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A concise route for the preparation of nucleobase-simplified cADPR mimics by click chemistry

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ABSTRACT

Novel nucleobase-modified cADPR mimics were synthesized by the application of click chemistry. Cu(I)-Huisgen cycloaddition (click reaction) was used to construct 4-amide-1,2,3-triazole nucleobase and connect two building blocks efficiently. A concise protection strategy was used for the synthesis of the corresponding cyclo-pyrophosphate, and the target compounds **6a** and **6b** were prepared within four steps.

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Recently, cyclic ADP-ribose (cADPR) (Fig. 1) has been shown to control Ca^{2+}-dependent cellular responses in numerous cell systems.[1] The cADPR mimics are valuable tools for the investigation of the mechanisms of cADPR-mediated Ca^{2+} signaling.[1b,2] As a complex 18-atom macrocycle molecule, the preparation of cADPR and its mimics is synthetically challenging.[3] cADPR mimics can be prepared from NAD^+ derivatives by enzymatic and chemo-enzymatic methods using soluble ADP-ribosyl cyclase from *Aplysia californica* to catalyze the cyclization reaction.[4] However, the mimics obtained by these methods are limited because of the substrate specificity of ADP-ribosyl cyclase. Shuto et al. developed a method for the total chemical synthesis of cADPR mimics through building N-1 substituted purine nucleoside and intra-molecular cyclization for the formation of pyrophosphate linkage.[5] The chemical synthesis method allowed the extensive modifications of both ribose and nucleobase moieties, and has been well applied for the synthesis of cADPR mimics.[6–9] However, this method has too many steps for the protection and deprotection strategies in building the substituted nucleoside and pyrophosphate linkage. Thus, one of the main objectives resulting from our (and others) previous work was to develop a concise synthetic pathway to novel cADPR mimics and at the same time, to reduce the chemical complexity of the naturally occurring cADPR without major loss of biological activity (Fig. 1).[7–9]

It was found in our laboratory that cADPR mimics with ribose moieties replaced by simple ether strand or carbon strand, such as cIDPRE and cADPRE could act as the membrane-permeant calcium agonist.[8,9] Further, Walseth and colleagues have demonstrated that 3-deaza-cADPR, containing a 3-deazapurine moiety, is a potent agonist, which is about 70 times more active than

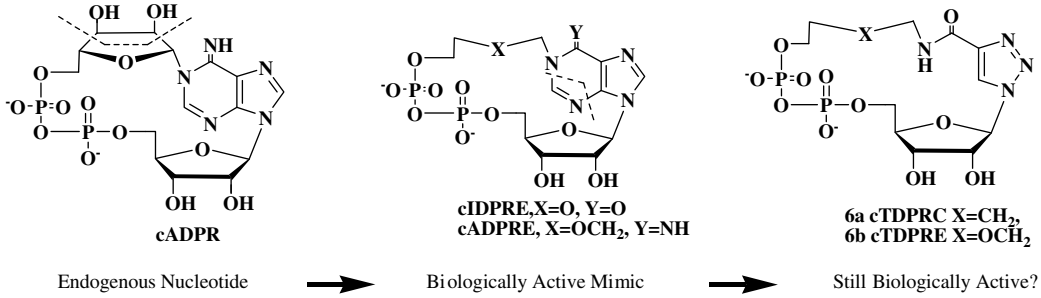

Figure 1. cADPR and cADPR mimics with simplified structures.

