SYNTHESIS OF CYCLIC ADP-CARBOCYLCIC-XYLOSE AND ITS 3”-*O*-METHYL ANALOGUE AS STABLE AND POTENT Ca^{2+}-MOBILIZING AGENTS

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This paper is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.

ABSTRACT

We previously showed that 3”-deoxy-cyclic ADP-carbocyclic-ribose (3”-deoxy-cADPcR, **2**) is a stable and highly potent analogue of cyclic ADP-ribose (cADPR, **1**), a Ca^{2+}-mobilizing second messenger. From these results, we newly designed another 3”-modified analogues of cADPcR and identified the N1-“xylo”-type carbocyclic analogue, i.e., cADPcX (**4**), as one of the most potent cADPR-related compounds reported so far.

INTRODUCTION

Much attention has been focused on cyclic ADP-ribose (cADPR, **1**, **FIGURE 1**), a naturally occurring metabolite of NAD^+,[2] due to the biological interest.[3] cADPR has been shown to mobilize intracellular Ca^{2+} in various cells, and is now recognized as a general mediator involved in Ca^{2+} signaling.[3] Under neutral conditions, cADPR is in a zwitterionic form with a positive charge around the N(1)-C(6)-N^6 moiety (pK_a = 8.3),

making the molecule unstable. The charged adenine moiety attached to the anomeric carbon of the N1-ribose can be an efficient leaving group. Accordingly, cADPR is readily hydrolyzed at the unstable *N*-1-ribosyl linkage of its adenine moiety to produce ADP-ribose (ADPR), even in neutral aqueous solution.[4] Under physiological conditions, cADPR is also hydrolyzed at the *N*-1-ribosyl linkage by cADPR hydrolase to give the inactive ADPR.[4]

cADPR analogues can be used in proving the mechanism of cADPR-mediated Ca^{2+} signaling pathways and are also expected to be lead structures for the development of drugs, since cADPR has been shown to play important physiological roles.[3] Therefore, the synthesis of cADPR analogues has been extensively investigated by enzymatic and chemo-enzymatic methods using ADP-ribosyl cyclase-catalyzed cyclization. However, the analogues obtained by these methods are limited due to the substrate-specificity of the ADP-ribosyl cyclase.[3]

On the other hand, in the chemical synthesis of cADPR and its analogues, construction of the large 18-membered ring structure is the key step, and we recently developed an efficient method for forming the 18-membered ring employing phenylthiophosphate-type substrates.[5] When these substrates were activated by $AgNO_3$ or I_2 in the presence of molecular sieves in pyridine, the corresponding 18-membered ring products were obtained in high yields.[5b,c] Using this method, we successfully synthesized cyclic ADP-carbocyclic-ribose (cADPcR, **2**),[5c] designed as a stable mimic of cADPR, in which the oxygen atom in the *N*-1-ribose ring of cADPR is replaced by a methylene group. Biological evaluation of cADPcR showed that it actually act as biologicically and chemically stable equivalent of cADPR.[5c]

Based on these results, we have investigated further synthetic and biological studies on N1-carbocyclic derivatives of cADPR.[6] In the course of these studies, we describe here the synthesis and biological evaluation of newly designed analogues of cADPcR, which are cyclic ADP-carbocyclic-xylose (cADPcX, **4**) and the corresponding 3"-*O*-methyl derivative (3"-OMe-cADPcX, **5**).

RESULTS AND DISCUSSION

Design and Synthetic Plan. We previously showed 1) that cADPcR (**2**) is actually resistant to both enzymatic and chemical hydrolysis, since it has a chemically and biologically stable *N*-alkyl linkage instead of the unstable N1-glycosidic linkage of cADPR,[5b] 2) that cADPcR has a conformation similar to that of cADPR,[6c] and 3) that cADPcR, like cADPR, effectively mobilizes intracellular Ca^{2+} in sea urchin eggs and neuronal cells.[5c,6b,c] Furthermore, we have investigated SRA of the N1-ribose moiety of cADPcR and clarified that modification at the N1-ribose moiety changes the biological potency.[6b,c] Throughout these studies, we also found that although deletion of the 2"-hydroxy group resulted in a marked reduction of potency, deletion of the adjacent 3"-hydroxy group (3"-deoxy-cADPcR, **3**) greatly potentiated the Ca^{2+}-mobilizing ability in sea urchin eggs.[6c] These results suggest that modification at the 3"-position may

improve the biological potency of cADPR and its analogues. Thus, we newly designed another 3"-modified analogues of cADPcR, which were the N1-"xylo"-type carbocyclic analogue, i.e., cADPcX (**4**) and the corresponding *O*-methyl analogue **5**.

As described above, we have developed an efficient total synthetic method for cADPR analogues,[5] which we[6] and other groups[7] have effectively used in the synthesis of a variety of cADPR analogues. Thus, we planned to synthesize the target compounds based on the previous total synthetic method.

The synthetic plan is shown in **SCHEME 1** as a retrosynthetic analysis. The chiral carbocyclic–xylosyl amines **III**, composing the N1-substituted moiety in the targets **4** and **5**, could be prepared from commercially available (1*R*)-(–)-2-azabicyclo[2.2.1]hept-5-en-3-one (**8**). From these carbocyclic amines **III** and the known imidazole nucleoside derivative **6**,[5b] the 5'-phenylthiophosphate-type substrates **II** for the key intramolecular condensation could be prepared. Treatment of **II** with $AgNO_3$/MS 3A as a promoter[5a,b] was expected to form the cyclized products **I**, and subsequent acidic treatment for deprotection would furnish the desired cADPcR analogues **4** and **5**.

Synthesis. The carbocyclic-xylosyl amine units **14a** and **14b** were synthesized from the optically active bicyclic lactam **8**, as summarized in Scheme 2. After protection of the primary hydroxyl with a triisopropysilyl (TIPS) group of cabocylicilc-ribose derivative **9**, prepared from **8** according to the previously reported method,[6c] treatment of the resulting **10** under Mitsunobu reaction conditions with $ClCH_2CO_2H$/DIAD/Ph_3P gave the corresponding 3-position-inverted product **11**, and subsequent removal the 3-*O*-chloroacetly group with NaOMe/MeOH to afford the carbocyclic-xylose derivative **12**. The 3-hydroxy of **12** was protected with a MOM group under usual conditions or methylated with MeOTf and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), followed by removal of the TIPS group, to give the desired 3-*O*-MOM-ptotected unit **14a** or the 3-*O*-methyl unit **14b**, respectively.

The target 3"-modified cADPcR analogues **4** and **5** were successfully synthesized from the carbocyclic amines **14a** or **14b** and the imidazole nucleoside **6**, as shown in **SCHEME 3**.

The *N*-1-substituted adenosine derivatives **15a** and **15b** were obtained in high yield by the treatment of a mixture of **6** and either amine **14a** or amine **14b** with K_2CO_3 in MeOH at room temperature. The 5"-hydroxy group of **15a** or **15b** was protected with a dimethoxytrityl (DMTr) group, and the 5'-*O*-TBS group of the product was subsequently removed with TBAF to give **16a** or **16b**. Treatment of **16a** or **16b** under the conditions reported by Hata and co-workers with an *S*,*S'*-diphenylphosphorodithioate (PSS)/2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl)/pyridine system[8] gave the 5'-bis(phenylthio)phosphate **17a** or **17b**, respectively. The 5"-*O*-DMTr group of **17a** or **17b** was removed to give **18a** or **18b**, respectively. A phosphoryl group was introduced at the resulting 5"-primary hydroxyl of **18a** or **18b** by Yoshikawa's method with $POCl_3$/$(EtO)_3PO$,[9] followed by treatment of the product with

H_3PO_2 and Et_3N[10] in the presence of *N*-methylmaleimide (NMM)[5c] in pyridine, to afford the corresponding 5'-phenylthiophosphate **19a** or **19b**, respectively, which was the substrate of the key intramolecular condensation reaction. When a solution of **19a** in pyridine was added slowly to a mixture of a large excess of $AgNO_3$ and Et_3N in the presence of MS 3A in pyridine at room temperature,[5b,c] the intramolecular pyrophosphate linkage was successfully formed as the previously reported cases to give the desired cyclization product **20a** in 46% yield. The other substrate **19b** was similarly condensed and the cyclization product **19b** was obtained. Finally, the protecting groups of **19a** and **19b** were simultaneously remove by acidic treatment with aqueous HCO_2H furnished the target cADPcX (**4**) and 3"-OMe-cADPcX (**5**).

Ca^{2+}-mobilizing Activity in Sea Urchin Egg Homogenate. The Ca^{2+}-mobilizing ability of the newly synthesized compounds **4** and **5** was evaluated by the fluorometrically Ca^{2+}-monitoring method with *H. pulcherrimus* sea urchin egg homogenate,[6b,11] and the results were compared those of the natural second messenger cADPR (**1**) and the related carbocyclic analogues cADPcR (**2**) and 3"-deoxy-cADPcR (**3**). Both of the two newly synthesized compounds **4** and **5** released Ca^{2+} from the homogenate in a dose-dependent manner, as shown in **FIGURE 2**, where the maximal Ca^{2+}-mobilizing activity was almost equal to that of cADPR. Thus, **4** and **5** were shown to be full agonists as cADPcR (**2**) and 3"-deoxy-cADPcR (**3**). cADPcX (**4**), the 3"-epimer of cADPcR, showed marked Ca^{2+}-mobilizing activity with an EC_{50} value of 69 nM, which was similar to that of cADPcR with an EC_{50} value of 79 nM and was 3 times more potent than the natural ligand cADPR (EC_{50} = 220 nM). The 3"-*O*-methyl cADPcX (**5**) showed an EC_{50} value of 740 nM, which was about 10 times weaker than cADPcX. Similar to the case of cADPcR, 3"-methylation of the 3"-hydroxy group of cADPcX markedly reduced the activity.

