Concise Syntheses of Trifluoromethylated Cyclic and Acyclic Analogues of cADPR

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Received: 6 November 2010; in revised form: 17 November 2010 / Accepted: 22 November 2010 / Published: 30 November 2010

Abstract: A novel trifluoromethylated analogue of cADPR, 8-CF_3-cIDPDE (5) was designed and synthesized via construction of N_1,N_9-disubstituted hypoxanthine, trifluoromethylation and intramolecular condensation. A series of acyclic analogues of cADPR were also designed and synthesized. These compounds could be useful molecules for studying the structure-activity relationship of cADPR analogues and exploring the cADPR/RyR Ca^{2+} signalling system.

Keywords: cADPR analogue; acyclic cADPR analogue; trifluoromethylation; synthesis

1. Introduction

Cyclic adenosine diphosphate ribose (cADPR, 1, Figure 1), isolated from sea urchin eggs [1], is a metabolite of β-nicotinamide adenine dinucleotide (NAD^+). It has been proved that cADPR is a signalling molecule, which regulates calcium mobilization via ryanodine receptor (RyR) in a wide variety of Ca^{2+}-dependent cellular responses such as fertilization, secretion, contraction, proliferation and so on [2]. Since the discovery of cADPR, numerous works have been done on the synthesis of cADPR analogues to search for agonists or antagonists of cADPR/RyR Ca^{2+} signalling system [3-5].

In our previous work, a series of cADPR analogues in which the southern and/or northern ribose was replaced by an ether chain were synthesized [6,7]. Most of those compounds, such as cIDPRE (2) and cIDPDE (3), are membrane permeate agonists in Jurkat T cells.
Figure 1. Structures of cADPR and its analogues.

Moreover, it was found that those agonists antagonize the hydrolysis of CD38. Substitution at C-8 of purine affects the agonistic activity of cADPR analogues. For example, 8-Br or 8-Cl substituted cIDPRE loses activity; however, the activity is retained for 8-N3 or 8-NH2 substituted cIDPRE. These results indicate that the effect of substitution at 8-position depends on the property of the substituent group. The trifluoromethyl group, possessing high electronegativity and lipophilicity, usually alters considerably the overall charge distribution and enhances the membrane permeability of molecules. Since the trifluoromethyl group imparts a variety of special physical and chemical properties to molecules, a number of trifluoromethylated compounds exhibit enhanced biological activity [8]. Taking these points into account, we synthesized 8-CF3-cIDPRE (4, Figure 2). We found that this compound was also a membrane permeate calcium agonist in Jurkat T cells [9]. In this study, the trifluoromethyl group is introduced to cIDPDE (8-CF3-cIDPDE, 5, Figure 2). This compound provides a complementary agent for understanding the effect of 8-substitution on calcium signalling property.

Figure 2. Structures of compounds 4-8.

cADPR can be hydrolyzed either in vivo or in vitro [10,11]. The cyclic pyrophosphate moiety, as one of the most vulnerable linkages in cADPR, can be hydrolyzed by Mn2+-dependent ADP-ribose/CDP-alcohol pyrophosphatase to afford the bisphosphate metabolite [12]. Recently, a series of acyclic analogues of cADPR, in which the pyrophosphate moiety is cleaved to give a bisphosphate, have been synthesized [13]. The primary pharmacological research revealed that some of them could inhibit cIDPRE-induced Ca2+ release. To further explore the Ca2+-modulating activities of this novel
class of cADPR mimics and their mechanism further, we have designed and synthesized acyclic analogues of cIDPDE and the trifluoromethylated analogues 6-8 (Figure 2).

2. Results and Discussion

2.1. Synthesis of 8-CF₃-cIDPDE (5)

The synthesis of 8-CF₃-cIDPDE is summarized in Scheme 1. Starting from 8-bromoadenine [14], N⁹-substitution was carried out with (2-acetoxyethoxy)methyl bromide [15] in the presence of potassium tert-butoxide (t-BuOK) and 18-crown-6 [16] to afford 10 in 44% yield. It is noteworthy that when (2-acetoxyethoxy)methyl chloride was employed instead, replacement of the 8-bromo group with a chlorine atom was observed. The structure of compound 10 was confirmed by ¹H-NMR, ¹³C-NMR, HMBC and HR-ESI-MS spectra. In the HMBC spectrum of 10, the correlation between H-1’ of the ether chain and C-4 and C-8 of adenine base were observed, which verified that the substitution was on N-9.

Scheme 1. Synthesis of 8-CF₃-cIDPDE (5).