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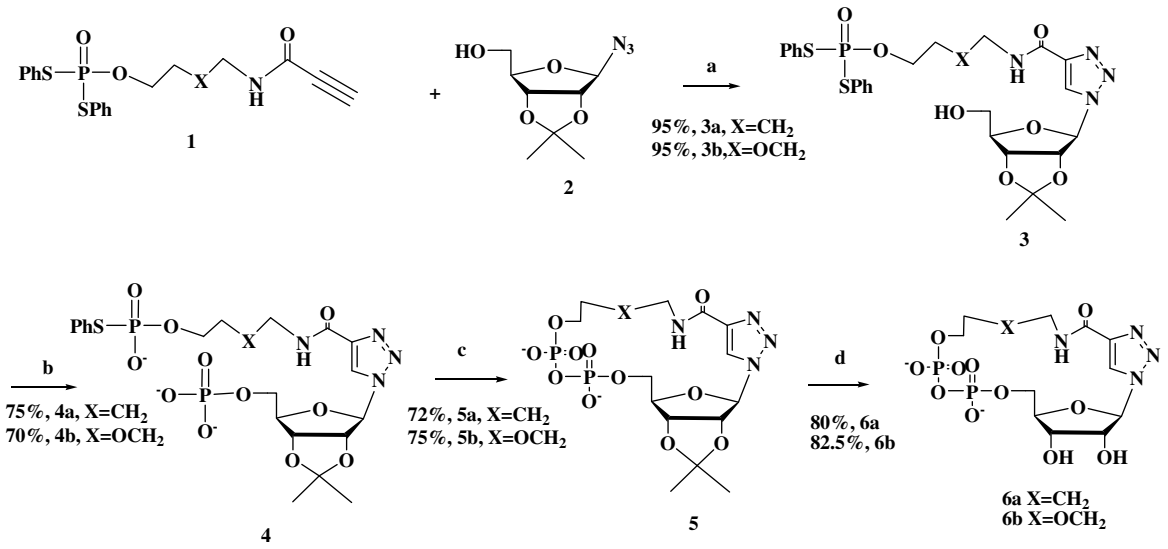


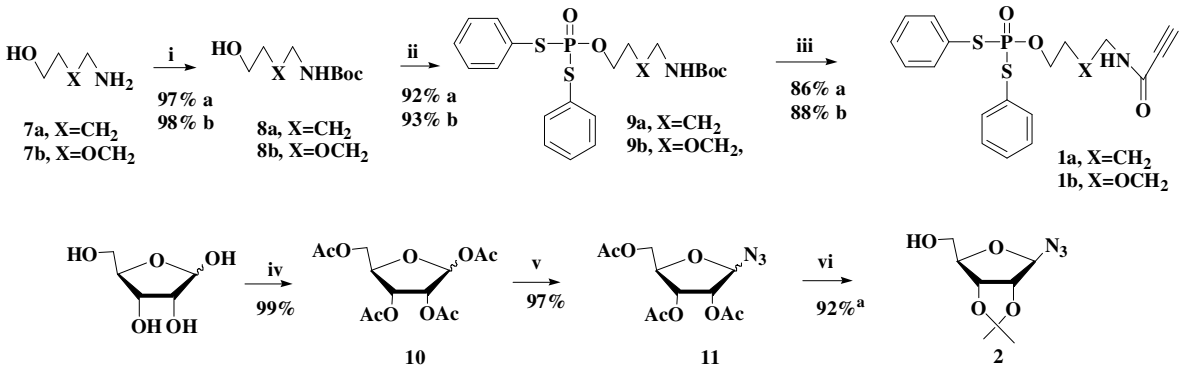
Scheme 1. The synthetic route of cTDPRE and cTDPRC. Reagents and conditions: (a) CuI, DIPEA, CH_3CN; (b) (1) $POCl_3$, DIPEA, CH_3CN, 0 °C, 12 h; (2) 0.1 M TEAB; (c) I_2, 3 Å MS, pyridine; (d) 50% HCOOH.

cADPR itself.[10] Thus, starting from the structure of cIDPRE and 3-deaza-cADPR, we designed and synthesized a new type of triazole-based cADPR analogues, abbreviated cTDPRC **6a** and cTDPRE **6b** (Fig. 1), in which 1,2,3-triazole-4-amide was constructed instead of the adenine moiety and the northern ribose was replaced by an ether or carbon strand. We report here the concise synthetic route for the preparation of this kind of cADPR mimics through Cu(I)-catalyzed Huisgen [3+2] cycloaddition.

Cu(I)-catalyzed Huisgen [3+2] cycloaddition between terminal alkyne and azide has significant advantages as an ideal synthetic reaction such as efficiency, versatility and selectivity.[11] It was applied well in biological conjugation and drug design as a click reaction.[12] In our approach, the efficient Huisgen 1,3-dipolar cycloaddition was applied to build the 4-amide-1,2,3-triazole moiety thereby connecting the northern strand and the southern ribose. The *S*,*S*-diphenylphosphate group, later on used for the formation of the pyrophosphate ring, was already introduced during the synthesis of building block **1**. After phosphorylation of the 5′-OH in the southern ribose, compound **4** was used to construct the cyclopyrophosphate ring by intra-molecular cyclization. Finally, deprotection of the 2′ and 3′ hydroxyl groups on the southern ribose resulted in the desired target compounds. Thus, the novel cADPR analogues were obtained by four synthetic steps and the protection strategy used for synthesizing such cADPR mimics was simplified significantly (Scheme 1).

