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Synthesis of disaccharide nucleoside analogues as potential poly(ADP-ribose) polymerase-1 inhibitors

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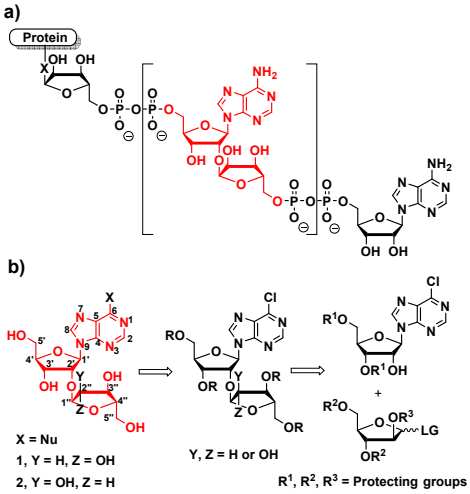
Poly(ADP-ribose) polymerase-1 (PARP-1) is an important target in cancer therapy. We present the synthesis of novel disaccharide nucleoside analogues that resemble the central motif of poly(ADP-ribose) and tested their inhibitory effects on human PARP-1. Some compounds show inhibition on enzymatic activity *in vitro* and thus might be interesting for further investigations.

Poly(ADP-ribos)ylation (PARylation), a highly dynamic post-translational modification of proteins, is involved in a wide range of biological processes in eukaryotic cells, including DNA repair, maintenance of genomic stability and transcriptional regulation.[1,2] Nuclear poly(ADP-ribos)ylation[3,4] is crucial for the repair of DNA strand breaks.[5] As DNA damage sensor and signal transducer in the nucleus,[6,7] poly(ADP-ribose) polymerase-1 (PARP-1) acts as central enzyme in this process. Inspired by the discovery of synthetic lethality between PARP-1 and BRCA-1/2[8,9], a number of PARP inhibitors (PARPi) were developed[10,11] which have been used for the treatment of ovarian cancer.[12] Currently, these PARPi are derived from the nicotinamide core structure.[13,14] Since resistance against this type of reagents has been multiply reported about, new generations of PARPi with novel core structures would be highly beneficial for the future treatment of those cancer types.[12]

Poly(ADP-ribose) is built of successively connected ADP-ribose moieties which are linked via α-glycosidic bonds of the 2'-*O* of adenosine and the 1-*C* of ribose forming the disaccharide nucleoside 2'-*O*-α-D-ribofuranosyladenosine as repeated structural motif of the polymer (Scheme 1a).[15-17] Inspired by this structural pattern, we supposed
that structurally similar disaccharide nucleosides with variations in functional groups (Compounds **1** and **2**, Scheme 1b) might be a suitable platform for the identification of novel molecules that inhibit PARP-1.



Scheme 1 a) Structure of poly(ADP-ribose) and the central motif 2'-*O*-α-D-ribofuranosyladenosine (in red); b) The target molecules **1** and **2** (in red) in this study and their retro-synthetic analysis.

In this study, we report the synthesis of a series of disaccharide nucleosides based on analogues **1** and **2,** starting from protected 6-chloropurine-9-riboside (Scheme 1b). Various substituents were introduced at the 6-position of the purine ring. Subsequently, these analogues were investigated for their ability to inhibit PARP-1 activity *in vitro*.

In order to access target molecules **1** and **2** in a concise reaction pathway and to minimize conversion steps, we aimed for a common intermediate that could provide the arabinosyl-(**1**), as well as the ribosyl-nucleoside analogues (**2**). To achieve this, the glycosylation donor has to meet several requirements. First, a neighbouring-group assisted glycosylation is needed, which requires ester-based protecting groups on the arabinose to guarantee the *trans*-configuration of the product. Additionally, a selective liberation of the hydroxyl group at the 2-position of arabinose moiety should be possible to provide the opportunity to directly convert the arabinose moiety into a ribose moiety. Therefore, we decided **3** to be the ideal glycosylation donor since acetate protection