Reagents and conditions: (a) t-BuOK, 18-crown-6, BrCH₂OCH₂CH₂OAc, THF, 0 °C; (b) K₂CO₃, MeOH, rt; (c) NaNO₂, AcOH, rt; (d) TBDPSCl, imidazole, DMF, rt; (e) DBU, ClCH₂OCH₂CH₂OAc, CH₂Cl₂, rt; (f) FSO₂CF₂CO₂Me, Cul, HMPA, DMF, 70 °C; (g) 70% HF·Py, THF; (h) PSS, TPSCI, tetrazole, Py, rt; (i) AcCl, MeOH; (j) i. POCI₃/DIPEA, CH₃CN, 0 °C; ii. 1 M TEAB, pH 7.5, rt; (k) I₂, 3Å MS, Py, rt.
Deacetylation of 10 with K₂CO₃/MeOH gave compound 11, and after diazotization, and protection of the 5′-hydroxyl group with a tert-butylidiphenylsilyl (TBDPS) group, 13 was obtained. An N₁-substitution was carried out on compound 13 with (2-acetoxyethoxy)methyl chloride in the presence of excess 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford 14 in 61% yield. Since both of the N₁ and the O₆ have nucleophilicity, the N₁-isomer and O₆-isomer were obtained (Figure 3). The structure of 14 was confirmed by ¹H-NMR, ¹³C-NMR, HMBC and HR-ESI-MS spectra. In the HMBC spectrum of 14, the correlation between H-1′ of the northern ether chain and C-2 of hypoxanthine base, and that between C-1′′ of the northern ether chain and H-2 of hypoxanthine base were both observed, which were similar to that of N₁-isomer. Corresponding correlations were not found in the HMBC spectrum of the O₆-substituted side product.

The unstable glycosyl bond in nucleosides is sensitive to certain conditions, which causes great difficulties in the trifluoromethylation of nucleosides. In our previous work, methyl fluorosulphonyldifluoroacetate/copper iodide (FSO₂CF₂CO₂Me/CuI) [17] was initially applied to the synthesis of 8-CF₃-purine nucleosides [9]. Adopting this strategy, trifluoromethylation of 14 was achieved successfully, and optimization of this reaction was carried out (Table 1). Under the optimal reaction conditions, 15 was obtained in 42% yield, and 14 was recovered in 17% yield. Interestingly, compound 16 was also obtained in a yield of 14%. It is known that the tert-butyldimethylsilyl (TBDMS) group and TBDPS group could be removed by tetrabutylammonium fluoride/tetrahydrofuran (TBAF/THF), potassium fluoride and other agents containing fluoride [18]. Accordingly, we deduced it was the fluoride ion generated in the process of trifluoromethylation [17] that facilitated the removal of the 5′-O-TBDPS group. The trifluoromethylated product 15 was characterized by ¹H-NMR, ¹³C-NMR, ¹⁹F-NMR and HR-ESI-MS spectra. In the ¹³C-NMR spectrum of compound 15, signals of the CF₃ group and C-8 were spilt into two quartets, with ¹J_CF = 270 Hz and ²J_CF = 41 Hz, respectively, and the singlet at −63.358 ppm was observed in the ¹⁹F-NMR spectrum. These data strongly support the incorporation of the trifluoromethyl group.

![Figure 3. Structures of 14 and its O6-isomer.](image-url)

**Table 1.** Optimization of the reaction conditions of trifluoromethylation.

| Entry | FSO₂CF₂CO₂Me /HMPA | Yield |
|-------|---------------------|-------|
| 1     | 5 equiv             | trace |
| 2     | 10 equiv            | 12%   |
| 3     | 15 equiv            | 42%   |
| 4     | 20 equiv            | 31%   |
| 5     | 30 equiv            | 18%   |
The 5′-O-TBDPS group in compound 15 was removed by employing 70% HF-pyridine [19]. The strong electronegativity of trifluoromethyl group at C-8 of hypoxanthine makes the glycosyl bond rather sensitive to acid conditions. Hence, 70% HF-pyridine was added dropwise to the reaction mixture at −20 °C. Compound 16 was successfully converted to 17 by the reaction with S,S-diphenylphosphorodithioate (PSS) [20] in the presence of triisopropylbenzenesulfonyl chloride (TPSCI) and tetrazole in pyridine, in a yield of 79%. Considering the instability of phenylthio group under basic conditions [21], acetyl chloride in methanol (AcCl/MeOH) [22] was applied to the deacetylation of 17. When 1.2 equivalent of AcCl was utilized, compound 17 was successfully converted to 18. Phosphorylation of the 5′′-hydroxyl in 18 was carried out in the presence of excess POCl3 and N,N-diisopropylethylamine (DIPEA) at 0 °C. After being stirred for 14 h, the mixture was treated with 1 M triethylammonium bicarbonate (TEAB) for 6 h at room temperature [23], which facilitated the semi-deprotection of the S,S-diphenylphosphate. Purified by high performance liquid chromatography (HPLC), compound 19 was obtained as its triethylammonium salt.