CONCLUSION

We successfully synthesized cADPcX (**4**), the 3"-epimer of cADPcR (**2**), and the corresponding 3"-*O*-methyl analogue (3"-OMe-cADPcX, **5**) and identified cADPcX as one of the most potent cADPR-related compounds reported so far. Therefore, irrespective of the 3"-configuration, both cADPcX and cADPcR have strong Ca^{2+}-mobilizing activity to suggest that the 3"-hydroxy group seems to be unnecessary in their binding with the target biomolecule, which is in accord with the previous results on the 3"-deoxy-cADPcR with remarkable activity.[6c]

EXPERIMENTAL

General Methods. Chemical shifts are reported in ppm downfield from Me_4Si (^1H), MeCN (^{13}C) or H_3PO_4 (^{31}P). All of the ^1H NMR assignments described were in agreement with COSY spectra. Thin-layer chromatography was done on Merck coated

plate $60F_{254}$. Silica gel chromatography was done on Merck silica gel 5715. Reactions were carried out under an argon atmosphere.

(1*R*,2*S*,3*R*,4*R*)-1-*tert*-Butoxycarbonylamino-2-(methoxymethyloxy)-3-hydroxy-4-(triisopropylsilyloxymethyl)cyclopentane (10). A mixture of **9** (1.20 g, 4.12 mmol), TIPSCl (2.12 mL, 9.88 mmol), imidazole (1.01 g, 14.8 mmol) and DMAP (0.604 g, 4.94 mmol) in DMF (40 mL) was stirred at room temperature for 4 h. After addition of MeOH (10 mL), the resulting mixture was evaporated. The residue was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 25% EtOAc in hexane) to give **10** (1.68 g, 91%) as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 4.98 (brs, 1H, NH), 4.78 (d, 1H, MOM-CH_2, J = 6.4 Hz), 4.72 (d, 1H, MOM-CH_2, J = 6.4 Hz), 4.08 (m, 1H, H-3), 3.99 (m, 1H, H-1), 3.88 (dd, 1H, H-6a, $J_{6a,6b}$ = 9.6 Hz, $J_{6a,4}$ = 2.8 Hz), 3.80 (m, 1H, H-2), 3.69 (dd, 1H, H-6b, $J_{6b,6a}$ = 9.6 Hz, $J_{6b,4}$ = 3.2 Hz), 3.41 (s, 3H, MOM-CH_3), 2.66 (brs, 1H, OH), 2.37 (m, 1H, H-5a), 2.04 (m, 1H, H-4), 1.42 (s, 9H, *tert*-Bu), 1.33 (m, 1H, H-5b), 1.07-1.18 (m, 21H, TIPS); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 155.10, 96.53, 84.21, 79.13, 72.64, 63.66, 55.73, 53.62, 45.40, 30.33, 28.36, 18.00, 12.05; HRMS (FAB, positive) calcd for $C_{22}H_{46}NO_6Si$ 448.3094 (MH^+), found 448.3109.

(1*R*,2*S*,3*S*,4*R*)-1-*tert*-Butoxycarbonylamino-2-(methoxymethyloxy)-3-(chloroacethoxy)-4-(triisopropylsilyloxymethyl)cyclopentane (11). To a mixture of **10** (0.160 g, 0.357 mmol), chloroacetic acid (0.135 g, 1.43 mmol), and Ph_3P (0.374 g, 1.43 mmol) in toluene (2 mL), a solution of DIAD (281 μL, 1.43 mmol) in toluene (1.5 mL) was added slowly at 0 °C, and the mixture was stirred at room temperature for 6 h and then evaporated. The residue was purified by column chromatography (SiO_2, 10% EtOAc in hexane) to give **11** (0.139 g, 74%) as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 5.21 (dd, 1H, H-3, $J_{3,2}$ = 3.9 Hz, $J_{3,4}$ = 6.0 Hz), 4.86 (m, 1H, NH), 4.72 (d, 1H, MOM-CH_2, J = 6.6 Hz), 4.69 (d, 1H, MOM-CH_2, J = 6.6 Hz), 4.05 (s, 2H, Cl-Ac), 3.97 (m, 1H, H-1), 3.92 (m, 1H, H-2), 3.70 (m, 2H, H-6 x 2), 3.36 (s, 3H, MOM-CH_3), 2.48 (m, 1H, H-4), 2.38 (m, 1H, H-5a), 1.43 (m, 10H, H-5b, *tert*-Bu), 1.09 (m, 21H, TIPS); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 166.33, 155.16, 95.70, 85.43, 79.78, 73.06, 70.82, 61.80, 55.48, 54.77, 41.55, 40.78, 32.43, 28.35, 17.95, 11.89; HRMS (FAB, positive) calcd for $C_{24}H_{47}ClNO_7Si$ 524.2810 (MH^+), found 524.2816.

(1*R*,2*S*,3*S*,4*R*)-1-*tert*-Butoxycarbonylamino-2-(methoxymethyloxy)-3-hydroxy-

4-(triisopropylsilyloxymethyl)cyclopentane (12). A mixture of **11** (0.173 g, 0.330 mmol) and NaOMe (1 M in MeOH, 50 μL, 50 μmol) in MeOH (3 mL) was stirred at room temperature for 30 min. After addition of aqueous saturated NH_4Cl (1 mL) at 0 °C, the resulting mixture was extracted with EtOAc, and the organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 25% EtOAc in hexane) to give **12** (0.130 g, 88%) as a colorless oil: ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 6.69 (d, 1H, NH, J = 7.3 Hz), 4.70 (d, 1H, OH, J = 4.6 Hz), 4.63 (d, 1H, MOM-CH_2, J = 6.4 Hz), 4.60 (d, 1H, MOM-CH_2, J = 6.4 Hz), 3.84 (m, 2H, H-6a, H-3), 3.62 (m, 1H, H-1), 3.58 (m, 2H, H-2, H-6b), 3.22 (s, 3H, MOM-CH_3), 2.02 (m, 1H, H-4), 1.96 (m, 1H, H-5a), 1.36 (s, 10H, H-5b, *tert*-Bu), 1.03 (m, 21H, TIPS); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 156.04, 95.36, 88.30, 78.17, 74.92, 63.31, 55.46, 55.04, 43.62, 32.28, 28.56, 18.16, 11.67; HRMS (FAB, positive) calcd for $C_{22}H_{46}NO_6Si$ 448.3094 (MH^+), found 448.3092.

(1*R*,2*S*,3*S*,4*R*)-1-*tert*-Butoxycarbonylamino-2,3-bis(methoxymethyloxy)-4-hydroxy methylcyclopentane (13a). A mixture of **12** (0.871 g, 1.95 mmol), MOMCl (739 μL, 9.73 mmol) and *i*-Pr_2NEt (3.39 mL, 19.5 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 75 h. After addition of MeOH (10 mL), the resulting mixture was evaporated. The residue was partitioned between EtOAc and 0.1 M HCl, and the organic layer was washed H_2O and brine, dried (Na_2SO_4), and evaporated. A mixture of the residue and TBAF (1.0 M in THF, 3.0 mL, 3.0 mmol) in THF (20 mL) was stirred at room temperature for 1 h and then evaporated. The residue was purified by column chromatography (SiO_2, EtOAc) to give **13a** (0.634 g, 97%) as a yellow oil: ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 6.85 (d, 1H, NH, J = 7.9 Hz), 4.64 (d, 1H, MOM-CH_2, J = 6.5 Hz), 4.59 (m, 3H, MOM-CH_2 x 3), 4.34 (m, 1H, OH), 3.84 (m, 1H, H-3), 3.80 (m, 1H, H-2), 3.66 (m, 1H, H-1), 3.50 (m, 1H, H-6a), 3.38 (m, 1H, H-6b), 3.26 (s, 3H, MOM-CH_3), 3.23 (s, 3H, MOM-CH_3), 2.06 (m, 1H, H-4), 1.93 (m, 1H, H-5a), 1.33 (s, 9H, *tert*-Bu), 1.32 (m, 1H, H-5b); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 155.15, 95.14, 94.64, 85.42, 80.11, 77.69, 60.05, 55.65, 54.98, 54.72, 42.69, 32.25, 28.39; FAB-MS m/z 336 (MH^+); Anal. Calcd for $C_{15}H_{29}NO_7$: C, 53.72; H, 8.72; N, 4.18. Found; C, 53.49; H, 8.57; N, 4.00.