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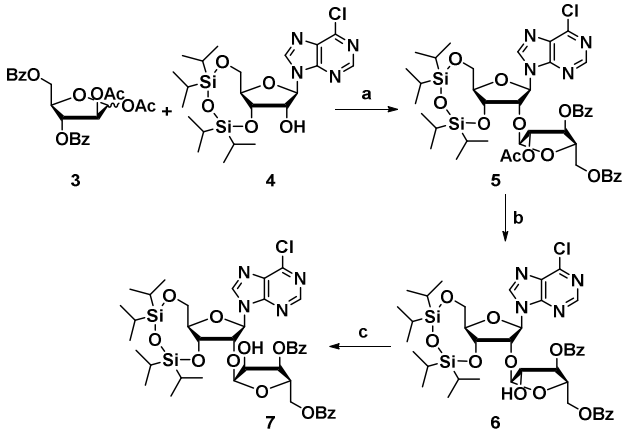
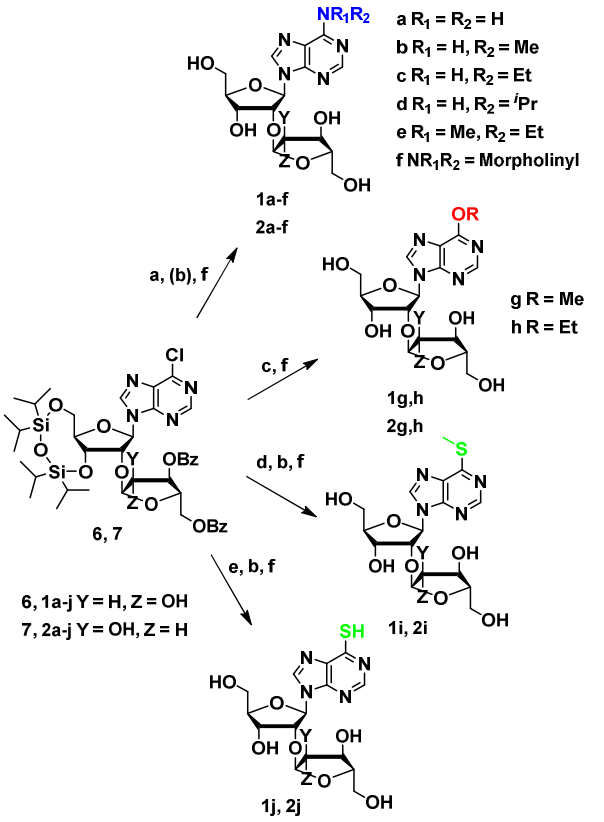
groups are less stable than benzoate groups under basic conditions,[18] allowing selective cleavage of the acetyl ester following 1,2-*trans* glycosylation.



Scheme 2 Synthesis of precursors **6** and **7**. a) $SnCl_4$, DCE, 4 °C, 36 h, 76%; b) 1% DIPEA in MeOH, 0 °C, 3 h, 74%; c) (1) Ac_2O, DMSO, r.t., 24 h; (2) $NaBH_4$, EtOH, 0 °C, 2 h; 60% over two steps.

Access to the disaccharide nucleoside scaffold was achieved through arabinosylation of the protected 6-chloropurine-9-riboside **4**[19] by stereoselective glycosylation, using **3**[20] according to Mikhailov and Herdewijn[17] (Scheme 2) to afford **5** in 76% yield. Selective removal of the 2''-acetate group was achieved in 74% yield to form **6,** using 1% DIPEA in methanol. In order to obtain the 2'-*O*-ribosyl precursor **7**, the 2''-OH group was inverted via an oxidation-reduction protocol. The arabinosyl precursor **6** was oxidized under Albright-Goldman conditions,[21] and then reduced *in situ* with sodium borohydride at 0 °C, following Robins' procedure[22] yielding the 2'-*O*-ribosyl precursor **7** in 60% yield over two steps (Scheme 2).