Following the Matsuda strategy [24], with excess I2 and 3Å molecular sieves as promoters, the intramolecular cyclization was performed in pyridine by adding a solution of compound 19 slowly over 20 h utilizing a syringe pump. Purification by HPLC afforded cyclic product 5 as its triethylammonium salt in 71% yield, which was characterized by 1H-NMR, 19F-NMR, 31P-NMR and HR-ESI-MS spectra.

2.2. Syntheses of Compounds 6-8

Deacetylation of 16 with K2CO3/MeOH afforded compound 20 (Scheme 2), then both of the free hydroxyl groups in 20 were phosphorylated by employing POCl3/DIPEA in CH3CN at 0 °C for 16 h, followed by the treatment with 1 M TEAB for 6 h. Purified by HPLC, the target molecule 6 was obtained as its triethylammonium salt in 62% yield for two steps.

Scheme 2. Syntheses of compounds 6-8.

Reagents and conditions: (a) K2CO3, MeOH, rt; (b) i. POCl3/DIPEA, CH3CN, 0 °C; ii. for 20 and 25, 1M TEAB, pH 7.5, rt; for 22, 1 M NaOH, rt; (c) for 23, 60% HCOOH, rt; for 26, 10% HCOOH, rt.
Compound 23 was synthesized from 21 [6] in a yield of 71% for two steps by a similar method as used for the preparation of 6. After removing the 2',3'-O-isopropylidene group using 60% HCOOH solution, compound 7 was obtained as its triethylammonium salt in 85% yield. Starting from compound 24 [9], 26 was synthesized by a similar procedure. Considering the sensitivity of 8-CF₃-purine nucleosides to acid conditions, we performed the deprotection of 26 by employing 10% rather than 60% HCOOH solution, which afforded compound 8, with little de-glycosylated side product being generated. After purification by HPLC, the target molecule 8 was obtained as its triethylammonium salt in 68% yield, with 26 recovered in a yield of 15%. The biological activity assay of all the compounds synthesized is underway.

3. Experimental

3.1. General

HR-ESI-MS and ESI-MS were performed with a Bruker BIFLEX III instrument. ¹H-NMR and ¹³C-NMR were recorded with a Bruker AVANCE III 400; CDCl₃, DMSO-d₆ or D₂O were used as a solvent. Chemical shifts are reported in parts per million downfield from TMS (¹H and ¹³C). ³¹P-NMR spectra were recorded at room temperature by use of a JEOL AL300 spectrometer (121.5 MHz) or JEOL ECA600 spectrometer (243 MHz). Orthophosphoric acid (85%) was used as external standard. ¹⁹F- NMR spectra were recorded on a Varian VXR-500 spectrometer (470 MHz). Chemical shifts of ¹⁹F- NMR are reported in ppm with reference to CF₃COOH as external standard. Compounds 19, 23, 26, and 5-8 were purified on an Alltech preparative C₁₈ reversed-phase column (2.2 × 25 cm) with a Gilson HPLC using MeCN/TEAB (pH 7.5) buffer system as eluent.

3.2. Synthesis

N⁹-[(5'-Acetoxyethoxy)methyl]-8-bromoadenine (10). To a stirred suspension of 8-bromoadenine (4.5 g, 21.03 mmol) [14] in anhydrous THF (400 mL) was added potassium tert-butoxide (2.59 g, 23.13 mmol) and 18-crown-6 (1.11 g, 4.20 mmol). The reaction mixture was stirred at room temperature for 15 min, and then BrCH₂OCH₂CH₂OAc (3.1 mL, 23.13 mmol) [15] was added dropwise at 0 °C. After being stirred for 30 min at 0 °C, the mixture was filtered and the filtrate is evaporated under reduced pressure. The residue was purified by silica gel column chromatography (PE-EA = 1:2) to afford compound 10 (3.02 g, 44%). ¹H-NMR (400 MHz, DMSO-d₆) δ 1.92 (s, 3H, OAc), 3.69-3.72 (m, 2 H, H₄'), 4.04-4.17 (m, 2 H, H₅'), 5.51 (s, 2H, H₁'), 7.48 (s, 2H, NH₂), 8.16 (s, 1H, H₂). ¹³C-NMR (100 MHz, DMSO-d₆) δ 170.1, 154.8, 153.2, 151.2, 126.5, 118.7, 72.3, 67.0, 62.7, 20.5. MS (ESI-TOF⁺): m/z = 330.0 [(M + H)+].