(1*R*,2*S*,3*S*,4*R*)-1-*tert*-Butoxycarbonylamino-2-(methoxymethyloxy)-3-methoxy -4-hydroxymethylcyclopentane (13b). A mixture of **12** (0.513 g, 1.15 mmol), MeOTf (648 μL, 5.73 mmol) and DTBMP (1.29 g, 6.30 mmol) in CH_2Cl_2 (11 mL) was stirred at

room temperature for 12 h. After addition of aqueous saturated $NaHCO_3$ (10 mL) at 0 °C, the mixture was extracted with EtOAc, and the organic layer was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 10% EtOAc in hexane) to give the 3-*O*-methylated product (0.259 g, 49%) as a colorless oil. A mixture of the oil and TBAF (1.0 M in THF, 0.84 mL, 0.84 mmol) in THF (5 mL) was stirred at room temperature for 2 h and then evaporated. The residue was purified by column chromatography (SiO_2, 75% EtOAc in hexane) to give **13b** (0.154 g, 90%) as a yellow oil: ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 6.84 (d, 1H, NH, J = 8.0 Hz), 4.65 (d, 1H, MOM-CH_2, J = 6.5 Hz), 4.58 (d, 1H, MOM-CH_2, J = 6.5 Hz), 4.29 (m, 1H, OH), 3.77 (dd, 1H, H-2, $J_{2,1}$ = 5.1 Hz, $J_{2,3}$ = 3.0 Hz), 3.65 (m, 1H, H-1), 3.55 (m, 1H, H-6a), 3.47 (dd, 1H, H-3, $J_{3,2}$ = 3.0 Hz, $J_{3,4}$ = 5.8 Hz), 3.32 (m, 1H, H-6b), 3.24 (s, 6H, OMe, MOM-CH_3), 2.04 (m, 1H, H-4), 1.91 (m, 1H, H-5a), 1.36 (s, 9H, *tert*-Bu), 1.30 (m, 1H, H-5b); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 155.16, 94.70, 84.84, 84.45, 77.68, 59.93, 56.82, 55.69, 54.74, 42.65, 32.30, 28.39; FAB-MS *m/z* 306 (MH^+); Anal. Calcd for $C_{14}H_{27}NO_6$: C, 55.07; H, 8.91; N, 4.59. Found; C, 55.09; H, 8.76; N, 4.51.

(1*R*,2*S*,3*S*,4*R*)-1-Amino-2,3-bis(methoxymethyloxy)-4-hydroxymethylcyclopentane (14a). A solution of **13a** (0.614 g, 1.83 mmol) in H_2O (18 mL) was stirred at 100 °C for 12 h and then evaporated. The residue was azeoptroped with toluene (10 mL x 3) to give **14a** (0.430 g, quant.) as a brown oil: ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 4.67 (d, 1H, MOM-CH_2, J = 6.5 Hz), 4.59 (m, 3H, MOM-CH_2 x 3), 3.85 (dd, 1H, H-3, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 6.0 Hz), 3.57 (m, 1H, H-2), 3.50 (dd, 1H, H-6a, $J_{6a,6b}$ = 10.3 Hz, $J_{6a,4}$ = 7.0 Hz), 3.37 (dd, 1H, H-6b, $J_{6b,6a}$ = 10.3 Hz, $J_{6b,4}$ = 6.5 Hz), 3.26 (s, 6H, MOM-CH_3 x 2), 2.96 (m, 1H, H-1), 2.06 (m, 1H, H-4), 1.92 (m, 1H, H-5a), 1.15 (s, 1H, H-5b); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 95.24, 95.18, 89.75, 80.81, 60.41, 56.84, 54.98, 54.86, 42.57, 35.50; HRMS (FAB, positive) calcd for $C_{10}H_{22}NO_5$ 236.1498 (MH^+), found 236.1508.

(1*R*,2*S*,3*S*,4*R*)-1-Amino-2-(methoxymethyloxy)-3-methoxy-4-hydroxymethylcyclopentane (14b). Compound **14b** (0.239 g, quant.) was obtained from **13b** (0.351 g, 1.15 mmol) as described for the synthesis of **14a**: ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 4.68 (d, 1H, MOM-CH_2, J = 6.6 Hz), 4.62 (d, 1H, MOM-CH_2, J = 6.6 Hz), 3.53 (m, 1H, H-2), 3.49 (m, 2H, H-3, H-6a), 3.33 (m, 1H, H-6b), 3.27 (s, 3H, MOM-CH_3 or OMe), 3.25 (s, 3H, MOM-CH_3 or OMe), 2.95 (m, 1H, H-1), 2.06 (m, 1H, H-4), 1.91 (m, 1H, H-5a), 1.14 (s, 1H, H-5b); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 95.30, 89.03, 85.62, 60.28,

56.96, 56.91, 54.87, 42.50, 35.44; HRMS (FAB, positive) calcd for $C_9H_{20}NO_4$ 206.1392 (MH^+), found 206.1390.

***N*-1-{(1*R*,2*S*,3*S*,4*R*)-2,3-Bis(methoxymethyloxy)-4-(hydroxymethyl)cyclopentyl}-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-*O*-isopropylideneadenosine (15a).** A mixture of **6** (0.765 g, 1.75 mmol), **14a** (0.417 g, 1.77 mmol), and K_2CO_3 (12 mg, 88 μmol) in MeOH (18 mL) was stirred at room temperature for 12 h and then evaporated. The residue was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 25% MeOH in EtOAc) to give **15a** (0.930 g, 83%) as a white foam: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.91 (s, 1H, H-2 or H-8), 7.82 (s, 1H, H-2 or H-8), 6.02 (d, 1H, H-1', $J_{1',2'}$ = 2.7 Hz), 5.37 (m, 1H, H-1"), 5.09 (dd, 1H, H-2', $J_{2',1'}$ = 2.7 Hz, $J_{2',3'}$ = 6.1 Hz), 4.89 (dd, 1H, H-3', $J_{3',2'}$ = 6.1 Hz, $J_{3',4'}$ = 2.4 Hz), 4.81 (d, 1H, MOM-CH_2, *J* = 6.6 Hz), 4.76 (d, 1H, MOM-CH_2, *J* = 6.6 Hz), 4.69 (d, 1H, MOM-CH_2, *J* = 6.9 Hz), 4.65 (d, 1H, MOM-CH_2, *J* = 6.9 Hz), 4.48 (m, 1H, H-2"), 4.39 (ddd, 1H, H-4', $J_{4',3'}$ = 2.4 Hz, $J_{4',5'a}$ = 6.4 Hz, $J_{4',5'b}$ = 3.7 Hz), 4.19 (dd, 1H, H-3", $J_{3",2"}$ = 3.5 Hz, $J_{3",4"}$ = 5.8 Hz), 3.83 (m, 2H, H-5'a, H-5"a), 3.77 (m, 2H, H-5'b, H-5"b), 3.45 (s, 3H, MOM-CH_3), 3.21 (s, 3H, MOM-CH_3), 2.47 (m, 1H, H-4"), 2.39 (m, 1H, H-6"a), 2.02 (m, 1H, H-6"b), 1.61, 1.39 (each s, each 3H, isopropylidene), 0.86 (s, 9H, *tert*-Bu), 0.046, 0.035 (each s, each 3H, TBS-Me x 2); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 154.45, 146.66, 140.62, 136.78, 123.40, 114.16, 96.39, 95.63, 91.26, 87.03, 85.32, 84.38, 81.76, 81.33, 63.46, 61.56, 60.08, 56.02, 55.49, 42.10, 31.22, 27.23, 25.86, 25.38, 18.32, –5.43, –5.54; FAB-MS *m/z* 640 (MH^+); UV (MeOH) λ_{max} = 261, 295 (sh) nm; Anal. Calcd for $C_{29}H_{49}N_5O_9Si$: C, 54.44; H, 7.72; N, 10.95. Found; C, 54.24; H, 7.66; N, 10.84.

***N*-1-[(1*R*,2*S*,3*S*,4*R*)-2,3-Bis(methoxymethyloxy)-4-(dimethoxytrityloxymethyl)cyclopentyl]-2',3'-*O*-isopropylideneadenosine (16a).** A mixture of **15a** (0.884 g, 1.38 mmol) and DMTrCl (1.40 g, 4.14 mmol) in pyridine (15 mL) was stirred at room temperature for 10 min. After addition of MeOH (10 mL), the resulting mixture was evaporated. The residue was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. A mixture of the residue, TBAF (1.0 M in THF, 2.76 mL, 2.76 mmol) and AcOH (79 μL, 1.38 mmol) in THF (10 mL) was stirred at room temperature for 3 h and then evaporated. The residue was purified

by column chromatography (SiO_2, 25% MeOH in EtOAc) to give **16a** (1.19 g, quant.) as a white foam: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.00 (s, 1H, H-2 or H-8), 7.62 (s, 1H, H-2 or H-8), 6.82-7.43 (m, 13H, DMTr), 5.79 (m, 2H, H-1', H-1"), 5.02 (m, 2H, H-2', H-3'), 4.81 (d, 1H, MOM-CH_2, J = 6.9 Hz), 4.67 (d, 1H, MOM-CH_2, J = 6.9 Hz), 4.61 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.54 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.47 (m, 1H, H-4'), 4.20 (m, 1H, H-3"), 4.12 (m, 1H, H-2"), 3.91 (m, 1H, H-5'a), 3.80 (s, 6H, DMTr-OMe x 2), 3.72 (m, 1H, H-5'b), 3.33 (s, 3H, MOM-CH_3), 3.29 (m, 1H, H-5"a), 3.25 (s, 3H, MOM-CH_3), 3.13 (m, 1H, H-5"b), 2.68 (m, 1H, H-4"), 2.56 (m, 1H, H-6"a), 1.62, 1.35 (each s, each 3H, isopropylidene), 1.49 (m, 1H, H-6"b); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 158.45, 146.70, 144.96, 137.95, 136.31, 136.11, 129.97, 129.93, 128.12, 127.79, 126.76, 114.20, 113.09, 113.06, 96.41, 94.93, 93.90, 86.00, 85.82, 83.61, 81.37, 63.19, 61.56, 60.37, 58.81, 55.98, 55.60, 55.20, 42.94, 35.23, 27.55, 25.18, 21.02, 14.19; HRMS (FAB, positive) calcd for $C_{44}H_{54}N_5O_{11}$ 828.3820 (MH^+), found 828.3818; UV (MeOH) λ_{max} = 260, 295 (sh) nm.