With the two precursors **6** and **7** in hand, the substitution of chlorine and the subsequent deprotection was investigated. It was reported that the substitution of 6-chlorine with *N*-nucleophiles often required refluxing with corresponding amines in alcoholic solvent for hours or even days.[23-25] To accelerate this reaction, microwave irradiation was tested.[26] Thus, we treated **6** and **7** with ammonia, primary (methylamine, ethylamine and *iso*-propylamine) and secondary amines (ethylmethylamine and morpholine) at 160 °C in methanol under microwave irradiation, successfully converting the chlorine into the corresponding amines (Scheme 3). This conversion was accompanied by cleavage of the benzoic acid esters except for the reaction with morpholine as a nucleophile due to its relatively weaker nucleophilicity. In this case, the benzoic acid esters were cleaved by treatment with methylamine in methanol subsequent to the substitution reaction (Scheme 3, condition b). The 1,3-(1,1,3,3-tetraisopropyldisiloxanylidene) (TIPDS) group was removed by treatment of crude product with triethylamine trihydrofluoride (Scheme 3, condition f), resulting in the fully deprotected disaccharide nucleoside analogues **1a**-**1f** and **2a**-**2f** (Scheme 3).



Scheme 3 Substitution and deprotection of **6** and **7** to obtain target molecules **1a**-**1j** and **2a**-**2j**: a) Amine, MeOH, 160 °C (mw), 0.5 h; b) $MeNH_2$, MeOH, r.t., 18 h; c) RONa, ROH, 0 °C; d) MeSNa, DMSO, 55 °C, 48 h; e) thiourea, EtOH, r.t., 24 h; f) TEA·3HF, THF, 0 °C, 4 h.

Alkoxy groups were introduced at 6-position by the reaction of precursors **6** and **7** with either sodium methoxide, or sodium ethoxide in the corresponding alcohol (Scheme 3, condition c).[27] Simultaneously, the benzoate groups were removed in one-pot. The obtained crude products were converted to the desired disaccharide nucleoside analogues **1g**-**1h** and **2g**-**2h,** using the already mentioned protocol for removing silyl-based protecting groups (Scheme 3, condition f).

The 6-methylthio substituted compounds **1i**, **2i** were obtained by heating of **6** and **7** with sodium thiomethoxide in DMSO (Scheme 3, condition d).[28] The 6-mercaptopurine compounds **1j**, **2j** were obtained via treatment of **6** and **7** with thiourea in ethanol (Scheme 3, condition e).[23] The resulting crude products were further treated with methylamine in methanol (Scheme 3, condition b) and subsequently triethylamine trihydrofluoride (Scheme 3, condition f), providing compounds **1i, 2i** and **1j**, **2j**. All disaccharide nucleoside analogues were purified by reverse phase MPLC. A summary of all yields is shown in Table 1.

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Table 1 Summarized yields of **1a-j** and **2 a-j** (*yields were calculated over 2 or 3 steps starting from **6** or **7**).

Entry	Substituent on purine base	Yield* 1	2
a	-NH_2	53%	65%
b	-NHMe	39%	53%
c	-NHEt	75%	66%
d	-NH^iPr	77%	72%
e	-NMeEt	97%	Quant.
f	-morpholinyl	83%	32%
g	-OMe	96%	Quant.
h	-OEt	68%	43%
i	-SMe	42%	79%
j	-SH	58%	67%

After successful synthesis of the two clusters of disaccharide nucleoside analogues **1** and **2** with different substituents at the 6-position of the purine base, their effects on PARP-1 were investigated, employing a commercially available chemi-luminescent assay. The quantification is based on the PARylation of immobilized histone as PAR-acceptor using biotinylated NAD^+. In turn, the incorporated biotin is coupled to the formation of a chemiluminescent signal that correlates to PAR synthesis.[29]