N⁹-[(5'-Hydroxyethoxy)methyl]-8-bromohypoxanthine (12). Compound 10 (1.43 g, 4.34 mmol) was dissolved in methanol (120 mL). To the solution was added K₂CO₃ (73 mg, 0.53 mmol) and stirred for 6 h at room temperature. The mixture was neutralized by addition of 0.1 M HCl solution, and evaporated under reduced pressure. The residue was dissolved in AcOH (70 mL), and a solution of NaNO₂ (2.52 g, 36.4 mmol) in H₂O (17 mL) was added. The resulting mixture was stirred at room temperature for 24 h. After the mixture was evaporated in vacuo, the residue was partitioned between CHCl₃ and H₂O. The aqueous phase was extracted again with CHCl₃, the organic layer was combined...
and washed with brine, dried (Na₂SO₄), filtered and concentrated in vacuo. Flash chromatography (CH₂Cl₂-MeOH = 40:1) afforded 12 (792 mg, 63% for two steps). ¹H-NMR (400 MHz, DMSO-­d₆) δ 3.46-4.51 (m, 4H, H₄', H₅'), 4.63 (s, 1H, OH), 5.32 (s, 2H, H₁'), 8.14 (s, 1H, H₂), 12.56 (s, 1H, NH). MS (ESI-TOF⁺): m/z = 289.2 [(M + H)⁺].

N⁰-[5′-tert-Butyldiphenylsilyloxyethoxy)methyl]-8-bromohypoxanthine (13). To a solution of 12 (700 mg, 2.42 mmol) in anhydrous DMF (10 mL) was added imidazole (1.86 g, 24.2 mmol) and tert-butyldiphenylsilyl chloride (3.4 mL, 12.1 mmol) under argon, and the mixture was stirred at room temperature for 12 h. And the mixture was evaporated in vacuo, the residue was partitioned between CH₂Cl₂ and H₂O. The aqueous phase was extracted again with CH₂Cl₂, the organic layer was combined and washed with brine, dried (Na₂SO₄), filtered and concentrated in vacuo. Flash chromatography (PE-acetone = 5:1) afforded compound 13 (1.21 g, 95%). ¹H-NMR (400 MHz, CDCl₃) δ 1.06 (s, 9H, (CH₃)₃C-), 3.71-3.73 (m, 2H, H₄'), 3.82-3.84 (m, 2H, H₅'), 5.66 (s, 2H, H₁'), 7.37-7.69 (m, 10H, ArH), 8.44 (s, 1H, H₂), 13.19 (s, 1H, NH). ¹³C-NMR (100 MHz, CDCl₃) δ 157.8, 150.8, 146.3, 135.5, 133.3, 129.6, 127.6, 126.4, 126.4, 77.3, 77.0, 76.7, 73.5, 71.1, 62.9, 26.7, 19.0. HRMS (ESI-TOF⁺): calcd for C₂₄H₂₇BrN₄O₃Si [(M + H)⁺] 527.1109, [(M + Na)⁺] 549.0928, [(M + K)⁺] 565.0662; found, 527.1109, 549.0931, 565.0667.

N¹-[5′-Acetoxyethoxy)methyl]-N⁰-[5′-tert-butyldiphenylsilyloxyethoxy)methyl]-8-bromohypoxanthine (14). To the solution of 13 (1.23 g, 2.33 mmol) and DBU (3.5 mL, 23.3 mmol) in anhydrous CH₂Cl₂ (25 mL) was added ClCH₂OCH₂CH₂OAc (1.8 mL, 11.65 mmol) [15] dropwise at 0 °C. After being stirred for 40 min at room temperature, the solvent was evaporated in vacuo and the residue was purified by silica gel column chromatography (PE-acetone = 5:1) to afford compound 14 (916 mg, 61%). ¹H-NMR (400 MHz, CDCl₃) δ 1.04 (s, 9H, (CH₃)₃C-), 2.03 (s, 3H, OAc), 3.68-3.70 (m, 2H, H₄'), 3.79-3.82 (m, 2H, H₅'), 3.85-3.87 (m, 2H, H₄''), 4.18-4.20 (m, 2H, H₅''), 5.54 (s, 2H, H₁''), 5.61 (s, 2H, H₁'), 7.35-7.66 (m, 10H, ArH), 8.09 (s, 1H, H₂). ¹³C-NMR (100 MHz, CDCl₃) δ 170.7, 155.3, 149.2, 147.8, 135.5, 133.2, 129.7, 126.7, 126.4, 75.0, 73.5, 71.0, 68.1, 62.9, 26.7, 20.7, 19.0. HRMS (ESI-TOF⁺): calcd for C₂₉H₃₅BrN₄O₆Si [(M + H)⁺] 643.1582, [(M + Na)⁺] 665.1401, [(M + K)⁺] 681.1135; found, 643.1563, 665.1377, 681.1117.