For the synthesis of cADPR mimics **6a** and **6b**, the northern carbon and ether strand moiety **1a** and **1b** and southern 1-β-azido-2,3-*O*-isopropylidene-ribose **2** were obtained by the reactions shown in Scheme 2. Beginning with the commercial reagent **7a** or **7b**, compounds **8a** and **8b** were prepared according to the reported method.[13a] Compound **8a** or **8b** was treated with TPSCl and PSS in the presence of tetrazole in pyridine at room temperature to obtain **9a** or **9b** in 92% or 93% yield, respectively. After deprotection of **9a** or **9b** by TFA, the intermediate was reacted with propiolic acid in the presence of DCC to provide the building block **1a** in 86% yield or **1b** in 87% yield. The total yields for the preparation of building blocks **1a** and **1b** were 76% and 80% after three steps, respectively, and the building block **2** was prepared in 88% total yield based on the Carrington' method.[13b] The ratio of α:β isomer of **2** was 1:8 and the β isomer was easily separated by silica gel column chromatography.

Several classical Cu(I) systems were chosen to catalyze the Huisgen [3+2] cycloaddition reaction between **1a** and **2** (Table 1). The reaction of **1a** with **2** catalyzed by CuI/DIPEA in CH_3CN at 20 °C for 3 h afforded compound **3a** in the yield of 95%, which was higher than the yields by using other catalytic systems. The β-configuration in ribofuranoside moiety of **3a** was retained during this reaction condition. Moreover, the click reaction is highly regioselective and only 1,2,3-triazole-4-amide derivative **3a** was formed. The structure of compound **3a** was confirmed by HMBC



Scheme 2. Synthetic route of building blocks **1** and **2**. Reagents and conditions: (i) $(Boc)_2O$, $(CH_3CH_2)_3N$; (ii) PSS, TPSCl, Tetrazole; (iii) (a) CF_3COOH, CH_2Cl_2; (b) propiolic acid, DCC, CH_2Cl_2; (iv) Ac_2O, Py, 80 °C; (v) (a) HCl (gas), $(CH_3CH_2)_2O$, 0 °C; (b) NaN_3, CH_3CN, reflux; (vi) (a) K_2CO_3, CH_3OH; (b) H^+/acetone. [a]For β and α isomer.

Table 1
Cu(I)-catalyzed Huisgen 1,3-cycloaddition reaction between **1** and **2a** to **3a** in different catalyst systems

Entry	Catalyst system	Temperature (°C)	Time (h)	Yield of **3a** (%)
1	$CuSO_4{\cdot}5H_2O$, Cu, H_2O/*t*BuOH	20	24	81[a]
2	$CuSO_4{\cdot}5H_2O$, sodium ascorbate H_2O/*t*BuOH	20	24	65[a]
3	CuI, TEA, CH_3CN	20	24	85[a]
4	CuI, DIPEA, CH_3CN	20	3	95[a]
5	None catalyst, toluene	80	48	50[b]

[a] Separated yield.
[b] The 5-triazole amide-isomer was given in 25% yield from ^{1}H NMR.

NMR. A coupling peak between 5C in the 1,2,3-triazole ring and 1′H in southern ribose was observed, which supported the formation of 1,2,3-triazole-4-amide of compound **3a**. However, 1,2,3-triazole-5-amide, an isomer of compound **3a**, was obtained with 25% yield in Huisgen dipolar cycloaddition reaction when the reaction was carried out in toluene at 80 °C without Cu^+ as catalyst (Table 1). Compound **3b** was obtained under the same condition in 95% yield.

The precursor **4a** for the formation of pyrophosphate was obtained from compound **3a** in 75% yield by using $POCl_3$/DIPEA in CH_3CN at 0 °C for 12 h, followed by treatment of 0.1 M TEAB. More interestingly, the phosphorylation of 5′-OH of the southern ribose and the partial deprotection of *S*,*S*-diphenylphosphate were completed by a one-pot reaction. The same procedure was applied for the synthesis of **4b** resulting in the yields of 70%.