***N*-1-[(1*R*,2*S*,3*S*,4*R*)-2,3-Bis(methoxymethyloxy)-4-(dimethoxytrityloxymethyl) cyclopentyl]-5'-*O*-{bis(phenylthio)phospholyl}-2',3'-*O*-isopropylideneadenosine (17a).** After stirring a mixture of PSS (1.56 g, 4.09 mmol) and TPSCl (1.12 g, 3.69 mmol) in pyridine (14 mL) at room temperature for 1 h, **16a** (1.13 g, 1.36 mmol) was added, and the resulting mixture was stirred at room temperature for further 2 h and then evaporated. The residue was partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 2% MeOH in $CHCl_3$) to give **17a** (0.890 g, 65%) as a white foam: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.95 (s, 1H, H-2 or H-8), 7.64 (s, 1H, H-2 or H-8), 6.81-7.51 (m, 23H, DMTr, SPh x 2), 5.96 (d, 1H, H-1', $J_{1',2'}$ = 2.6 Hz), 5.76 (m, 1H, H-1"), 5.07 (dd, 1H, H-2', $J_{2',1'}$ = 2.6 Hz, $J_{2',3'}$ = 6.3 Hz), 4.85 (dd, 1H, H-3', $J_{3',2'}$ = 6.3 Hz, $J_{3',4'}$ = 2.7 Hz), 4.76 (d, 1H, MOM-CH_2, J = 6.8 Hz), 4.64 (d, 1H, MOM-CH_2, J = 6.8 Hz), 4.57 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.53 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.41 (m, 1H, H-4'), 4.38 (m, 2H, H-5' x 2), 4.20 (d, 1H, H-3", $J_{3",4"}$ = 4.0 Hz), 4.14 (d, 1H, H-2", $J_{2",1"}$ = 2.6 Hz), 3.79 (s, 6H, DMTr-OMe x 2), 3.30 (m, 1H, H-5"a), 3.27 (s, 3H, MOM-CH_3), 3.19 (s, 3H, MOM-CH_3), 3.16 (m, 1H, H-5"b), 2.61 (m, 1H, H-4"), 2.54 (m, 1H, H-6"a), 1.59, 1.34 (each s, each 3H, isopropylidene), 1.54 (m, 1H, H-6"b); ^{13}C-NMR ($CDCl_3$, 125 MHz)

δ 158.43, 154.52, 146.61, 144.99, 140.49, 136.94, 136.35, 136.17, 135.34, 135.29, 135.21, 135.17, 129.98, 129.96, 129.66, 129.63, 129.46, 129.44, 128.13, 127.76, 126.72, 125.93, 125.87, 125.79, 125.74, 123.52, 114.69, 113.05, 113.02, 95.88, 94.99, 90.61, 86.00, 85.87, 84.67, 84.61, 84.45, 81.06, 80.78, 66.42, 66.36, 61.80, 58.76, 55.86, 55.51, 55.19, 42.79, 35.17, 27.13, 25.30, 21.02, 14.19; ^{31}P-NMR ($CDCl_3$, 202 MHz) δ 50.74 (s); HRMS (FAB, positive) calcd for $C_{56}H_{63}N_5O_{12}PS_2$ 1092.3652 (MH^+), found 1092.3660; UV (MeOH) λ_{max} = 295 (sh) nm.

***N*-1-[(1*R*,2*S*,3*S*,4*R*)-2,3-Bis(methoxymethyloxy)-4-(hydroxymethyl)cyclopentyl]-5'-*O*-[bis(phenylthio)phospholyl]-2',3'-*O*-isopropylideneadenosine (18a).** A solution of **17a** (0.916 g, 0.839 mmol) in aqueous 60% AcOH (8 mL) was stirred at room temperature for 4 h. After addition of aqueous saturated $NaHCO_3$ (60 mL) at 0 °C, the mixture was extracted with EtOAc, and the organic layer was washed with aqueous saturated $NaHCO_3$, H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (SiO_2, 10% MeOH in $CHCl_3$) to give **18a** (0.551 g, 83%) as a white foam: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.93 (s, 1H, H-2 or H-8), 7.69 (s, 1H, H-2 or H-8), 7.30-7.52 (m, 10H, SPh x 2), 5.99 (m, 1H, H-1'), 5.32 (m, 1H, H-1"), 5.11 (m, 1H, H-2'), 4.90 (dd, 1H, H-3', $J_{3',2'}$ = 6.2 Hz, $J_{3',4'}$ = 2.5 Hz), 4.76 (d, 1H, MOM-CH_2, J = 6.5 Hz), 4.72 (d, 1H, MOM-CH_2, J = 6.5 Hz), 4.66 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.62 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.45 (m, 1H, H-2"), 4.43 (m, 2H, H-4', H-5'a), 4.38 (m, 1H, H-5'b), 4.18 (m, 1H, H-3"), 3.84 (dd, 1H, H-5"a, $J_{5"a,5"b}$ = 11.2 Hz, $J_{5"a,4"}$ = 3.8 Hz), 3.76 (dd, 1H, H-5"b, $J_{5"b,5"a}$ = 11.2 Hz, $J_{5"b,4"}$ = 5.9 Hz), 3.41 (s, 3H, MOM-CH_3), 3.19 (s, 3H, MOM-CH_3), 2.47 (m, 1H, H-4"), 2.40 (m, 1H, H-6"a), 2.04 (m, 1H, H-6"b), 1.60, 1.36 (each s, each 3H, isopropylidene); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 154.27, 146.89, 137.38, 135.31, 135.27, 135.17, 135.13, 129.69, 129.66, 129.64, 129.62, 129.44, 129.42, 125.87, 125.82, 125.77, 125.72, 114.66, 96.35, 95.77, 90.74, 84.74, 84.68, 84.43, 81.64, 81.12, 66.39, 66.32, 61.49, 55.95, 55.50, 42.02, 31.10, 27.11, 25.29; ^{31}P-NMR ($CDCl_3$, 202 MHz) δ 51.10 (s); FAB-MS *m/z* 790 (MH^+); UV (MeOH) λ_{max} = 259, 295 (sh) nm; Anal. Calcd for $C_{35}H_{44}N_5O_{10}PS_2$: C, 53.22; H, 5.61; N, 8.87. Found; C, 53.20; H, 5.66; N, 8.68.

***N*-1-[(1*R*,2*S*,3*S*,4*R*)-2,3-Bis(methoxymethyloxy)-4-(phosphonoxymethyl)cyclopentyl]-5'-*O*-[(phenylthio)phospholyl]-2',3'-*O*-isopropylideneadenosine (19a).** A mixture of $POCl_3$ (93 μL, 1.0 mmol) and **18a** (79 mg, 0.10 mmol) in $PO(OEt)_3$ (1.0 mL)

