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Four of a Kind: A Complete Collection of ADP-Ribosylated Histidine Isosteres Using Cu(I)- and Ru(II)-Catalyzed Click Chemistry

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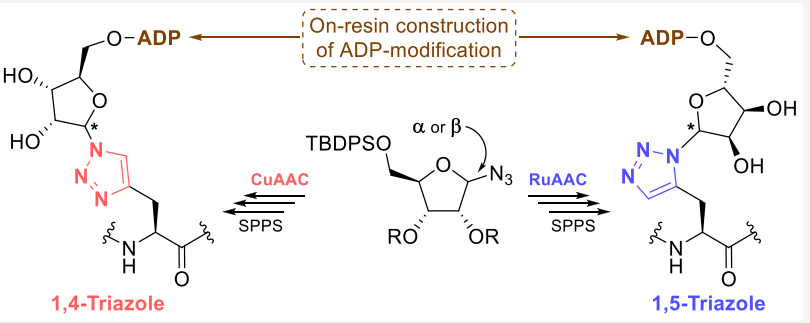

ABSTRACT: Adenosine diphosphate ribosylation (ADP-ribosylation) is a crucial post-translational modification involved in important regulatory mechanisms of numerous cellular pathways including histone maintenance and DNA damage repair. To study this modification, well-defined ADP-ribosylated peptides, proteins, and close analogues thereof have been invaluable tools. Recently, proteomics studies have revealed histidine residues to be ADP-ribosylated. We describe here the synthesis of a complete set of triazole-isosteres of ADP-ribosylated histidine to serve as probes for ADP-ribosylating biomachinery. By exploiting Cu(I)- and Ru(II)-catalyzed click chemistry between a propargylglycine building block and an α- or β-configured azidoribose, we have successfully assembled the α- and β-configured 1,4- and 1,5-triazoles, mimicking N(τ)- and N(π)-ADP-ribosylated histidine, respectively. The ribosylated building blocks could be incorporated into a peptide sequence using standard solid-phase peptide synthesis and transformed on resin into the ADP-ribosylated fragments to provide a total of four ADP-ribosyl triazole conjugates, which were evaluated for their chemical and enzymatic stability. The 1,5-triazole analogues mimicking the N(π)-substituted histidines proved susceptible to base-induced epimerization and the ADP-ribosyl α-1,5-triazole linkage could be cleaved by the (ADP-ribosyl)hydrolase ARH3.

INTRODUCTION

Adenosine diphosphate ribosylation is a highly versatile and dynamic post-translational modification (PTM) in which the well-known redox co-factor nicotinamide dinucleotide adenine (NAD^+) is used to covalently link an adenosine diphosphate ribose (ADPr) molecule to a nucleophilic amino acid functionality. It is a ubiquitously expressed modification that allows spatiotemporal regulation of important cellular pathways including adipogenesis,[1] DNA damage repair, gene expression,[2] and apoptosis.[3] ADP ribosylation is affected by a family of (ADP-ribosyl)transferase enzymes termed PARPs. Most members transfer a single ADPr moiety to a nucleophilic acceptor, which is referred to as mono-ADP-ribosylation (MARylation), although a small subset of PARPs (PARP1, 2, 5a and 5b) are able to mediate poly-ADP-ribosylation (PARylation) to create a linear polymer[4] with occasional branches.[5] The resulting poly-ADPr chains can be truncated by poly(ADP-ribosyl) glycohydrolase (PARG)[6,7] to yield a MARylated protein, after which a collection of (ADP-ribosyl)hydrolases and macrodomain proteins with distinct substrate specificity remove the final protein-linked ADPr moiety.[8−12]

The most common amino acid residue to be ADP-ribosylated is serine,[13,14] but glutamate, aspartate,[1,15−17] arginine,[18] cysteine,[13,19] lysine, and more recently tyrosine[13,20] and histidine[21,22] have been found to be ADP-ribosylated as well. Synthetic, well-defined MARylated peptides and ADPr-oligomers have been shown to be valuable molecular tools to investigate ADP-ribosylation, informing on the exact structure of ADPr polymers and modified peptides, the chemical and enzymatic stability of the PTMs, and the binding with