All 20 compounds were screened in duplicates at a concentration of 250 µM (Figure 1) towards their propensity to interfere with PAR synthesis. The enzymatic activity of PARP-1 in the absence of any potential inhibitor was normalized to 100%, and PAR formation in presence of compounds **1**, **2** measured and normalized to the reaction in the absence of any compound. Some of the compounds showed inhibitory effects on PARP-1. Compounds **2a-j,** bearing the natural 2'-*O*-ribosyl moiety (Figure 1, in red), exhibited predominantly stronger inhibitory effects than compounds **1a-j,** bearing a 2'-*O*-arabinosyl moiety (Figure 1, in blue). Different properties were observed when nucleobase modifications were studied. The natural occurring amine **2a** merely showed poor inhibition of PAR formation. The most promising candidate was the methylamine modified analogue **2b**, which showed inhibition of more than 90%. However, the inhibitory potential does not correlate with steric demand since **2c,** bearing the -NHEt substituent, is less potent than **2d,** comprising the sterically more demanding $-NH^iPr$ substituent, which was also a promising candidate with over 60% inhibition.

Compared to compounds **2b** (-NHMe), the reduced inhibition potential of structurally similar compound **2g** (-OMe) and **2i** (-SMe) is striking. A possible explanation for this might be the presence of a hydrogen atom at the amine in **2b,** providing the possibility to act as H-bond donor. Comparable compounds without this capability (**2g**, **2i**) show less pronounced inhibition than **2b**.

To determine the IC_{50} values of compounds **2b** and **2d** against PARP-1, we utilized the same assay as before with varying concentrations of the respective inhibitor (Figure S1, S2). It was found that compounds **2b** and **2d** inhibit the polymerization function of PARP-1 dose-dependently with an IC_{50} value of 93.4 ± 3.7 µM (**2b**) and 158.2 ± 17.7 µM (**2d**). In comparison to the reported IC_{50} value of Olaparib which is 5 nM[30] the herein reported novel compounds were less potent. Considering the efforts invested in the development of a commercial drug like Olaparib such results were expected. However, the here presented compounds may serve as promising lead structure in further studies.

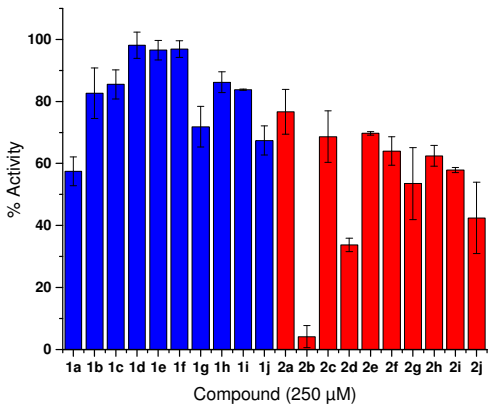

Figure 1 Normalized enzymatic activity of PARP-1 in the presence of compound **1a-j** (in blue) and **2a-j** (in red).

Conclusions

In this study, inspired by the central structural motif of PAR, a versatile and robust synthetic approach to synthesize disaccharide nucleoside analogues was developed, bearing different substituents on the purine base as well as different epimers as pentose moieties. Combined with a careful manipulation of the protecting groups, this gave us the opportunity to successfully synthesize a selection of disaccharide nucleoside analogues **1** and **2,** starting from protected arabinose donor **3** and protected 6-chloropurine-9-ribonucleoside **4**. The effects of these 20 disaccharide nucleoside analogues on PARP-1-mediated poly(ADP-ribos)ylation of histones were studied *in vitro*. Two analogues showed promising inhibitory effects on PARP-1. The subtle dependence of the inhibitory effect on the modification indicates selective recognition processes of the analogues by the enzyme that need to be further elucidated. However, since these compounds are based on a different molecular scaffold compared to PARPi, which derived from pyridine amide core structures,[13,14] we consider the presented disaccharide nucleoside skeleton to be a promising candidate for further development towards novel PARP inhibitors.

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Conflicts of interest

There are no conflicts to declare.

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