N¹-[5′-Acetoxyethoxy)methyl]-N⁰-[5′-tert-butyldiphenylsilyloxyethoxy)methyl]-8-trifluoromethyl-hypoxanthine (15). To a solution of compound 14 (576 mg, 0.895 mmol) and CuI (206 mg, 1.074 mmol) in anhydrous DMF (33 mL), hexamethyl phosphoric triamide (2.39 mL, 13.425 mmol) and FSO₂CF₂CO₂Me (1.71 mL, 13.425 mmol) were added successively. The reaction mixture was stirred for 20 h at 70 °C under argon, then cooled to room temperature, 22 mL of saturated aq. NH₄Cl was added and the mixture was extracted with 200 mL of EA-hexanes (7:3). The organic layer was washed successively with sat. aq. NaHCO₃, water and brine, dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE-acetone = 9:2) to afford compound 15 (238 mg, 42%) and compound 16 (48 mg, 14%), with compound 14 recovered (96 mg, 17%). ¹H-NMR (400 MHz, CDCl₃) δ 1.03 (s, 9H, (CH₃)₃C-), 2.04 (s, 3H, OAc), 3.68-3.70 (m, 2H, H₄'), 3.79-3.81 (m, 2H, H₅'), 3.87-3.89 (m, 2H, H₄''), 4.20-4.22 (m, 2H, H₅''), 5.56 (s, 2H, H₁''), 5.76 (s, 2H, H₁'), 7.35-7.66 (m, 10H, ArH), 8.18 (s, 1H, H₂). ¹³C-NMR (100 MHz, CDCl₃) δ 170.7,
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156.3, 149.4, 149.3, 138.6 (d, $^2J_{CF} = 41$ Hz), 135.5, 133.2, 129.7, 127.7, 122.7, 118.2 (q; $^1J_{CF} = 270$ Hz), 75.1, 73.5, 71.4, 68.3, 63.1, 63.0, 26.7, 20.8, 19.1. $^{19}$F-NMR (470 MHz, CDCl$_3$) δ -63.4 (s). HRMS (ESI-TOF$^+$): calcd for C$_{30}$H$_{35}$F$_3$N$_4$O$_6$Si [(M + Na)$^+$] 655.2170, [(M + K)$^+$] 671.1904; found, 655.2169, 671.1913.

N$_1$-[5′′-Acetoxyethoxy)methyl]-N$_9$-[5′-hydroxyethoxy)methyl]-8-trifluoromethylhypoxanthine (16). A solution of 15 (182 mg, 0.288 mmol) in anhydrous THF (35 mL) was added 70% HF·Py 1.3 mL at −20 °C. The mixture was stirred at 0 °C for 1 h and at room temperature over night. The reaction mixture was quenched with saturated aq. NaHCO$_3$ at 0 °C and diluted with ethyl acetate, then partitioned and the water layer was washed with ethyl acetate again. The organic layer was combined, washed with brine, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE-EA = 1:5) to afford the compound 16 (91 mg, 82%). $^{1}$H-NMR (400 MHz, CDCl$_3$) δ 2.05 (s, 3H, OAc), 3.68-3.74 (m, 4H, H$_4^′$, H$_5^′$), 3.87-3.89 (m, 2H, H$_4^{′′}$), 4.19-4.22 (m, 2H, H$_5^{′′}$), 5.56 (s, 2H, H$_1^{′′}$), 5.76 (s, 2H, H$_1^′$), 8.23 (s, 1H, H$_2$). $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 170.8, 156.2, 149.6, 149.3, 138.4 (q, $^2J_{CF} = 41$ Hz), 122.7, 118.2 (q, $^1J_{CF} = 270$ Hz), 75.1, 73.2, 71.2, 68.3, 62.9, 61.4, 20.8. $^{19}$F-NMR (470 MHz, CDCl$_3$) δ -63.4 (s). HRMS (ESI-TOF$^+$): calcd for C$_{14}$H$_{17}$F$_3$N$_4$O$_6$ [(M + Na)$^+$] 417.0992, [(M + K)$^+$] 433.0726; found, 417.0991, 433.0730.

