmoc cleavage, capping and washing (DCM and NMP) obtained the protected 5-phosphate-ribose peptides **21** and **22**.

General procedure B, on-resin tBu removal

The resin was treated with 10 v/v% TFA in DCM for 30 minutes. The progress of the reaction was monitored by on-resin ³¹P-NMR analysis by the use of an acetone capillary. If the *t*Bu removal was not fully complete the treatment was repeated until full deprotection was observed. After full deprotection of the phosphate the resin was washed twice with pyridine.

General procedure C, on-resin ADP-ribosylation

The resin was washed thoroughly with dry MeCN and purged with nitrogen gas. To the resin a solution of **5** in MeCN (0.3 M, 3 eq.) was added together with ETT solution in MeCN (0.25 M, 6 eq.). The resin was shaken for 30 minutes before the resin was washed with dry MeCN. CSO in MeCN (0.5 M, 10 eq.) was added to the resin and shaken for 10 minutes after which the resin was washed with MeCN, DCM and DMF. A solution of DBU in DMF (0.5 M, 10 eq.) was added to the resin and shaken for 10 minutes. The resin was washed extensively with DMF, MeCN and DCM.

General procedure D, cleavage of the crude peptides from the resin

The resin was treated with 5 mL of a saturated solution of NH_3 in TFE for 4 hours. To the cleavage cocktail 5 mL of sat. aq. NH_4OH was added and the resin was shaken overnight. The resin was filtered, washed with milli-Q water and the filtrate was concentrated *in vacuo*. The crude peptide was dissolved in a minimal amount of MeOH with three drops of AcOH and precipitated in cold Et_2O . The precipitate was centrifuged and the liquids were decanted to obtain the crude peptide.

Ac-Pro-Ala-Lys-Ser(5-O-phosphate- α -D-ribosyl)-Ala-Pro-Ala-Pro-Lys-Lys-Gly-NH₂ (23)

General procedure A was applied with **6** to 200 mg HMBA-TentaGel® S resin (50 μmol) followed by general procedure B and D. RP-HPLC with a gradient of 0 $\rightarrow$ 20 % B in A (eluens A, 10 mM NH_4OAc in H_2O , eluens B MeCN) gave pure fractions which were concentrated, redissolved in H_2O and lyophilized to obtain the title compound as a white solid (3.20 mg, 2.45 μmol , 5%). **¹H NMR** (600 MHz, D_2O) δ 5.23 (d, J = 4.4 Hz, 1H, H1' ribose). **³¹P NMR** (202 MHz, D_2O) δ 1.33. **LC-MS**: (0 $\rightarrow$ 20% B in A): R_t = 7.56. **HRMS**: [$\text{C}_{54}\text{H}_{94}\text{N}_{15}\text{O}_{20}\text{P} + \text{H}$]⁺ found: 1304.6610, calculated: 1304.6610. [$\text{C}_{54}\text{H}_{94}\text{N}_{15}\text{O}_{20}\text{P} + 2\text{H}$]²⁺ found: 652.8356, calculated: 652.8341.

Ac-Pro-Ala-Lys-Ser(5-O-phosphate- β -D-ribosyl)-Ala-Pro-Ala-Pro-Lys-Lys-Gly-NH₂ (24)

General procedure A was applied with **7** to 200 mg HMBA-TentaGel® S resin (50 μmol) followed by general procedure B and D. RP-HPLC with a gradient of 0 $\rightarrow$ 20 % B in A (eluens A, 10 mM NH_4OAc in H_2O , eluens B MeCN) gave pure fractions which were concentrated, redissolved in H_2O and lyophilized to obtain the title compound as a white solid (4.48 mg, 3.43 μmol , 7%). **¹H NMR** (600 MHz, D_2O) δ 5.12 (s, 1H, H1' ribose). **³¹P NMR** (202 MHz, D_2O) δ 1.29. **LC-MS**: (0 $\rightarrow$ 20% B in A): R_t = 7.11. **HRMS**: [$\text{C}_{54}\text{H}_{94}\text{N}_{15}\text{O}_{20}\text{P} + 2\text{H}$]²⁺ found: 652.8345, calculated: 652.8341.

Ac-Pro-Ala-Lys-Ser(5-O-adenosine diphosphate- α -D-ribosyl)-Ala-Pro-Ala-Pro-Lys-Lys-Gly-NH₂ (1)

General procedure A was applied with **6** to 400 mg HMBA-TentaGel® S resin (100 μmol) followed by general procedure B, C and D. RP-HPLC with a gradient of 0 $\rightarrow$ 20 % B in A (eluens A, 10 mM NH_4OAc in H_2O , eluens B MeCN) gave pure fractions which were concentrated, redissolved in H_2O and lyophilized to obtain the title compound as a white solid (7.58 mg, 4.65 μmol , 5%). **¹H NMR** (600 MHz, D_2O) δ 8.48 (s, 1H, H-2), 8.23 (s, 1H, H-8), 6.10 (d, J = 6.0 Hz, 1H H1' adenosine), 4.94 (d, J = 4.3 Hz, 1H, H1' ribose). **³¹P NMR** (202 MHz, D_2O) δ -10.51, -10.61, -10.65, -10.76. **LC-MS**: (0 $\rightarrow$ 20% B in A): R_t = 6.01. **HRMS**: [$\text{C}_{64}\text{H}_{106}\text{N}_{20}\text{O}_{26}\text{P}_2 + 2\text{H}$]²⁺ found: 817.362, calculated: 817.3604.