was stirred at 0 °C for 1 h. After addition of aqueous saturated $NaHCO_3$ (3.0 mL), the resulting mixture was stirred at 0 °C for 10 min. To the mixture was added triethylammonium acetate (TEAA, 2.0 M, pH 7.0, 1.0 mL) buffer and H_2O (5.0 mL), and the resulting solution was applied to a C_{18} reversed phase column (1.1 × 14 cm). The column was developed using a linear gradient of 0–65% MeCN in TEAA buffer (0.1 M, pH 7.0, 400 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reversed phase column chromatography (1.1 × 17 cm, eluted with 70% aqueous MeCN). Appropriate fractions were evaporated, and the residue was co-evaporated with pyridine (2.0 mL × 3). A mixture of the residue, NMM (77 mg, 0.69 mmol), H_3PO_2 (70 μL, 1.39 mmol), and Et_3N (97 μL, 0.69 mmol) was stirred at 0 °C for 4 h under shading. After addition of TEAA buffer (1.0 M, pH 7.0, 2.0 mL), the resulting mixture was evaporated. The residue was partitioned between EtOAc and H_2O, and the aqueous layer was evaporated. A solution of the residue in H_2O (5.0 mL) was applied to a C_{18} reversed phase column (1.1 × 16 cm), and the column was developed using a linear gradient of 0–40% MeCN in TEAA buffer (0.1 M, pH 7.0, 400 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reversed phase column chromatography (1.1 × 17 cm, eluted with 50% aqueous MeCN). Appropriate fractions were evaporated, and the residue was lyophilized to give **19a** (41 mg, 46%) as a triethylammonium salt: ^{1}H-NMR (D_2O, 500 MHz) δ 8.71 (s, 1H, H-2 or H-8), 8.40 (s, 1H, H-2 or H-8), 7.01-7.30 (m, 5H, SPh), 6.31 (d, 1H, H-1', $J_{1',2'}$ = 2.1 Hz), 5.33 (dd, 1H, H-2', $J_{2',1'}$ = 2.1 Hz, $J_{2',3'}$ = 6.0 Hz), 5.00 (m, 1H, H-1"), 4.94 (dd, 1H, H-3', $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 1.2 Hz), 4.86 (s, 2H, MOM-CH_2), 4.72 (d, 1H, MOM-CH_2, J = 7.3 Hz), 4.70 (m, 1H, H-4'), 4.68 (d, 1H, MOM-CH_2, J = 7.3 Hz), 4.51 (m, 1H, H-2"), 4.33 (m, 1H, H-3"), 4.15 (m, 2H, H-5' x 2), 4.07 (m, 2H, H-5" x 2), 3.48 (s, 3H, MOM-CH_3), 3.27 (s, 3H, MOM-CH_3), 3.18 (q, 6H, Et_3NH-CH_2 x 3, J = 7.3 Hz), 2.74 (m, 1H, H-4"), 2.68 (m, 1H, H-6"a), 2.08 (m, 1H, H-6"b), 1.62, 1.39 (each s, each 3H, isopropylidene), 1.26 (t, 9H, Et_3NH-CH_3 x 3, J = 7.3 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 151.02, 146.31, 146.10, 143.91, 133.21, 133.17, 129.90, 129.85, 129.44, 128.28, 119.61, 115.17, 97.10, 96.75, 91.87, 87.05, 86.67, 86.59, 84.64, 81.91, 80.44, 66.27, 63.81, 56.40, 56.14, 47.12, 40.75, 40.68, 26.38, 24.73, 8.69; ^{31}P-NMR (D_2O, 202 MHz) δ 17.10 (s), 0.84 (s); HRMS (FAB, positive) calcd for $C_{29}H_{42}N_5O_{14}P_2S$ 778.1919 (MH^+), found 778.1934; UV (H_2O) λ_{max} 260 nm.

2",3"-Bis-*O*-Methoxymethyl-cyclic ADP-carbocyclic-xylose 2',3'-Acetonide

(20a). To a mixture of $AgNO_3$ (310 mg, 1.83 mmol), Et_3N (255 μL, 1.83 mmol), and MS 3A (400 mg) in pyridine (70 mL), a solution of **19a** (76 mg, 0.087 mmol) in pyridine (70 mL) was added slowly over 15 h, using a syringe-pump, at room temperature under shading. The MS 3A was filtered off with Celite and washed with H_2O. To the combined filtrate and washings was added TEAA buffer (2.0 M, pH 7.0, 2 mL), and the resulting solution was evaporated. The residue was partitioned between EtOAc and H_2O, and the aqueous layer was evaporated. A solution of the residue in H_2O (5.0 mL) was applied to a C_{18} reverse phase column (1.1 × 17 cm), and the column was developed using a linear gradient of 0–25% MeCN in TEAA buffer (0.1 M, pH 7.0, 400 mL). Appropriate fractions were evaporated, and excess TEAA was removed by C_{18} reverse phase column chromatography (1.1 × 17 cm, eluted with 40% aqueous MeCN). Appropriate fractions were evaporated, and the residue was lyophilized to give **20a** (40 mg, 46%) as a triethylammonium salt: ^{1}H-NMR (D_2O, 500 MHz) δ 9.20 (s, 1H, H-2 or H-8), 8.44 (s, 1H, H-2 or H-8), 6.41 (d, 1H, H-1', $J_{1',2'}$ = 1.6 Hz), 5.53 (dd, 1H, H-2', $J_{2',1'}$ = 1.6 Hz, $J_{2',3'}$ = 6.1 Hz), 5.46 (dd, 1H, H-3', $J_{3',2'}$ = 6.1 Hz, $J_{3',4'}$ = 2.5 Hz), 5.04 (m, 1H, H-1"), 4.80 (s, 2H, MOM-CH_2), 4.76 (d, 1H, MOM-CH_2, J = 6.9 Hz), 4.67 (d, 1H, MOM-CH_2, J = 6.9 Hz), 4.63 (m, 1H, H-4'), 4.62 (m, 1H, H-2"), 4.37 (m, 1H, H-3"), 4.23 (m, 1H, H-5"a), 4.16 (m, 1H, H-5'a), 4.09 (m, 2H, H-5'b, H-5"b), 3.42 (s, 3H, MOM-CH_3), 3.11 (s, 3H, MOM-CH_3), 3.19 (q, 6H, Et_3NH-CH_2 x 3, J = 7.3 Hz), 2.99 (m, 1H, H-6"a), 2.76 (m, 1H, H-4"), 2.36 (m, 1H, H-6"b), 1.64, 1.44 (each s, each 3H, isopropylidene), 1.27 (t, 9H, Et_3NH-CH_3 x 3, J = 7.3 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 151.93, 146.22, 146.28 144.80, 119.64, 115.13, 97.69, 96.82, 92.47, 88.26, 86.92, 86. 82, 85.13, 81.87, 81.29, 64.82, 64.61, 60.93, 56.25, 47.10, 37.29, 37.21, 28.70, 26.43, 2 4.74, 8.67; ^{31}P-NMR (D_2O, 202 MHz) δ –9.58 (d, J = 11.2 Hz), –10.56 (d, J = 11.2 Hz); HRMS (FAB, positive) calcd for $C_{23}H_{36}N_5O_{14}P_2$ 668.1729 (MH^+), found 668.1730; UV (H_2O) λ_{max} 259 nm.

Cyclic ADP-carbocyclic-xylose (4). A solution of **20a** (31 mg, 0.040 mmol) in aqueous 80% HCO_2H (1 mL) was stirred at room temperature for 48 h and then evaporated. After evaporation of the residue in H_2O and *i*-PrOH, aqueous 28% NH_3 (1 mL) was added to the residue, and the mixture was stirred at room temperature for 90 min. After evaporation of the residue in H_2O and *i*-PrOH, the resulting residue was dissolved in TEAB buffer (0.1 M, pH 7.0, 600 μL), and the solution was lyophilized to give **4** (21 mg, 90%) as a triethylammonium salt: ^{1}H-NMR (D_2O, 500 MHz, K^+ salt) δ

9.11 (s, 1H, H-2 or H-8), 8.42 (s, 1H, H-2 or H-8), 6.11 (d, 1H, H-1', $J_{1',2'}$ = 6.0 Hz), 5.20 (dd, 1H, H-2', $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 4.8 Hz), 4.95 (m, 1H, H-1"), 4.64 (dd, 1H, H-3', $J_{3',2'}$ = 4.8 Hz, $J_{3',4'}$ = 2.6 Hz), 4.62 (m, 1H, H-5'a), 4.51 (m, 1H, H-2"), 4.45 (m, 1H, H-4'), 4.32 (m, 1H, H-3"), 4.23 (m, 1H, H-5"a), 4.14 (m, 2H, H-5'b, H-5"b), 2.95 (m, 1H, H-6"a), 2.65 (m, 1H, H-4"), 2.46 (m 1H, H-6"b); ^{13}C-NMR (D_2O, 125 MHz) δ 152.20, 146.70, 145.43 144.83, 120.40, 91.07, 85.29, 82.52, 76.53, 73.89, 71.16, 65.13, 64.20, 63.12, 39.52, 28.02; ^{31}P-NMR (D_2O, 202 MHz) δ –9.41 (d, J = 11.4 Hz), –10.35 (d, J = 11.4 Hz); HRMS (FAB, positive) calcd for $C_{16}H_{24}N_5O_{12}P_2$ 540.0891 (MH^+), found 540.0875; UV (H_2O) λ_{max} 259 nm.