N$_1$-[5′′-Phosphonoxyethoxy)methyl]-N$_9$-[5′-(phenylthio)phosphoryloxyethoxy)methyl]-8-trifluoromethylhypoxanthine (17). To a solution of 16 (66 mg, 0.167 mmol) in anhydrous pyridine (5 mL) was added TPSCl (302 mg, 1.00 mmol), PSS (571 mg, 1.50 mmol) [20], and tetrazole (105 mg, 1.50 mmol), and the mixture was stirred at room temperature for 12 h. The mixture was evaporated, and the residue was purified by silica gel column chromatography (PE-EA = 1:2) to give compound 17 (86 mg, 79%). $^{1}$H-NMR (400 MHz, CDCl$_3$) δ 2.05 (s, 3H, OAc), 3.82-3.84 (m, 2H, H$_4^′$), 3.86-3.88 (m, 2H, H$_5^′$), 4.18-4.21 (m, 2H, H$_4^{′′}$), 4.31-4.35 (m, 2H, H$_5^{′′}$), 5.56 (s, 2H, H$_1^{′′}$), 5.68 (s, 2H, H$_1^′$), 7.33-7.52 (m, 10H, ArH), 8.22 (s, 1H, H$_2$). $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 170.8, 156.2, 149.6, 149.3, 138.4 (q, $^2J_{CF} = 41$ Hz), 122.7, 118.2 (q, $^1J_{CF} = 270$ Hz), 75.1, 73.2, 71.2, 68.3, 62.9, 61.4, 20.8. $^{19}$F-NMR (470 MHz, CDCl$_3$) δ-63.4 (s). $^{31}$P-NMR (D$_2$O, 243 MHz, decoupled with $^1$H) δ 50.41 (s). HRMS (ESI-TOF$^+$): calcd for C$_{30}$H$_{35}$F$_3$N$_4$O$_6$Si [(M + H)$^+$] 659.1005; found, 659.1006.

N$_1$-[5′′-Phosphonoxyethoxy)methyl]-N$_9$-[5′-(phenylthio)phosphoryloxyethoxy)methyl]-8-trifluoromethylhypoxanthine (19). Compound 17 (54 mg, 0.082 mmol) was dissolved in MeOH (4 mL), and a solution of acetyl chloride (7 µL, 0.098 mmol) in anhydrous CH$_2$Cl$_2$ (1 mL) was added at −20 °C. The mixture was stirred at 0 °C for 30 min and raised to room temperature for 24 h, then neutralized by sat. aq. NaHCO$_3$ solution. The mixture was evaporated, and the residue was partitioned between CH$_2$Cl$_2$ and H$_2$O. The aqueous phase was extracted again with CH$_2$Cl$_2$, the organic layers were combined and washed with brine, dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (PE-EA = 1:10) to give compound 18 (31 mg). The deacetylated product 18 (31 mg, 0.050 mmol) was dissolved in anhydrous CH$_3$CN (8 mL). DIPEA (65 µL, 0.375 mmol) and POCl$_3$ (28 µL, 0.300 mmol) were added successively to the solution at −20 °C, and the mixture was stirred at 0 °C for 14 h, and then added 5 mL of TEAB (1 M, pH 7.5) at 0 °C and stirred at room
temperature for 6 h. After evaporation under reduced pressure, the residue was partitioned between H2O and CHCl3, and the aqueous layer was washed with CHCl3 and evaporated in vacuo. The residue was dissolved in 5 mL of TEAB buffer (0.05 M, pH 7.5), then applied to a C18 reversed-phase column (2.2 × 25 cm). The column was developed using a linear gradient of 0-40% CH3CN in TEAB buffer (0.05 M, pH 7.5) within 30 min to afford 19 (27 mg, 41% for two steps) as its triethylammonium salt. 1H-NMR (400 MHz, D2O) δ 3.70-3.73 (m, 4H, H4′, H5′), 3.85-3.89 (m, 2H, H4′′), 3.94-3.98 (m, 2H, H5′′), 5.45 (s, 2H, H1′′), 5.64 (s, 2H, H1′), 7.09-7.29 (m, 5H, ArH), 8.41 (s, 1H, H2). 13C-NMR (100 MHz, D2O) δ 157.6, 150.9, 149.5, 138.8 (q, JCF = 41 Hz), 132.7, 129.6, 128.9, 127.7, 122.1, 117.8 (q, JCF = 270 Hz), 76.4, 73.3, 69.3, 64.9, 64.3, 46.6, 8.2. 19F-NMR (470 MHz, D2O) δ -63.0. 31P-NMR (D2O, 243 MHz, decoupled with 1H) δ −10.07 (d, JPP = 18.2 Hz), −10.42 (d, JPP = 18.2 Hz). HRMS (ESI-TOF−) calcd for C18H21N4O10P2SF3 [(M−H)−], 603.0333; found, 603.0331.