Ac-Pro-Ala-Lys-Ser(5-O-adenosine diphosphate- β -D-ribosyl)-Ala-Pro-Ala-Pro-Lys-Lys-Gly-NH₂ (2)

General procedure A was applied with **7** to 200 mg HMBA-TentaGel® S resin (50 μmol) followed by general procedure B, C and D. RP-HPLC with a gradient of 0 $\rightarrow$ 20 % B in A (eluens A, 10 mM NH_4OAc in H_2O , eluens B MeCN) gave pure fractions which were concentrated, redissolved in H_2O and lyophilized to obtain the title compound as a white solid (5.86 mg, 3.59 μmol , 7%). **¹H NMR** (600 MHz, D_2O) δ 8.40 (s, 1H, H-2), 6.26 (d, J = 5.5 Hz, 1H H1'adenosine), 4.87 (H1' ribose overlapped with D_2O). **³¹P NMR** (202 MHz, D_2O) δ -10.46, -10.56, -10.63, -10.74. **LC-MS**: (0 $\rightarrow$ 20% B in A): R_t = 6.77. **HRMS**: [$\text{C}_{64}\text{H}_{106}\text{N}_{20}\text{O}_{26}\text{P}_2 + 2\text{H}$]²⁺ found: 817.3615, calculated: 817.3604.

Biochemical evaluation

Plasmids and Proteins

The expression construct for wildtype ARH3 was a gift from Prof Paul Hergenrother (University of Illinois) and mutation was introduced by site directed mutagenesis as described earlier.^[29] For expression of recombinant hARH3 proteins plasmid were transformed in Rosetta (DE3) cells and grown to an OD₆₀₀ of 0.6 in LB medium supplemented with 2 mM MgCl₂ and appropriate antibiotics. Expression was induced with 0.4 mM isopropyl β -D-1-thiogalactopyranoside (IPTG), cells were grown overnight at 17 °C and harvested by centrifugation. Proteins were purified by Ni²⁺-NTA chromatography (Jena Bioscience) according to the manufacturer's protocol using the following buffers: all buffers contained 50 mM TrisHCl (pH 8), 500 mM NaCl and 10 mM MgCl₂; additionally, the lysis buffer contained 25 mM, the washing buffer 40 mM, and the elution buffer 500 mM imidazole. Proteins were dialysed overnight against 50 mM TrisHCl (pH 8), 200 mM NaCl, 1 mM dithiothreitol and 5% (v/v) glycerol. Recombinant human NUDT5 was a kind gift from the Protein Science Facility (PSF) at Karolinska Institute (Sweden).

Peptide demodification assay

20 μ M histone H2B peptides **1** and **2** (aa 4-14) were demodified by incubation with 1 μ M hARH3 for 20 minutes at rt in assay buffer (50 mM TrisHCl [pH 8], 200 mM NaCl, 10 mM MgCl₂ and 1 mM dithiothreitol). The released ADPr was converted into AMP by co-incubation with 0.2 μ M human NUDT5. Reactions were stopped and analyzed 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 analyzed with GraphPad Prism 7. Control reactions were carried out in absence of peptide.

Chemical degradation assay of peptide 1

Peptide **1** (0.6 mg) was dissolved in 300 μ L water to make a stock solution of peptide **1**. This stock solution was divided in three portions of 100 μ L and each portion was mixed respectively with 100 μ L 0.2 M TFA in water, 100 μ L 1.0 M NH₂OH in water or 100 μ L 0.2 M NaOH in water. Samples of 30 μ L were taken for LC-MS analysis at various time points (15 minutes, 1 hour, 2.5 hours, 5 hours and 24 hours). The samples treated with TFA or NH₂OH were injected directly, the sample treated with NaOH was quenched with 2 μ L AcOH prior to injection. Peptide degradation was monitored by UV-detection and mass fragments.