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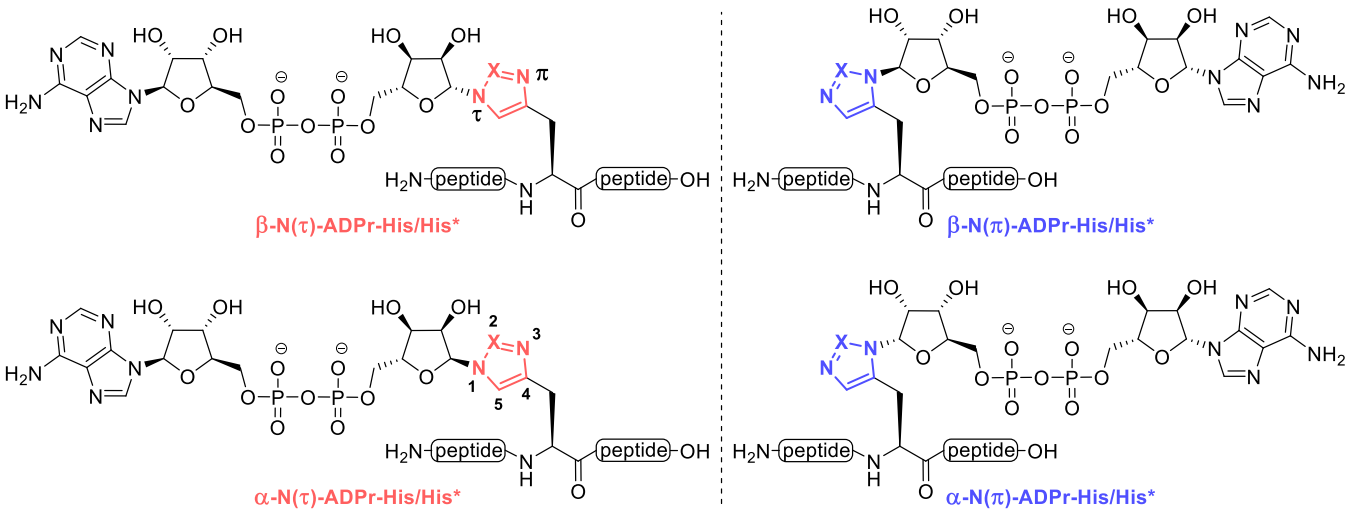

Figure 1. All four possible chemical structures for ADP-ribosylated histidine residues (X═CH), including the pros ("near," π) and tele ("far," τ) terminology for the imidazolium nitrogen atoms. The 1,4- and 1,5-disubstituted triazole-based isosteres (X═N, referred to as His*) mimic their N(τ)- and N(π)-ADP-ribosylated histidine counterparts, respectively. The numbering nomenclature of triazolyl-functionalities is highlighted.

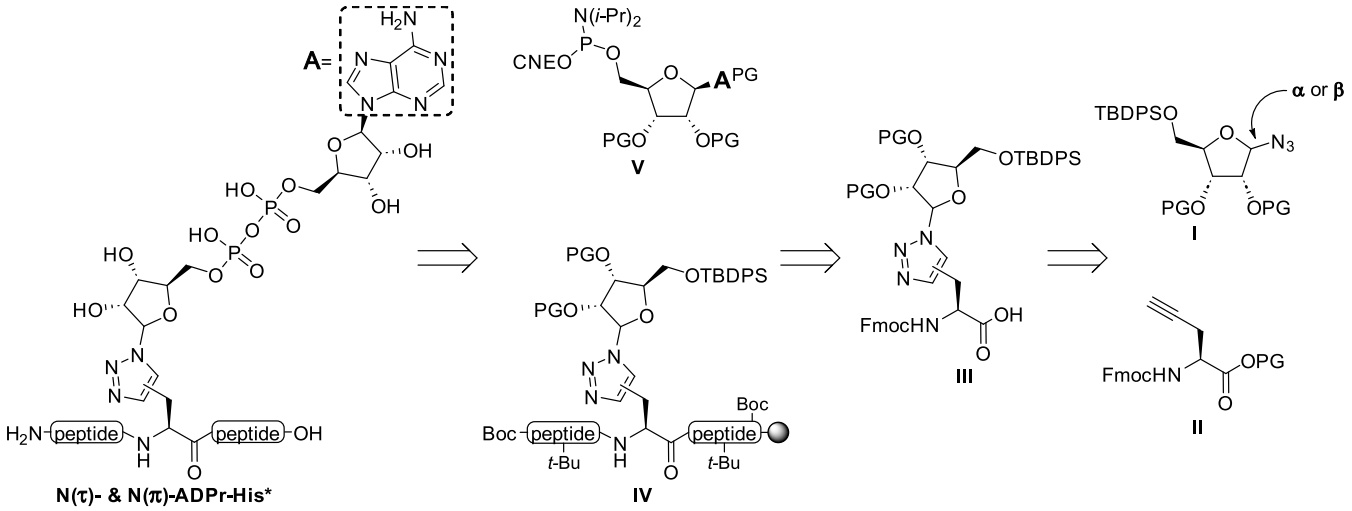

Figure 2. Retrosynthetic analysis of 1,4- and 1,5-disubstituted triazole-based isosteres for N(τ)- and N(π)-ADP-ribosylated histidine, respectively, referred to as N(τ)- and N(π)-ADPr-His*. $HPF1_{221-233}$ (T-F-H*-G-A-G-L-V-V-P-V-D-K, where H* refers to the triazole isostere) includes the histidine modification site that was recently identified in proteomic experiments.[21] Here, adenine is abbreviated as **A** and PG is used to depict an unspecified protecting group.

interaction partners.[23−26] Various isosteres of ADP-ribosylated amino acids have been introduced as ADPr chemical biology tools with special attention being paid to stabilizing the glycosidic linkage that connects the ADPr moiety to a protein and to expedite synthetic accessibility. Examples of the isosteric replacements for native ADPr-peptides include ADP-ribosylated glutamine and asparagine[27] and *N*-methyl aminooxy functionalized peptides[28] serving as base-stable substitutes for their glutamate and aspartate counterparts. Likewise, the urea functionality of citrulline has been introduced as a mimic for the guanidine group of arginine.[27] Click chemistry has been implemented to generate non-natural MARylated oligopeptides[29−31] and even full-length proteins, as was demonstrated by the synthesis of ADP-ribosylated ubiquitin, carrying the ADPr moiety at specific arginine residues.[32]