N1-[5″-O-Phosphorylethoxy)methyl]-N9-[5′-O-phosphorylethoxy)methyl]-8-trifluoromethylhypoxanthine-cyclic pyrophosphate (5). A solution of 19 (5 mg, 6.1 μmol) in anhydrous pyridine (4.5 mL) was added slowly over 20 h, utilizing a syringe pump, to a mixture of I2 (36 mg, 142 μmol) and 3 Å molecular sieves (0.36 g), in pyridine (40 mL) at room temperature in the dark. The molecular sieves were filtered off with Celite and washed with H2O. The combined filtrate was evaporated, and the residue was partitioned between CHCl3 and H2O. The aqueous layer was evaporated, and the residue was dissolved in 0.05 M TEAB buffer, which was applied to C18 reversed-phase column (2.2 × 25 cm). The column was developed using a linear gradient of 0-20% CH3CN in TEAB buffer (0.05 M, pH 7.5) within 30 min to give 5 as its triethylammonium salt (3.0 mg, 71%). 1H-NMR (400 MHz, D2O) δ 3.70-3.78 (m, 4H, H4′, H5′), 3.81-3.83 (m, 2H, H4′′), 3.88-3.90 (m, 2H, H5′′), 5.54 (s, 2H, H1′′), 5.75 (s, 2H, H1′), 8.49 (s, 1H, H2). 19F-NMR (470 MHz, D2O) δ −62.5. 31P-NMR (D2O, 121.5 MHz, decoupled with 1H) δ −10.07 (d, JPP = 18.2 Hz), −10.42 (d, JPP = 18.2 Hz). HRMS (ESI-TOF−) calcd for C12H15N4O10P2F3 [(M−H)−], 493.0143; found, 493.0146.

N1-[5″-Phosphonoxyethoxy)methyl]-N9-[5′-Phosphonoxyethoxy)methyl]-8-trifluoromethylinosine (6). Compound 16 (20 mg, 0.051 mmol) was dissolved in methanol (2 mL). To the solution was added K2CO3 (1 mg, 7.24 μmol) at room temperature and stirred for 6 h. The mixture was neutralized by addition of 0.01 M HCl solution, and removed of the solvent in vacuo. The residue was partitioned between CHCl3 and H2O, and the organic layer was washed with brine, dried (Na2SO4), and evaporated, affording compound 20 (16 mg). Compound 20 (16 mg, 0.045 mmol) was dissolved in anhydrous CH3CN (5 mL). DIPEA (94 μL, 0.54 mmol) and POCl3 (42 μL, 0.45 mmol) were added successively to the solution at −20 °C. The mixture was stirred at 0 °C for 16 h, and then added 5 mL of TEAB (1 M, pH 7.5) at 0 °C and stirred for 6 h at room temperature. After evaporation under reduced pressure, the residue was partitioned between H2O and CHCl3, and the aqueous layer was washed with CHCl3 and evaporated in vacuo. The residue was dissolved in 5 mL of TEAB buffer (0.05 M, pH 7.5), and applied to a C18 reversed-phase column (2.2 × 25 cm). The column was developed using a linear gradient of 0-40% CH3CN in TEAB buffer (0.05 M, pH 7.5) within 30 min to give 6 (22.3 mg, 62% for two steps) as its triethylammonium salt. 1H-NMR (400 MHz, D2O) δ 3.73-3.79 (m, 4H, H4′, H5′), 3.85-3.92 (m, 2H, H4′′, H5′′), 5.45 (s, 2H, H1′′), 5.77 (s, 2H, H1′), 8.51 (s, 1H, H2). 13C-NMR (100 MHz, D2O) δ 157.8, 151.0, 149.7, 138.6 (q, JCF = 41 Hz), 122.2, 117.9 (q, JCF = 270 Hz), 76.4, 73.3, 69.5,
71% for two steps. 1H-NMR (400 MHz, D2O) δ 1.31, 1.53 (each s, each 3H, 2 × CH3), 3.72-3.74 (m, 2H, H5), 3.85-3.89 (m, 2H, H2), 3.91-3.94 (m, 2H, CH2OP), 4.52-4.56 (m, 1H, H4), 5.06 (dd, 1H, JH3,H4= 1.6 Hz, JH3,H2=- 6.0 Hz, H3), 5.29 (dd, 1H, JH2,H1= 2.8 Hz, JH2,H3=- 6.0 Hz, H2), 5.47 (d, 1H, JH1,a,H1,a= 10.8 Hz, H1,a), 5.51 (d, 1H, JH1,a,H1,b= 10.8 Hz, H1,b), 6.17 (d, 1H, JH1,H2= 2.8 Hz, H1), 8.23, 8.34 (each s, each 1H, H8, H2). 31P-NMR (D2O, 121.5 MHz, decoupled with 1H) δ 1.79 (s), 1.91 (s). HRMS(ESI-TOF−): calcd for C14H24N4O13P2 [(M− H−)], 541.0742; found, 541.0733.