***N*-1-[(1*R*,2*S*,3*S*,4*R*)-2-(Methoxymethyloxy)-3-methoxy-4-(hydroxymethyl)cyclopentyl]-5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-*O*-isopropylideneadenosine (15b).** Compound **15b** (0.565 g, 84%) was obtained from **6** (0.480 g, 1.10 mmol) and **14b** (0.227 g, 1.11 mmol) as described for the synthesis of **15a**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.91 (s, 1H, H-2 or H-8), 7.83 (s, 1H, H-2 or H-8), 6.02 (d, 1H, H-1', $J_{1',2'}$ = 2.6 Hz), 5.54 (m, 1H, H-1"), 5.11 (dd, 1H, H-2', $J_{2',1'}$ = 2.6 Hz, $J_{2',3'}$ = 6.1 Hz), 4.89 (dd, 1H, H-3', $J_{3',2'}$ = 6.1 Hz, $J_{3',4'}$ = 2.5 Hz), 4.74 (d, 1H, MOM-CH_2, J = 6.8 Hz), 4.65 (d, 1H, MOM-CH_2, J = 6.8 Hz), 4.39 (ddd, 1H, H-4', $J_{4',3'}$ = 2.5 Hz, $J_{4',5'a}$ = 3.7 Hz, $J_{4',5'b}$ = 6.3 Hz), 4.35 (m, 1H, H-2"), 3.90 (dd, 1H, H-5"a, $J_{5"a,4"}$ = 3.0 Hz, $J_{5"a,5"b}$ = 11.3 Hz), 3.84 (dd, 1H, H-5'a, $J_{5'a,4'}$ = 3.8 Hz, $J_{5'a,5'b}$ = 11.2 Hz), 3.79 (m, 2H, H-3", H-5'b), 3.76 (m, 1H, H-5"b), 3.50 (s, 3H, MOM-CH_3 or OMe), 3.25 (s, 3H, MOM-CH_3 or OMe), 2.42 (m, 2H, H-4", H-6"a), 2.08 (m, 1H, H-6"b), 1.61, 1.39 (each s, each 3H, isopropylidene), 0.86 (s, 9H, *tert*-Bu), 0.048, 0.037 (each s, each 3H, TBS-Me x 2); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 154.48, 146.35, 140.70, 136.89, 123.26, 114.17, 95.33, 91.27, 87.04, 86.99, 85.28, 83.40, 81.34, 63.45, 61.54, 59.37, 57.70, 55.51, 42.02, 31.65, 27.22, 25.86, 25.37, 18.32, –5.43, –5.54; FAB-MS *m/z* 610 (MH^+); Anal. Calcd for $C_{28}H_{47}N_5O_8Si$: C, 55.15; H, 7.77; N, 11.48. Found; C, 54.95; H, 7.59; N, 11.26.

***N*-1-[(1*R*,2*S*,3*S*,4*R*)-2-(Methoxymethyloxy)-3-methoxy-4-(dimethoxytrityloxymethyl)cyclopentyl]-2',3'-*O*-isopropylideneadenosine (16b).** Compound **16b** (0.697 g, quant.) was obtained from **15b** (0.527 g, 0.864 mmol) as described for the synthesis of **16a**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.92 (brs, 1H, H-2 or H-8), 7.62 (brs, 1H, H-2 or H-8), 6.82-7.45 (m, 13H, DMTr), 5.78 (m, 2H, H-1', H-1"), 5.02 (m, 2H, H-2', H-3'), 4.82 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.68 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.49 (m, 1H,

H-4'), 4.11 (m, 1H, H-2"), 3.91 (m, 1H, H-3"), 3.80 (s, 7H, H-5'a, DMTr-OMe x 2), 3.75 (m, 1H, H-5'b), 3.37 (s, 3H, MOM-CH_3 or OMe), 3.35 (s, 3H, MOM-CH_3 or OMe), 3.25 (m, 1H, H-5"a), 3.19 (m, 1H, H-5"b), 2.56 (m, 1H, H-4"), 2.48 (m, 1H, H-6"a), 1.62, 1.36 (each s, each 3H, isopropylidene), 1.46 (m, 1H, H-6"b); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 180.59, 165.74, 163.74, 152.56, 152.49, 144.47, 142.10, 141.56, 140.21, 136.11, 124.30, 122.87, 122.84, 99.99, 98.40, 88.24, 87.29, 86.38, 85.52, 82.67, 59.34, 56.69, 54.38, 51.96, 49.88, 49.24, 33.87, 23.65, 18.31, 14.03, 11.04, 9.88, 4.19, –3.60, –21.04; HRMS (FAB, positive) calcd for $C_{43}H_{52}N_5O_{10}$ 798.3714 (MH^+), found 798.3721.

***N*-1-[(1*R*,2*S*,3*S*,4*R*)-2-(Methoxymethyloxy)-3-methoxy-4-(dimethoxytrityloxy methyl)cyclopentyl]-5'-*O*-[bis(phenylthio)phospholyl]-2',3'-*O*-isopropylideneadenosine (17b).** Compound **17b** (0.539 g, 62%) was obtained from **16b** (0.659 g, 0.826 mmol) as described for the synthesis of **17a**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.91 (s, 1H, H-2 or H-8), 7.64 (s, 1H, H-2 or H-8), 6.81-7.51 (m, 23H, DMTr, SPh x 2), 5.96 (d, 1H, H-1', $J_{1',2'}$ = 2.6 Hz), 5.76 (m, 1H, H-1"), 5.08 (dd, 1H, H-2', $J_{2',1'}$ = 2.6 Hz, $J_{2',3'}$ = 6.3 Hz), 4.86 (dd, 1H, H-3', $J_{3',2'}$ = 6.3 Hz, $J_{3',4'}$ = 2.7 Hz), 4.79 (d, 1H, MOM-CH_2, J = 6.8 Hz), 4.63 (d, 1H, MOM-CH_2, J = 6.8 Hz), 4.41 (m, 3H, H-4', H-5' x 2), 4.11 (m, 1H, H-2"), 3.79 (s, 6H, DMTr-OMe x 2), 3.78 (m, 1H, H-3"), 3.33 (s, 3H, MOM-CH_3 or OMe) , 3.30 (s, 3H, MOM-CH_3 or OMe), 3.25 (m, 1H, H-5"a), 3.18 (m, 1H, H-5"b), 2.54 (m, 1H, H-4"), 2.47 (m, 1H, H-6"a), 1.59, 1.35 (each s, each 3H, isopropylidene), 1.48 (m, 1H, H-6"b); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 158.38, 154.52, 146.79, 145.20, 140.52, 136.97, 136.42, 136.38, 135.34, 135.29, 135.22, 135.18, 130.04, 130.02, 129.65, 129.63, 129.43, 128.15, 127.69, 126.63, 125.94, 125.89, 125.82, 125.77, 123.51, 114.69, 113.00, 112.98, 95.09, 90.65, 85.79, 85.23, 84.71, 84.64, 84.45, 81.04, 66.45, 66.38, 61.23, 58.69, 57.39, 55.56, 55.19, 43.12, 35.07, 27.13, 25.30, 11.44; ^{31}P-NMR ($CDCl_3$, 202 MHz) δ 48.14 (s); FAB-MS *m/z* 1062 (MH^+); UV (MeOH) λ_{max} = 295 (sh) nm; Anal. Calcd for $C_{55}H_{60}N_5O_{11}PS_2$: C, 62.19; H, 5.69; N, 6.59. Found; C, 62.34; H, 5.83; N, 6.34.

***N*-1-[(1*R*,2*S*,3*S*,4*R*)-2-(Methoxymethyloxy)-3-methoxy-4-(hydroxymethyl)cyclopentyl]-5'-*O*-{bis(phenylthio)phospholyl}-2',3'-*O*-isopropylideneadenosine (18b).** Compound **18b** (0.312 g, 88%) was obtained from **17b** (0.496 g, 0.467 mmol) as described for the synthesis of **18a**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.92 (s, 1H, H-2 or H-8), 7.69 (s, 1H, H-2 or H-8), 7.31-7.52 (m, 10H, SPh x 2), 5.99 (d, 1H, H-1', $J_{1',2'}$ =

2.3 Hz), 5.48 (m, 1H, H-1"), 5.12 (dd, 1H, H-2', $J_{2',1'}$ = 2.3 Hz, $J_{2',3'}$ = 6.2 Hz), 4.91 (dd, 1H, H-3', $J_{3',2'}$ = 6.2 Hz, $J_{3',4'}$ = 2.4 Hz), 4.71 (d, 1H, MOM-CH_2, J = 6.7 Hz), 4.62 (d, 1H, MOM-CH_2, J = 6.5 Hz), 4.43 (m, 2H, H-4', H-5'a), 4.39 (m, 2H, H-2", H-5'b), 3.90 (dd, 1H, H-5"a, $J_{5"a,5"b}$ = 11.4 Hz, $J_{5"a,4"}$ = 2.8 Hz), 3.79 (m, 1H, H-3"), 3.76 (dd, 1H, H-5"b, $J_{5"b,5"a}$ = 11.4 Hz, $J_{5"b,4"}$ = 4.7 Hz), 3.46 (s, 3H, MOM-CH_3 or OMe), 3.21 (s, 3H, MOM-CH_3 or OMe), 2.41 (m, 2H, H-4", H-6"a), 2.06 (m, 1H, H-6"b), 1.60, 1.36 (each s, each 3H, isopropylidene); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 154.37, 146.65, 140.61, 137.38, 135.34, 135.30, 135.21, 135.17, 129.70, 129.68, 129. 66, 129.63, 129.44, 125.92, 125.86, 125.81, 125.76, 123.57, 114.70, 95.54, 90.76, 86.98 , 84.79, 84.73, 84.47, 83.46, 81.14, 66.44, 66.38, 61.55, 60.36, 59.69, 57.74, 55.55, 41.9 0, 31.44, 27.13, 25.30, 21.01, 14.18; ^{31}P-NMR ($CDCl_3$, 202 MHz) δ 51.05 (s); FAB-MS *m/z* 760 (MH^+); UV (MeOH) λ_{max} = 259, 295 (sh) nm; Anal. Calcd for $C_{34}H_{42}N_5O_9PS_2$: C, 53.75; H, 5.57; N, 9.22. Found; C, 53.59; H, 5.60; N, 8.97.