The imidazole ring of histidine has recently been identified as a potential ADP-ribosylation site.[21] Proteomics studies have however been unable to elucidate the nature of the ribosyl−histidine linkage. The imidazolyl side chain of histidine carries two possible modification sites that are commonly referred to as the N(π)- and N(τ)-positions (Figure 1). In addition, the chirality of the ribosyl anomeric center is unknown. Although all ADP-ribosyl linkages identified to date are α-configured (as a result of the substitution of the NAD^+ nicotinamide with inversion of stereochemistry), it cannot a priori be ruled that the linkage to the histidine imidazolyl group cannot be β-configured. For example, ADPr-Arg has been shown to spontaneously anomerize under physiological conditions via an endocyclic ring-opening pathway to transform the α-ribosyl linkage into the corresponding β-ribose.[33] We have previously reported on the synthesis of triazolyl-linked N(τ)-ADP-ribosylated conjugates that function as histidine isosteres, indicated here as ADPr-His*. We generated peptides with ADPr-His* via a convergent synthesis, introducing the 1,4-triazole moieties by exploiting the highly regioselective Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC) of azido-ADP-ribose to a propargylglycine residue, pre-installed in the peptide chain by solid-phase peptide synthesis.[31] We also showed that the glycosidic linkage of these

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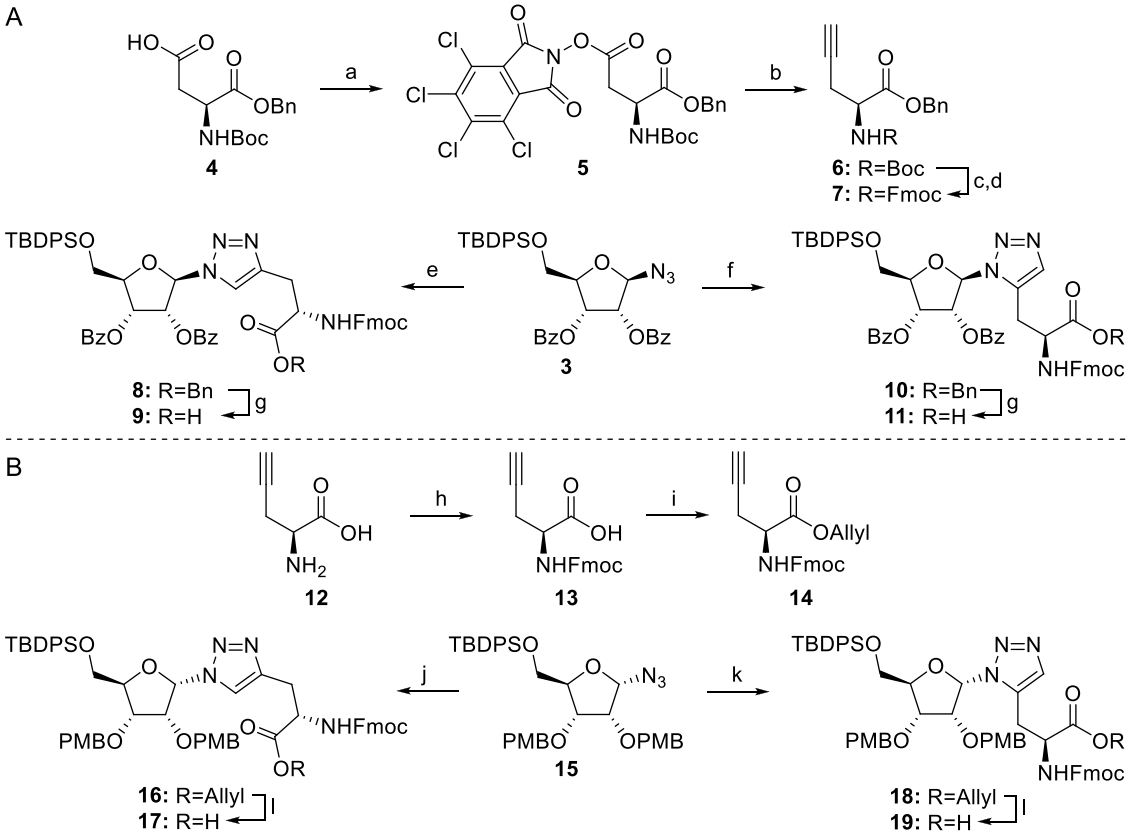
Scheme 1. Preparation of Triazolyl-Linked Ribosylated Building Blocks through Cu(I)- and Ru(II)-Catalyzed Cycloadditions between Suitably Protected Propargylglycine Analogues with β-Configured Azido-Ribofuranoside 3 (A) or α-Configured Azido-Ribofuranoside 15 (B)[a]