The intramolecular cyclization was performed in the presence of excess I_2 and 3 Å molecular sieves in pyridine by adding a solution of compound **4a** over 20 h using a syringe.[9] The cyclic product **5a** was purified by HPLC as its triethylammonium salt in 75% yield. The structure of **5a** was confirmed by ESI-MS$^+$, ^{31}P NMR and ^{1}H NMR. Finally, the removal of isopropylidene group of **5a** was carried out with 50% HCOOH in water at room temperature for 2 h to obtain the target compound **6a**, cTDPRC. **6a** was purified by HPLC as its triethylammonium salt in 80% yield. The total yield of cTDPRC **6a** was 42.7% from building blocks **1a** and **2**.[14]

Similarly, compound cTDPRE **6b** was prepared by the same strategy. The total yield of cTDPRE **6b** is 41.5% from building blocks **1b** and **2**.[15]

In summary, novel nucleobase-modified cADPR mimics **6a** and **6b** were synthesized. Cu(I)-Huisgen cycloaddition (click reaction) was used to construct 4-amide-1,2,3-triazole nucleobase and connect two building blocks efficiently. A concise protection strategy was used for the synthesis of the corresponding cyclo-pyrophosphate, and the target compounds **6a** and **6b** were prepared within four steps in more than 41.5% total yields.

Primary pharmacological research showed that **6a** cTDPRC and **6b** cTDPRE could introduce Ca^{2+} release in intact human Jurkat T cell,[16] and the further pharmacological study will be discussed elsewhere.