***N*-1-[(1*R*,2*S*,3*S*,4*R*)-2-(Methoxymethyloxy)-3-methoxy-4-(phosphonoxymethyl)cyclopentyl]-5'-*O*-[(phenylthio)phospholyl]-2',3'-*O*-isopropylideneadenosine (19b).** Compound **19b** (90 mg, 58%) was obtained from **18b** (140 mg, 0.184 mmol) as described for the synthesis of **19a**: ^{1}H-NMR (D_2O, 500 MHz) δ 8.65 (s, 1H, H-2 or H-8), 8.39 (s, 1H, H-2 or H-8), 7.12-7.30 (m, 5H, SPh), 6.30 (d, 1H, H-1', $J_{1',2'}$ = 2.2 Hz), 5.32 (dd, 1H, H-2', $J_{2',1'}$ = 2.2 Hz, $J_{2',3'}$ = 5.9 Hz), 5.01 (m, 1H, H-1"), 4.94 (dd, 1H, H-3', $J_{3',2'}$ = 5.9 Hz, $J_{3',4'}$ = 1.6 Hz), 4.74 (d, 1H, MOM-CH_2, J = 7.1Hz), 4.69 (m, 2H, H-4', MOM-CH_2), 4.50 (m, 1H, H-2"), 4.15 (m, 1H, H-5'a), 4.11 (m, 1H, H-5'b), 4.03 (m, 2H, H-5" x 2), 3.99 (m, 1H, H-3"), 3.54 (s, 3H, MOM-CH_3 or OMe), 3.26 (s, 3H, MOM-CH_3 or OMe), 3.18 (q, 6H, Et_3NH-CH_2 x 3, J = 7.3 Hz), 2.71 (m, 1H, H-4"), 2.60 (m, 1H, H-6"a), 2.06 (m, 1H, H-6"b), 1.62, 1.38 (each s, each 3H, isopropylidene), 1.26 (t, 9H, Et_3NH-CH_3 x 3, J = 7.3 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 150.82, 146.42, 146.30, 143.88, 133.18, 133.14, 129.92, 129.87, 129.42, 128.25, 119. 82, 119.61, 115.15, 97.02, 91.86, 86.61, 86.53, 84.62, 84.33, 81.89, 66.24, 63.67, 58.06, 56.12, 47.10, 40.69, 40.63, 26.39, 24.73, 8.68; ^{31}P-NMR (D_2O, 202 MHz) δ 16.70 (s), 0.55 (s); HRMS (FAB, positive) calcd for $C_{28}H_{40}N_5O_{13}P_2S$ 748.1813 (MH^+), found 748.1804; UV (H_2O) λ_{max} 260 nm.

2"-*O*-Methoxymethyl-3"-*O*-methyl-cyclic ADP-carbocyclic-xylose 2',3'-Acetonide (20b). Compound **20b** (37 mg, 47%) was obtained from **19b** (90 mg, 0.11 mmol) as described for the synthesis of **20a**: ^{1}H-NMR (D_2O, 500 MHz) δ 9.10 (s,

1H, H-2 or H-8), 8.45 (s, 1H, H-2 or H-8), 6.41 (s, 1H, H-1'), 5.55 (d, 1H, H-2', $J_{2',3'}$ = 6.1 Hz), 5.44 (dd, 1H, H-3', $J_{3',2'}$ = 6.1 Hz, $J_{3',4'}$ = 2.4 Hz), 5.02 (m, 1H, H-1"), 4.75 (d, 1H, MOM-CH_2, J = 6.8 Hz), 4.67 (d, 1H, MOM-CH_2, J = 6.8 Hz), 4.64 (m, 1H, H-4'), 4.57 (m, 1H, H-2"), 4.15 (m, 2H, H-5'a, H-5"a), 4.09 (m, 3H, H-5'b, H-5"b, H-3"), 3.48 (s, 3H, MOM-CH_3 or OMe), 3.16 (s, 3H, MOM-CH_3 or OMe), 3.19 (q, 6H, Et_3NH-CH_2 x 3, J = 7.3 Hz), 2.94 (m, 1H, H-6"a), 2.88 (m, 1H, H-4"), 2.37 (m, 1H, H-6"b), 1.64, 1.44 (each s, each 3H, isopropylidene), 1.27 (t, 9H, Et_3NH-CH_3 x 3, J = 7.3 Hz); ^{13}C-NMR (D_2O, 125 MHz) δ 152.18, 145.29 144.63, 119.65, 115.10, 97.24, 92.46, 87.79, 86.93, 86.83, 85.04, 84.82, 81.87, 64.60, 64.25, 58.47, 56.24, 47.10, 36.34, 36.26, 28.41, 26.42, 24.73, 8.67; ^{31}P-NMR (D_2O, 202 MHz) δ -9.56 (d, J = 11.4 Hz), -10.46 (d, J = 11.4 Hz); HRMS (FAB, positive) calcd for $C_{22}H_{34}N_5O_{13}P_2$ 638.1623 (MH^+), found 638.1627; UV (H_2O) λ_{max} 259 nm.

3"-*O*-Methyl-cyclic ADP-carbocyclic-xylose (5). Compound **5** (30 mg, quant.) was obtained from **20b** (37 mg, 0.050 mmol) as described for the synthesis of **4**: ^{1}H-NMR (D_2O, 500 MHz, K^+ salt) δ 9.04 (s, 1H, H-2 or H-8), 8.41 (s, 1H, H-2 or H-8), 6.10 (d, 1H, H-1', $J_{1',2'}$ = 6.1 Hz), 5.21 (m, 1H, H-2'), 4.95 (m, 1H, H-1"), 4.62 (m, 1H, H-5'a), 4.60 (m, 1H, H-3'), 4.56 (m, 1H, H-2"), 4.44 (m, 1H, H-4'), 4.11 (m, 3H, H-5'b, H-5" x 2), 4.03 (m, 1H, H-3"), 3.52 (s, 3H, OMe), 2.94 (m, 1H, H-6"a), 2.90 (m, 1H, H-4"), 2.52 (m 1H, H-6"b); ^{13}C-NMR (D_2O, 125 MHz) δ 152.19, 146.69, 145.43 144.52, 120.42, 91.00, 85.77, 85.34, 81.35, 73.82, 71.18, 65.14, 63.94, 63.46, 58.59, 36.81, 27.72; ^{31}P-NMR (D_2O, 202 MHz) δ -9.70 (d, J = 11.4 Hz), -10.32 (d, J = 11.4 Hz); HRMS (FAB, positive) calcd for $C_{17}H_{26}N_5O_{12}P_2$ 554.1048 (MH^+), found 554.1035; UV (H_2O) λ_{max} 259 nm.

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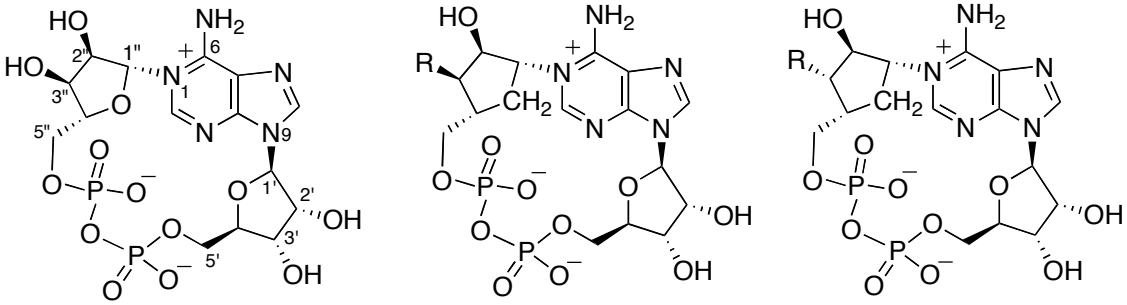


1 (cADPR)

2 (cADPcR): R = OH
3 (3"-deoxy-cADPcR): R = H

4: R = OH
5: R = OMe

FIGURE 1 cADPR (**1**), cADPcR (**2**), and the 3"-modified cADPcR analogues **3–5**.