[a]Reagents and conditions: (a) *N*-hydroxytetrachlorophthalimide, DCC, DMAP, DCM, rt, 16 h (81%). (b) Ethynylmagnesium bromide, 4,4-dimethoxy-2,2′-bipyridine, $NiCl_2$, $ZnCl_2$, LiCl, THF/DMF (1:1), rt, 16 h (73%). (c) TFA, DCM, rt, 2 h. (d) Fmoc-OSu, $NaHCO_3$, H_2O/MeCN (1:1), rt, 16 h (91% over 2 steps). (e) **7**, $CuSO_4$, sodium ascorbate, DMF, rt, 1 h (71%). (f) **7**, Cp*ClRu(COD), THF, microwave, 100 °C, 5 min (85%). (g) H_2 (1 atm), Pd/C, MeOH, rt, 3.5 h (80% for **9**, 90% for **11**). (h) Fmoc-OSu, $NaHCO_3$, H_2O/THF (1:1), rt, 16 h (quant). (i) AllylOH, DMAP, DIC, DCM, rt, 45 min (81%). (j) **14**, $CuSO_4$, sodium ascorbate, DMF, rt, 1 h (93%). (k) **14**, Cp*ClRu(COD), THF, microwave, 100 °C, 1 h (60%). (l) $Pd(PPh_3)_4$, DMBA, DCM, rt, 1.5 h (96% for **17**, 85% for **19**).

isosteres could be cleaved by ARH3, an (ADP-ribosyl)-hydrolase, capable of clipping off ADPr moieties from ADPr-Ser,[23] *O*-acetyl-ADPr,[34] α-NAD$^+$,[35] poly-ADPr,[36] as well as ADPr-5′-P DNA.[37] The 1,4-triazole ADPr-His* was however a relatively weak substrate for ARH3.[31]

To expand the set of probes to investigate ADPr-histidine biology, we report here on the assembly of the full set of four possible ADP-ribosyl triazole histidine mimics and describe a synthetic methodology to access both 1,4-triazoles, resembling N(τ)-ADPr-histidine and 1,5-triazoles mimicking the N(π)-ADPr- histidine, having either the α- or β-ribosyl configuration (Figure 1). We have incorporated the (α/β)-1,4/1,5-triazole ADPr-His* mimics in a peptide fragment originating from histone PARylation factor 1 (HPF1), as the histidine residue in this peptide has been identified as a ribosylation site in recent proteomics studies.[21] The peptides have been used for stability studies to probe the integrity of the fragments under conditions typically used in proteomics workflows, and we have subjected the ADPr-His* peptides to a panel of (ADP ribosyl)hydrolases. These studies have revealed the 1,5-triazole analogues to be less stable under basic conditions and to be better substrates for ARH3 than their 1,4-counterparts.

RESULTS AND DISCUSSION

We previously generated the α/β-(1,4)-triazole His-mimetics through a regioselective CuAAC-reaction, and we reasoned that we could exploit the less common Ru(II)-catalyzed azide–alkyne cycloaddition (RuAAC)[38] to access their (α/β)-1,5-triazole counterparts. While the RuAAC has been successfully applied to peptides before,[39] we found the conditions to be incompatible with the late-stage conjugation of an azido-ADP-ribose to an oligopeptide carrying an alkyne click handle and only observed degradation of the azido-ADP-ribose. Therefore, an alternative approach toward the 1,5-disubstituted triazole-isosteres for ADPr-histidine was required and we opted for a stepwise SPPS approach where a suitably protected ribosyl azide **I** was first conjugated to fluorenylmethyloxycarbonyl (Fmoc)-propargylglycine **II** (Figure 2). The resulting Fmoc-building block **III** could then be used in a SPPS protocol for the incorporation in a peptide sequence of choice **IV** through standard Fmoc-based peptide chemistry. Next, on resin phosphorylation and pyrophosphate formation following a well-established P(III)-P(V) coupling strategy[40] with suitably protected adenosine amidite **V** and deprotection should enable the synthesis of the ADPr-His* mimetics. Using Cu(I)- or Ru(II)-catalyzed click chemistry in combination with either α-