Acknowledgements

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14. Spectroscopic data of compound **3a–6a**. Compound **3a** ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.09 (s, 1H), 7.32–7.56 (m, 10H), 5.82 (d, J = 8.5 Hz, 1H), 5.41 (d, J = 7 Hz, 1H), 4.76–4.78 (m, 1H), 4.68–4.72 (m, 1H), 4.45–4.48 (m, 1H), 4.30–4.37 (m, 1H), 4.20–4.27 (m, 1H), 4.05 (dd, J = 3, 13 Hz, 1H), 3.78–3.81 (dd, J = 3, 13 Hz, 1H), 3.40–3.48 (m, 1H), 3.23–3.29 (m, 1H), 1.62–1.78 (m, 7H,), 1.43 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), 160.3, 141.8, 135.3, 129.5, 126.4, 126.2, 110.6, 86.2, 74.1, 73.4, 68.1, 66.8, 65.3, 38.5, 27.7, 26.5, 25.3, 25.0. ^{31}P NMR ($CDCl_3$, 121.5 Hz, decoupled with ^{1}H), 51.1 (s). Anal. Calcd for $C_{27}H_{33}N_4O_7P_1S_2$: C, 52.25; H, 5.36; N, 9.03. Found: C, 52.02; H, 5.54; N, 9.06. Compound **4a** ^{1}H NMR (500 MHz, DMSO-d_6), δ 8.87 (s, 1H), 8.48 (s, 1H), 7.11–7.51 (m, 5H), 4.96–4.97 (m, 1H), 5.89 (d, J = 8.5, 1H), 4.94 (d, J = 7 Hz, 1H), 4.46 (d, J = 7.5 Hz, 1H), 4.17 (d, J = 12.5 Hz, 1H), 3.70–3.72 (m, 2H), 3.62 (d, J = 12.5 Hz, 1H), 3.19–3.20 (m, 2H), 1.46 (m, 7H), 1.31 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6), 159.6, 142.8, 135.4, 131.5, 128.1, 125.3, 108.8, 84.3, 73.2, 72.4, 68.6, 64.5, 57.3, 38.1, 27.7, 26.3, 25.8, 24.9. ^{31}P NMR (D_2O, 121.5 Hz, decoupled with ^{1}H), δ 20.8 (s), 3.5 (s). HRMS (ESI) m/z calcd for $C_{21}H_{31}N_4O_{11}P_2S_1$: 609.1180; found, 609.1179. Compound **5a** ^{1}H NMR (500 MHz, CD_3OD) δ 9.13 (s, 1H), 6.09 (d, J = 9.5 Hz, 1H), 5.25 (dd, J = 3, 7 Hz, 1H), 5.37–5.41 (m, 1H), 4.77–4.80 (m, 1H), 4.51–4.57 (m, 3H), 4.12 (d, J = 12.5 Hz, 1H), 3.75–3.80 (m, 2H), 1.72–1.89 (m, 4H), 1.39, 1.55 (each s, each 3H). ^{31}P NMR (D_2O, 121.5 Hz, decoupled with ^{1}H), δ −6.78 (d, $J_{p,p}$ = 13.5 Hz), −8.69 (d, $J_{p,p}$ = 13.5 Hz). HRMS (ESI) m/z calcd for $C_{15}H_{25}N_4O_{11}P_2$: 499.0989; found, 499.0984. Compound **6a** ^{1}H NMR (500 MHz, D_2O) δ 8.68 (s, 1H), 6.04 (d, J = 9 Hz, 1H), 4.53–4.57 (m, 2H), 4.11–4.17 (m, 1H), 3.94–4.03 (m, 2H), 3.82–3.88 (m, 1H), 3.67–3.73 (m, 1H), 3.54–3.5 (m, 1H), 3.37–3.42 (m, 1H), 1.71–1.90 (m, 4H). ^{31}P NMR (81 MHz, D_2O) δ −10.64 (br s), −11.59 (br s). HRMS (ESI) m/z calcd for $C_{12}H_{19}N_4O_{11}P_2$: 457.0531; found, 457.0526.
15. Spectroscopic data of compound **3b–6b**. Compound **3b** ^{1}H NMR (500 MHz, $CDCl_3$), δ 8.15 (s, 1H), 7.34–7.55 (m, 10H), 5.82 (d, J = 8.5 Hz, 1H), 4.75 (dd, J = 3.5, 7 Hz, 1H), 4.56–4.59 (m, 1H), 4.52–4.48 (m, 1H), 4.33–4.36 (m, 2H), 3.00 (dd, J = 13, 3 Hz, 1H), 3.82 (dd, J = 3, 13 Hz, 1H), 3.69–3.71 (m, 2H), 3.55–3.65 (m, 4H), 1.43, 1.61 (each s, each 3H). ^{13}C NMR(75 MHz, $CDCl_3$), 160.2, 142.1, 135.3, 129.5, 126.3, 126.0, 121.5, 110.6, 86.2, 73.9, 73.3, 69.6, 67.2, 67.0, 66.9, 65.2, 39.0, 26.6, 25.0. ^{31}P NMR ($CDCl_3$, 121.5 Hz, decoupled with ^{1}H), 51.05 (s). Anal. Calcd for $C_{27}H_{33}N_4O_8P_1S_2$: C, 50.93; H, 5.22; N, 8.80. Found: C, 51.08; H 5.42; N, 8.71. Compound **4b** ^{1}H NMR (500 MHz, D_2O), δ 8.57 (s, 1H), 7.30–7.55 (m, 5H), 6.03 (d, J = 8 Hz, 1H), 5.08 (dd, J = 3, 8 Hz, 1H), 4.94–4.98 (m, 1H), 4.69 (d, J = 8 Hz, 1H), 4.26 (dd, J = 13.5, 1 Hz, 1H), 4.07–4.23 (m, 2H), 3.87 (dd, J = 1, 13.5 Hz, 1H), 3.72–3.74 (m, 2H), 3.70 (t, J = 11 Hz, 2H), 3.56 (t, J = 11 Hz, 2H), 1.46–1.60 (each s, each 3H). ^{31}P NMR (D_2O, 81 Hz), δ 18.31 (br s), 2.75 (br s). HRMS (ESI) m/z calcd for $C_{21}H_{29}N_4O_{12}P_2S_1$: 623.0983; found, 623.0984. Compound **5b** ^{1}H NMR (300 Hz, D_2O), δ 8.48 (s, 1H), 5.97 (d, J = 8 Hz, 1H), 4.81–4.89 (m, 2H), 4.57–4.60 (m, 1H), 4.23 (d, J = 13 Hz, 1H), 3.85 (d, J = 13 Hz, 1H), 3.39–3.60 (m, 8H), 1.30, 1.45 (each s, each 3H). ^{31}P NMR (D_2O, 81 Hz), δ −9.11 (br s), −11.4 (br s). HRMS (ESI) calcd for $C_{15}H_{25}N_4O_{12}P_2$ m/z: 515.0939; found, 515.0925. Compound **6b** ^{1}H NMR (500 MHz, D_2O), δ 8.67 (s, 1H), 6.04 (d, J = 9 Hz, 1H), 4.54–4.56 (m, 2H), 4.14–4.18 (m, 1H), 3.93–4.02 (m, 2H), 3.80–3.87 (m, 2H), 3.69–3.73 (m, 2H), 3.53–3.66 (m, 4H). ^{31}P NMR (D_2O, 81 Hz) −8.91 (br s), −11.39 (br s). HRMS (ESI) m/z calcd for $C_{12}H_{19}N_4O_{12}P_2$: 473.0480; found, 473.0477.
16. The biological activity of cTDPRC **6a** and cTDPRE **6b** was assessed in intact human Jurkat T-lymphocytes as described previously.[8,9]