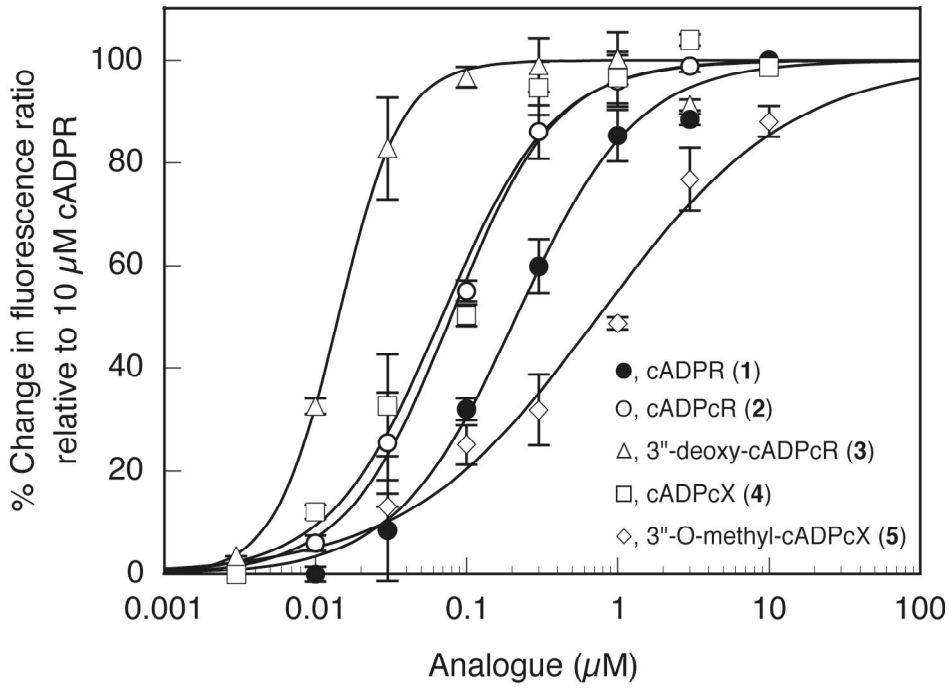
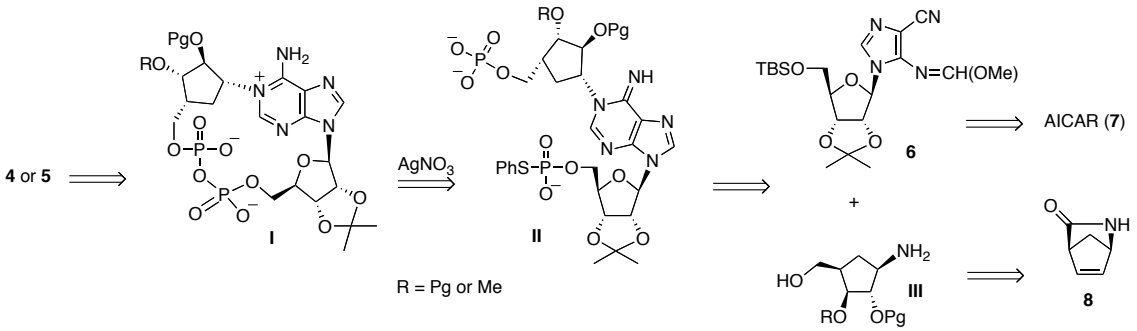
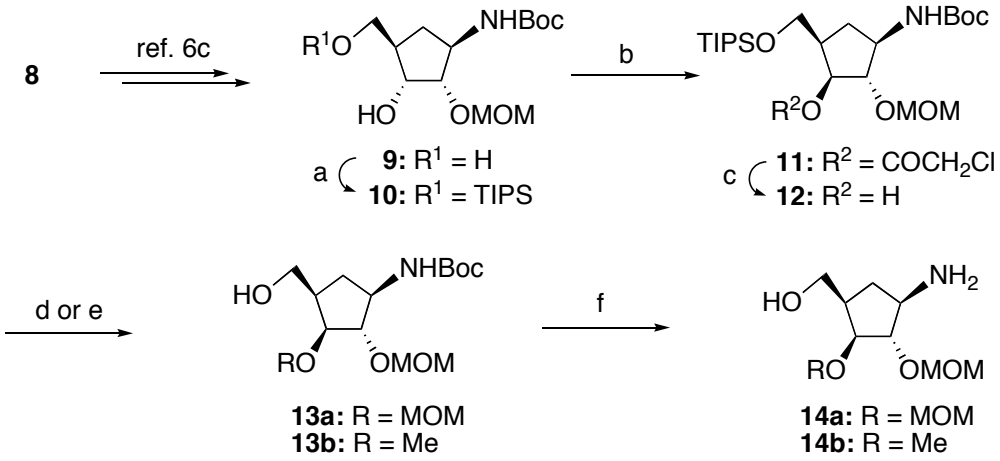


FIGURE 2 Dose-dependent Ca^{2+}-mobilizing activity of compounds in sea urchin egg homogenate. The Ca^{2+}-mobilizing activity of each compound was expressed as a percent change in ratio of fura-2 fluorescence (F340/F380) relative to that of 10 μM cADPR. The compounds examined are cADPR (**1**, filled circles), cADPcR (**2**, open circles), 3"-deoxy-cADPcR (**3**, open triangles), cADPcX (**4**, open squares), and 3"-*O*-methyl-cADPcX (**5**, open diamonds). Data are mean±SEM of 3-6 experiments.

SCHEME 1

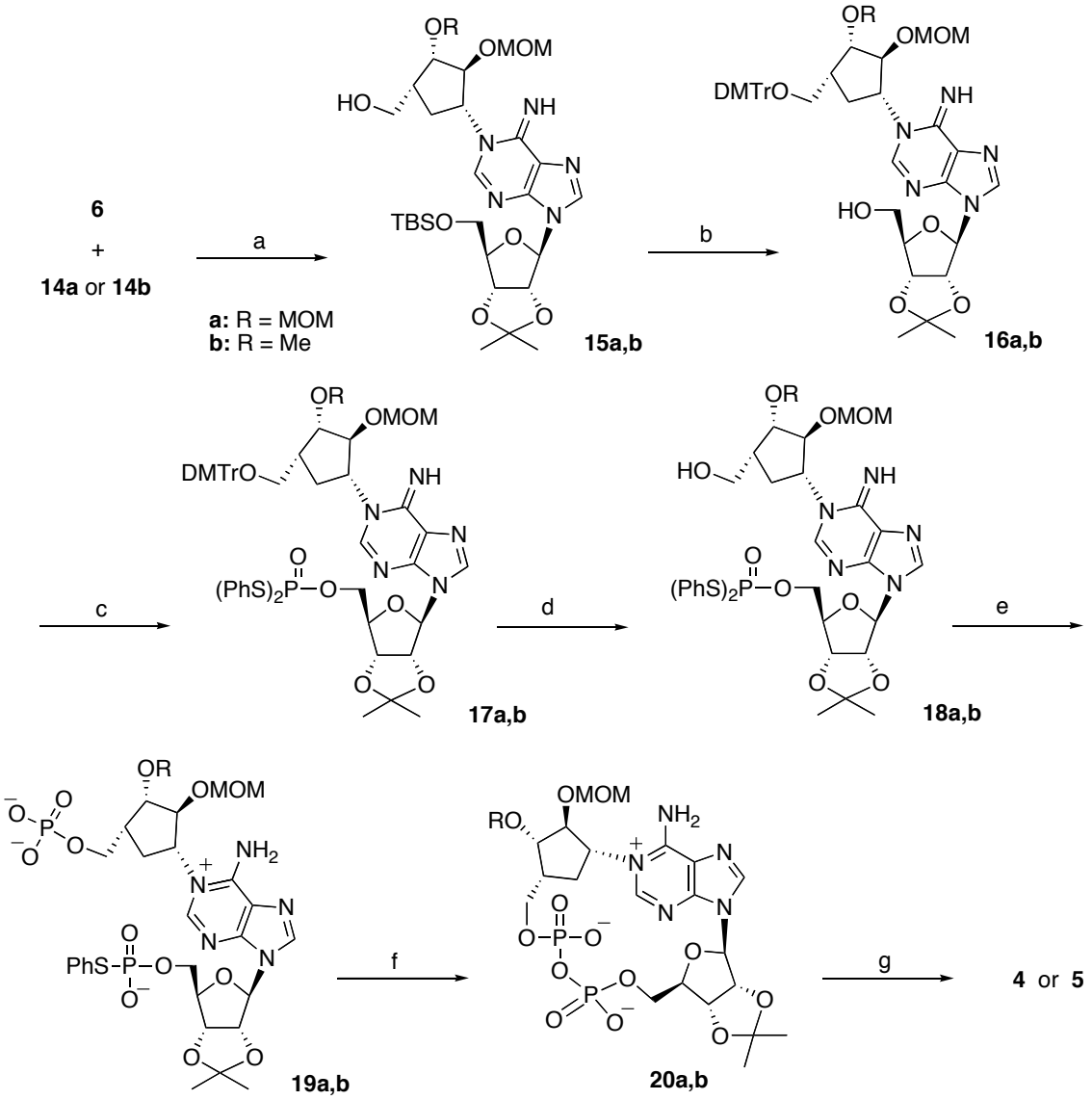


SCHEME 2



Reagents and conditions: a) TIPSCl, imidazole, DMAP, DMF, rt, 91%; b) $ClCH_2CO_2H$, DIAD, Ph_3P, toluene, 0 °C, 74%; c) NaOMe, MeOH, rt, 88%; d) 1) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 2) TBAF, THF, rt, 97% (**13a**) ; e) 1) MeOTf, DTBMP, CH_2Cl_2, rt, 2) TBAF, THF, rt, 45% (**13b**); f) H_2O, reflux, quant (**14a**, **14b**).

SCHEME 3



Reagents and conditions: a) K_2CO_3, MeOH, rt, 83% (**15a**), 84% (**15b**); :b) 1) DMTrCl, pyridine, rt, 2) TBAF, AcOH, THF, rt, quant. (**16a**), quant. (**16b**); c) PSS, TPSCl, py, rt, 65% (**17a**), 62% (**17b**); d) aq. 60% AcOH, rt, 83% (**18a**), 88% (**18b**); e) 1) $POCl_3$, $(EtO)_3PO$, 0 °C, 2) H_3PO_2, Et_3N, NMM, pyridine, 0 °C, 46% (**19a**), 58% (**19b**); f) $AgNO_3$, MS 3A, Et_3N, py, rt, 46% (**20a**), 47% (**20b**); h) aq. 80% HCO_2H, then aq. NH_3, rt, 90% (**4**), quant. (**5**).