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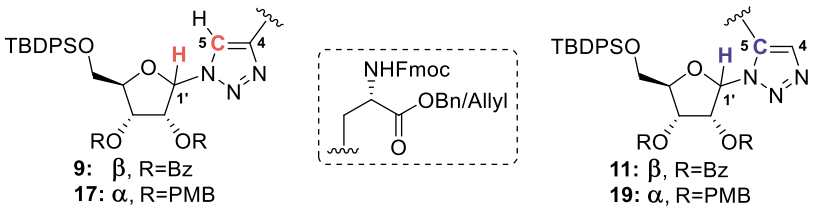
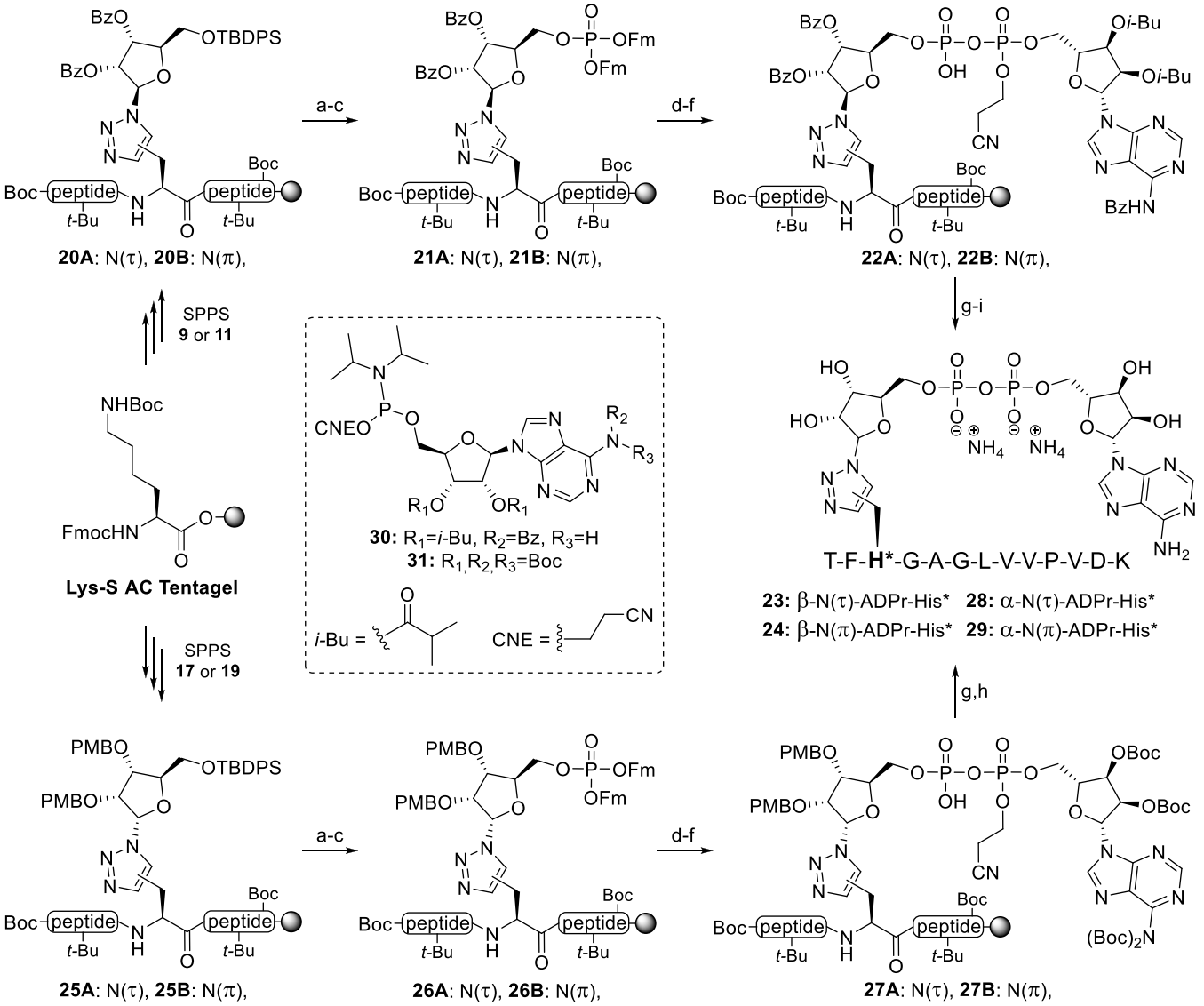


Figure 3. Characteristic proton−carbon three-bond couplings that have been observed in HMBC measurements for the here-described 1,4- and 1,5-disubstituted triazoles building blocks are highlighted in red and blue, respectively. No correlation between H-5 and C-1′ was observed for any of the 1,4-triazoles in the HMBC data sets.

Scheme 2. Incorporation of Ribosylated Amino Acids 9, 11, 17, and 19 in a Peptide Fragment Originating from HPF1, Where H* Refers to the Triazole Isostere, Resulting in (τ)- and (π)-His* Isosteres in Both α- and β-Configuration[a]



[a]Reagents and conditions: (a) HF-pyridine, pyridine, rt, 2 × 45 min. (b) $(FmO)_2PN(i\text{-}Pr)_2$, ETT, MeCN, rt, 30 min. (c) CSO, MeCN, rt, 30 min. (d) DBU, DMF, rt, 2 × 15 min. (e) **30** or **31**, ETT, MeCN, rt, 30 min. (f) CSO, MeCN, rt, 30 min. (g) DBU, DMF, rt, 2 × 10 min. (h) TFA, DCM, rt, 1 h (36 and 25% over 8 steps for **28** and **29**, respectively). (i) NH_4OH (28%), rt, 16 h (26 and 9% over 9 steps for **23** and **24**, respectively).

and β-azidoribose grants access to the full set of (α/β)-N(τ)/N(π)-ADPr-His* isosteres.

β-Ribosyltriazolylalanine Building Blocks 9 and 11. The preparation of 1,4- and 1,5-disubstituted triazole building blocks that are compatible with Fmoc-based SPPS commenced with the synthesis of β-configured azide **3**, which was derived from the commercially available ribose tetraacetate over four steps according to previously reported literature procedures (Scheme S1).[31,41] The required propargylglycine could be accessed from commercially available Boc−Asp−OBn **4**