References

- [1] B. Lüscher, M. Bütepage, L. Eckei, S. Krieg, P. Verheugd, B. H. Shilton, *Chem. Rev.* **2018**, 118, 1092–1136.
- [2] B. A. Gibson, W. L. Kraus, *Nat. Rev. Mol. Cell Biol.* **2012**, 13, 411–424.
- [3] M. O. Hottiger, P. O. Hassa, B. Lüscher, H. Schüler, F. Koch-Nolte, *Trends Biochem. Sci.* **2010**, 35, 208–219.
- [4] S. Laing, M. Unger, F. Koch-Nolte, F. Haag, *Amino Acids* **2011**, 41, 257–269.
- [5] N. Ogata, K. Ueda, H. Kagamiyama, O. Hayaishi, *J. Biol. Chem.* **1980**, 255, 7616–7620.
- [6] N. Ogata, K. Ueda, O. Hayaishi, *J. Biol. Chem.* **1980**, 255, 7610–7615.
- [7] C. M. Daniels, S. Ong, A. K. L. Leung, *Mol. Cell* **2015**, 58, 911–924.
- [8] O. Leidecker, J. J. Bonfiglio, T. Colby, Q. Zhang, I. Atanassov, R. Zaja, L. Palazzo, A. Stockum, I. Ahel, I. Matic, *Nat. Chem. Biol.* **2016**, 12, 998–1000.
- [9] L. Palazzo, O. Leidecker, E. Prokhorova, H. Dauben, I. Matic, I. Ahel, *Elife* **2018**, 7, 1–12.
- [10] J. J. Bonfiglio, P. Fontana, Q. Zhang, T. Colby, I. Gibbs-Seymour, I. Atanassov, E. Bartlett, R. Zaja, I. Ahel, I. Matic, *Mol. Cell* **2017**, 0, 1–9.
- [11] L. Palazzo, O. Leidecker, E. Prokhorova, H. Dauben, I. Matic, I. Ahel, *Elife* **2018**, 1–12.
- [12] I. Gibbs-Seymour, P. Fontana, J. G. M. Rack, I. Ahel, *Mol. Cell* **2016**, 62, 432–442.
- [13] E. E. Alemasova, O. I. Lavrik, *Nucleic Acids Res.* **2019**, 47, 3811–3827.
- [14] H. A. V Kistemaker, A. P. Nardoza, H. S. Overkleeft, G. A. van der Marel, A. G. Ladurner, D. V. Filippov, *Angew. Chemie - Int. Ed.* **2016**, 55, 10634–10638.
- [15] G. J. van der Heden van Noort, H. S. Overkleeft, G. A. van der Marel, D. V. Filippov, *J. Org. Chem.* **2010**, 75, 5733–5736.
- [16] H. A. V Kistemaker, G. J. van der Heden van Noort, H. S. Overkleeft, G. A. van der Marel, D. V. Filippov, *Org. Lett.* **2013**, 15, 2306–2309.
- [17] A. G. Volbeda, H. A. V. Kistemaker, H. S. Overkleeft, G. A. van der Marel, D. V. Filippov, J. D. C. Codée, *J. Org. Chem.* **2015**, 80, 8796–8806.
- [18] A. Bérces, D. M. Whitfield, T. Nukada, I. S. Z, A. Obuchowska, J. J. Krepinsky, *Can. J. Chem.* **2004**, 82, 1157–1171.
- [19] G. J. van der Heden van Noort, P. van Delft, N. J. Meeuwenoord, H. S. Overkleeft, G. a van der Marel, D. V Filippov, *Chem. Commun.* **2012**, 48, 8093–5.
- [20] P. Fontana, J. J. Bonfiglio, L. Palazzo, E. Bartlett, I. Matic, I. Ahel, *Elife* **2017**, 6, 1–20.
- [21] J. Moss, S. chen Tsai, R. Adamik, H. chia Chen, S. J. Stanley, *Biochemistry* **1988**, 27, 5819–5823.
- [22] J. Moss, N. J. Oppenheimer, R. E. West, S. J. Stanley, *Biochemistry* **1986**, 25, 5408–5414.
- [23] L. Palazzo, B. Thomas, A. S. Jemth, T. Colby, O. Leidecker, K. L. H. Feijs, R. Zaja, O. Loseva, J. C. Vert, I. Matic, T. Helleday, I. Ahel, *Biochem. J.* **2015**, 468, 293–301.
- [24] Y. Zhang, J. Wang, M. Ding, Y. Yu, *Nat. Methods* **2013**, 10, 981–984.
- [25] K. Wakabayashi, W. Pigman, *Carbohydr. Res.* **1974**, 35, 3–14.
- [26] M. B. Goshe, T. P. Conrads, E. A. Panisko, N. H. Angell, T. D. Veenstra, R. D. Smith, *Anal. Chem.* **2001**, 73, 2578–2586.
- [27] Y. Oda, T. Nagasu, B. T. Chait, *Nat. Biotechnol.* **2001**, 19, 379–382.
- [28] L. Wells, K. Vosseller, R. N. Cole, J. M. Cronshaw, M. J. Matunis, G. W. Hart, *Mol. Cell. Proteomics* **2002**, 1, 791–804.
- [29] R. K. Morgan, M. S. Cohen, *ACS Chem. Biol.* **2015**, 10, 1778–1784.