31P-NMR (D2O, 243 MHz, decoupled with 1H) δ 0.81 (s), 0.92 (s). HRMS (ESI-TOF−): calcd for C14H24N4O13P2 [(M− H−)], 501.0426; found, 501.0429.
decoupled with $^1$H) $\delta$ 0.54 (s), 0.70 (s). HRMS (ESI-TOF$^-$): calculd for C$_{17}$H$_{23}$F$_3$N$_4$O$_{13}$P$_2$ [(M – H)$^-$], 609.0612; found, 609.0615.

N,l-[(5r'-Phosphonoxyethoxy)methyl]-5'-O-phosphoryl-8-trifluoromethylinosine (8). A solution of 26 (15 mg, 18.47 $\mu$mol) in 10% HCOOH (7.5 mL) was stirred at room for 60 h, and then 11 mL of TEAB (1 M, pH 7.5) was added. The solution was evaporated in vacuo. The residue was dissolved in 0.05 M TEAB buffer (0.2 mL), which was applied to C$_{18}$ reversed-phase column (2.2 cm $\times$ 25 cm). The column was developed using a linear gradient of 0-40% CH$_3$CN in TEAB buffer (0.05 M, pH 7.5) within 30 min to afford compound 8 (9.7 mg, 68%) as its triethylammonium salt, with the compound 26 (2.2 mg, 15%) recovered. $^1$H-NMR (400 MHz, D$_2$O) $\delta$ 3.76-3.78 (m, 2H, H$_5$'), 3.87-3.91 (m, 2H, CH$_2$O), 4.02-4.13 (m, 2H, CH$_2$OP), 4.21-4.25 (m, 1H, H$_4$'), 4.55-4.57 (m, 1H, H$_3$'), 5.20-5.23 (m, 1H, H$_2$'), 5.46 (d, 1H, $J_{H1''b,H1''a} = 10.6$ Hz, H$_{1''b}$), 5.59 (d, 1H, $J_{H1''a,H1''b} = 10.6$ Hz, H$_{1''a}$), 5.99 (d, 1H, $J_{H1''a,CH}$ = 5.6 Hz, H$_{1''a}$), 8.47 (s, 1H, H$_2$). $^{13}$C-NMR (100 MHz, D$_2$O) $\delta$ 157.9, 150.3, 149.4, 138.9 (q, $^1$J$_{CF} = 269$ Hz), 123.2, 117.8 (q, $^1$J$_{CF} = 269$ Hz), 89.8, 84.4, 76.3, 72.0, 70.0, 69.3, 64.4, 64.1. $^{19}$F-NMR (470 MHz, D$_2$O) $\delta$ -61.7 (s). $^{31}$P-NMR (D$_2$O, 121.5 MHz, decoupled with $^1$H) $\delta$ 6.25(s), 6.27 (s). HRMS (ESI-TOF$^-$): calculd for C$_{14}$H$_{19}$F$_3$N$_4$O$_{13}$P$_2$ [(M – H)$^-$], 569.0303; found, 569.0315.

4. Conclusion

In conclusion, we have successfully synthesized 8-CF$_3$-cIDPDE (5) via construction of N,l, N,o-disubstituted hypoxanthine, trifluoromethylation and intramolecular condensation. A series of novel acyclic analogues of cADPR, compounds 6-8, were also synthesized by concise synthetic routes. With the special properties of trifluoromethyl, 8-CF$_3$-cIDPDE and the acyclic derivatives are expected to provide useful agents to explore the cADPR/RyR Ca$^{2+}$ signalling system and illuminate the structure-activity relationship of cADPR analogues.

Acknowledgements

This study was supported by the National Natural Sciences Foundation of China (Grant no. 90713005, 20832008) and the Ministry of Education of China (Grant no. 200800010078).

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