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through a radical-mediated[42,43] decarboxylative alkynylation, as described by Baran et al. for the synthesis of homopropargylglycine from Boc−Glu−OBn.[44] To this end, Boc−Asp−OBn **6** was transformed in redox-active ester **5** via a Steglich esterfication (Scheme 1A). Ethynylzinc chloride was prepared *in situ* from its Grignard precursor and an equimolar mixture of zinc chloride and lithium chloride in THF. To ensure the efficient and consistent formation of alkyne **6**, it was found that the bipyridine−Ni(II) complex solution and a large excess of the ethynylzinc chloride had to be added to phthalimide **5** in quick succession. This way the fully protected propargylglycine **6** was obtained in 73% yield. Protecting group manipulations then provided Fmoc-propargylglycine benzyl ester **7**, which could be conjugated to β-configured azide **3** in a Cu(I)- or Ru(II)-catalyzed cycloaddition. Successive addition of $CuSO_4$ and sodium ascorbate to a solution containing equimolar amounts of azide **3** and propargylglycine **7** in DMF provided a single product (**8**) that was conveniently isolated by silica gel column chromatography. Formation of 1,4-disubstituted triazole **8** was confirmed using heteronuclear multiple bond correlation (HMBC) NMR spectroscopy, which revealed a clear coupling between the ribosyl H-1′ and the tertiary C-5 of the triazole unit (Figure 3).

To generate the alternative 1,5-triazole, azide **3** was clicked to alkyne **7** using the chloro(pentamethylcyclo-pentadienyl)(cyclo octadiene)ruthenium (II) (Cp*RuCl(COD)) catalyst. An almost immediate conversion of the click partners **3** and **7** was realized by microwave heating the components in THF at 100 °C in the presence of the Cp*RuCl(COD) catalyst to provide 1,5-triazole **10** in 85% yield.[45] The isolated product clearly differed from the previously isolated 1,4-regioisomer **8** according to both ^1H and ^{13}C NMR. HMBC measurements revealed a strong coupling between the ribosyl H-1′ and the quaternary C-5 of the triazole moiety proving the regioselective formation of the 1,5-disubstituted triazole **10** (Figure 3). Deprotection of the benzyl esters in **8** and **10** provided the β-configured building blocks **9** and **11** for the planned Fmoc-based SPPS endeavors.

α-Ribosyltriazolylalanine Building Blocks 17 and 19. In the syntheses of the corresponding 1,4- and 1,5-triazole α-ribosyl building blocks, non-participating benzyl-type protecting groups are required to install the α-azido ribosyl linkages. Therefore, the benzyl ester **7** described above cannot be used to generate the triazole amino acid and we switched to the use of an allyl ester to mask the amino acid carboxylate. Since glutamic acid allyl esters are not readily commercially available, the route described above for the benzyl ester could not be followed and we generated the required building block **14** from commercially available propargylglycine **12** (Scheme 1B). Introduction of the Fmoc group under basic conditions in a H_2O/THF solvent system gave carboxylic acid **13**, which was converted into **14** via a Steglich esterification with DIC/DMAP and allyl alcohol. α-Configured azide **15** was prepared as described previously,[31] and both click partners could be joined by applying the same reaction conditions as discussed above to give 1,4-triazole **16** in good yield. The RuAAC-reaction to generate the 1,5-regioisomer **18** however required some optimization. Incomplete conversion of azide **15** was observed when a small excess of alkyne **14** (1.3 equiv) was used, and even when an extended reaction time (>1 h) was used, the yield did not exceed 35%. Using a 2-fold excess of alkyne **14** eventually led to the formation of 1,5-triazole **18** in a satisfactory yield of 60%. The regiochemistry in **16** and **18** was again substantiated by HMBC data. Removal of the allyl ester functionality in **16** and **18** was mediated using a catalytic amount of tetrakis-(triphenylphosphine)palladium(0) in the presence of 1,3-dimethylbarbituric acid (DMBA) as an allyl scavenger to provide the desired α-configured SPPS building blocks **17** and **19**. With both anomeric configurations of ribosylated 1,4- and 1,5-triazoles in hand, we next set out to generate the set of target ADPr-His* peptides using SPPS.

Solid-Phase Synthesis of ADPr-His* Peptides. As described above, peptides from histone PARylation factor 1 (HPF1) have been identified as the ADP-ribosylation site in recent proteomic studies,[21] and therefore, we incorporated the α/β-N(τ)/N(π)-ADPr-His* analogues in the corresponding peptide fragment derived from this protein.[31] Starting from Tentagel S AC resin, preloaded with *tert*-butyloxycarbonyl (Boc)-protected lysine, β-ribosyltriazolides **9** and **11** were incorporated using standard Fmoc-based SPPS conditions to provide fully protected intermediates **20A/B** (Scheme 2). Next, we planned to install the pyrophosphate moiety by unmasking the primary alcohol, installing the primary phosphate, and extending this using a P(V)-P(III) coupling with a suitably protected adenosine phosphoramidite. Initially, we explored the removal of the silyl protecting group with tetrabutylammonium fluoride (TBAF) in THF,[46] but LC-MS analysis indicated that desilylation was accompanied by loss of the 2′- and/or 3′-benzoyl moieties. This also implied that the C-terminal ester linkage to the resin might be at risk. Fortunately, HF-pyridine proved to be an adequate and the milder alternative, leaving the ester functionalities unscathed while efficiently removing the TBDPS group. The liberated alcohol was conjugated to a 9-fluorenylmethyl (Fm)-protected phosphoramidite[47] in the presence of 5-(ethylthio)-tetrazole (ETT) as an activator. The resulting phosphotriester was subsequently oxidized with (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) to provide protected phosphates **21A/B**. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) allowed for effective removal of the Fm-moieties, after which construction of the pyrophosphate was realized with adenosine amidite **30**[48] using P(III)-P(V) coupling chemistry under the *aegis* of ETT.[40] CSO-mediated oxidation of the phosphate-phosphite intermediate into the pyrophosphate provided fully protected ADPr-conjugates **22A/B**. After deprotection of the 2-cyanoethyl group with DBU, the oligopeptide was treated with 50% trifluoroacetic acid (TFA) to ensure cleavage of the construct from the Tentagel resin while simultaneously removing the *t*-Bu and Boc protecting groups. Global deprotection of the remaining base sensitive groups was affected by the treatment of the crude material with aqueous ammonia overnight, and this was followed by purification using preparative HPLC (NH_4OAc buffered) to yield the β-configured N(τ)- and N(π)-ADPr-His* peptides **23** and **24** in 36 and 25% yield, respectively.

In a similar manner as described above, the α-configured building blocks **17** and **19** were incorporated in the peptide backbone, after which the resin-bound intermediates were desilylated with HF-pyridine and phosphorylated in a two-step fashion to give the Fm-phosphates **26A/B**. After treatment with DBU, the liberated phosphate was coupled to fully Boc-protected adenosine amidite **31** to yield ADPr-peptides **27A/B**.[49] After the elimination of the 2-cyanoethyl functionality, all remaining protecting groups were successfully removed during the final cleavage with TFA. Identical preparative HPLC conditions allowed for the isolation of the two remaining α-configured N(τ)- and N(π)-histidine isosteres **28** and **29**.

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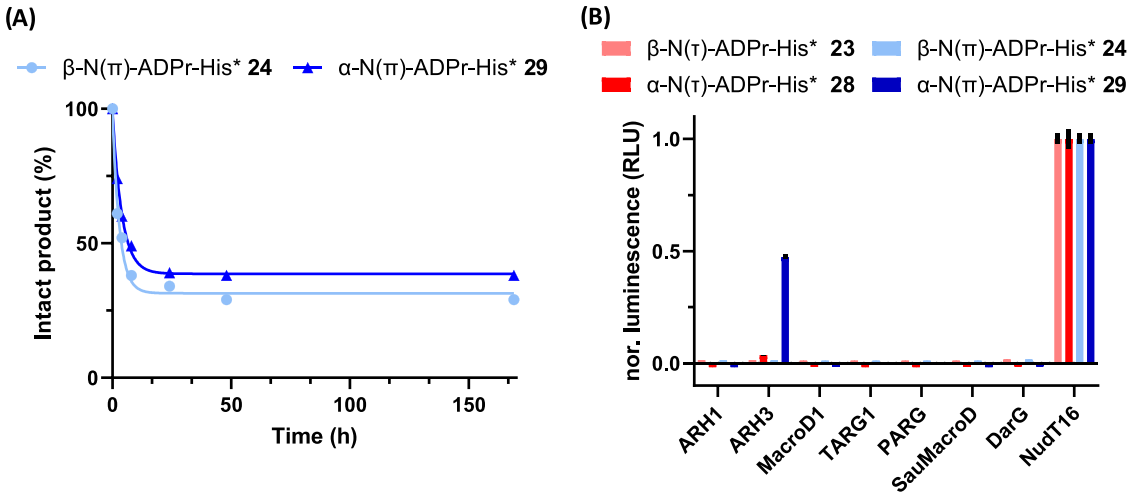

Figure 4. (A) Chemical stability of 1,5-disubstitued triazoles **24** and **29** under basic conditions (NaOH, 0.1 M). Samples were extracted at different time points (2, 4, 8, 24, 48, and 169 h) and quenched with TFA prior to LC-MS injection. Peptide degradation was quantified by analyzing the UV-trace (260 nm) using Xcalibur software. Including an exponential one-phase decay trendline (R^2 values are 0.986 and 0.997 for **24** and **29**, respectively). (B) Enzymatic hydrolysis of the ribosyl linkages in ADP-ribosylated histidine peptides **23, 24, 28**, and **29**. Enzymatic turnover of the various peptides was assessed by monitoring AMP release directly (NudT16) or converting released ADPr via NudT5 to AMP. AMP was measured using the AMP-Glo assay (Promega). Samples are background corrected and normalized to NudT16 activity.

With all four ADPr-His* conjugates available, we next evaluated their stability under various conditions in a liquid-chromatography mass-spectrometry (LC-MS) based assay. The conditions surveyed (NH_2OH, TFA, and NaOH) were selected because of their frequent occurrence in ADP-ribosylome-focused proteomics studies. In these studies, NH_2OH elimination steps are implemented to identify acidic ADP-ribosylated residues[1,16] while basic conditions are applied for the pre-fractionation of peptides, which is generally followed by a subsequent acid treatment.[13,22] All four isosteres remained unaffected under the TFA (0.1 M) or NH_2OH (0.5 M) conditions for at least 24 h, as no sign of degradation was observed by LC-MS. After 24 h of 0.1 M NaOH treatment, LC-MS analysis showed additional peaks for the β-N(τ)-ADPr-His* **23** and α-N(τ)-ADPr-His* **28** peptides. These peaks (5 and 12%) corresponded to products having an identical mass as the parent compounds, indicating an isomerization reaction. As the newly formed peaks did not correspond to the anomeric counterparts of the starting compounds, we assume these products to originate from epimerization of the His* α-carbon, as histidine has been observed to be relatively prone to epimerization. The steric hindrance in the more crowded 1,5-triazoles possibly makes these peptides more susceptible to the base-mediated epimerization than their 1,4-counterparts. We monitored the isomerization of the 1,5-triazoles β-N(π)-ADPr-His* **24** and α-N(π)-ADPr-His* **29** in a follow-up time course experiment (Figure 4A) and observed rapid consumption of the starting peptides leading to a mixture of products in which 38% and 30% of the original peptides **24** and **29**, respectively, were present after 12 h.

Finally, we assessed the susceptibility of the peptides toward (ADP-ribosyl)hydrolase-mediated hydrolysis (Figure 4B). Each of triazole conjugates **23, 24, 28**, and **29** was incubated with a set of purified human (ADP-ribosyl)hydrolases. Any ADPr freed by the hydrolase in these reactions was converted by the NudT5 enzyme into adenosine monophosphate (AMP and quantified using the AMP-Glo assay (Promega)).[50] As a positive control, the samples were treated with NudT16, which is able to hydrolyze the pyrophosphate linkage of both free and peptide-bound ADPr.[51] None of the hydrolases were able to cleave the *N*-glycosidic linkage of any of the four isosteres, except for ARH3, which was capable of hydrolyzing α-N(π)-ADPr-His* **29** and very weakly α-N(τ)-ADPr-His* **28**. Both β-anomers **23** and **24** remained unaffected by ARH3 under the given conditions. The slow but enzyme-dependent cleavage of α-N(τ)-ADPr-His* **28** corresponds to the hydrolysis we described in our previous study.[31] Of note, the hydrolysis rate for α-N(π)-ADPr-His* **29** was substantially higher than its N(τ)-regioisomer **28**, leading to ~50% conversion within the 1 h reaction. This may indicate that N(π)-ADPr-His is the naturally occurring isomer and that ARH3 could be the hydrolase involved in trimming ADP-ribosyl units from ADP-ribosylated histidine-containing proteins. It should be pointed out, however, that ARH3 is a rather promiscuous hydrolase[23,35] and the susceptibility to ARH3-mediated cleavage of the 1,5-triazoles can also be due to enhanced lability of these substrates as inferred from the stability studies described above.

CONCLUSIONS

A comprehensive SPPS-based methodology toward both 1,4- and 1,5-disubstituted triazole ADPr-peptide conjugates has been developed to mimic ADP-ribosyl histidine peptides. To this end, the regioselectivity of Cu(I)- and Ru(II)-catalyzed click reactions has been exploited to join a propargylglycine and an α- or β-configured azido ribosyl building block to furnish a complete set of (α/β)-N(τ)/N(π)-ADPr-His mimetics. Incorporation of these novel building blocks in a peptide sequence of interest was accomplished uneventfully using standard Fmoc-based SPPS conditions. Desilylation of the resulting resin-bound intermediates was performed with a slightly acidic HF-pyridine instead of TBAF, which we previously employed,[46] to minimize the degradation of the ester linkages. The adenosine diphosphate moiety could be readily introduced through phosphoramidite chemistry to effectively deliver, after a sequence of deprotection steps, the target triazolyl peptide conjugates **23, 24, 28**, and **29** in good to satisfactory yields. The stability of the peptides under nucleophilic (0.5 M NH_2OH), acidic (0.1 M TFA), and basic (0.1 M NaOH) conditions,

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commonly employed in ADP-ribosyl proteomics protocols, was assessed, revealing the 1,5-triazoles to be sensitive to base-assisted epimerization. A NudT-based luminescent assay enabled the quantification of (ADP-ribosyl)hydrolase-mediated degradation of the *N*-glycosidic linkage in the triazole constructs. Both α-N(τ)-ADPr-His* **28 and** α-N(π)-ADPr-His* **29** proved to be susceptible to ARH3-mediated hydrolysis, with the 1,5-triazole **29** being significantly more labile than its 1,4-triazole counterpart **28**. These findings may suggest the α-N(π)-His to be the naturally occurring isomer, although the chemical stability studies also point to the intrinsic lability of the 1,5-triazole conjugates. Our findings warrant the development of a synthetic methodology to access ADP-ribosylated histidines, featuring the natural linkages, to determine whether the observed hydrolytic activity of ARH3 is of real biological relevance and applies to the natural imidazolyl−glycosidic linkages. It is expected that the methodology described here can be transposed to many other peptide sequences to effectively deliver ADPr-His* tools for (structural) biology purposes.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its online Supporting Material.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.3c00827.

Procedures for the expression of plasmids and the following protein purification; procedure for the (ADP-ribosyl)hydrolase activity assay; chemical procedures including the relevant ^{1}H, ^{13}C, ^{31}P, HMBC NMR spectra for new compounds; spectra of the LC-MS-based stability assay performed for peptides **23**, **24**, **28**, and **29** (PDF)

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Notes

The authors declare no competing financial interest.

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