HETEROCYCLES, Vol. 91, No. 6, 2015, pp. 1164 - 1169. © 2015 The Japan Institute of Heterocyclic Chemistry
Received, 9th April, 2015, Accepted, 12th May, Published online, 26th May, 2015
DOI: 10.3987/COM-15-13223

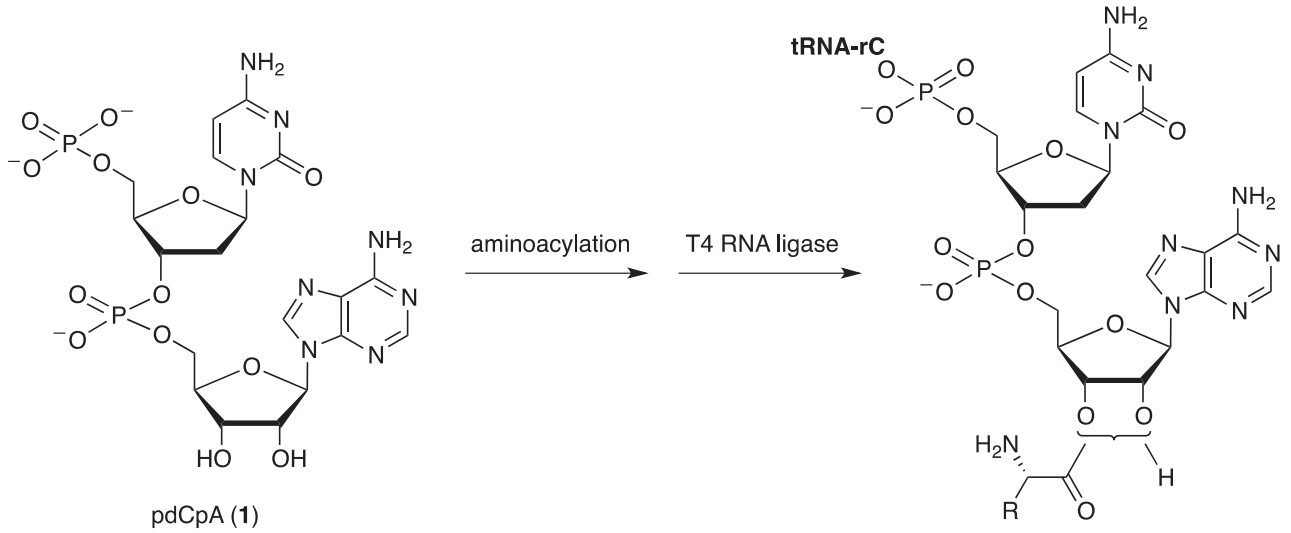
A NEW STRATEGY FOR SYNTHESIS OF THE DINUCLEOTIDE pdCpA: A CONVENIENT METHOD FOR THE DEPROTECTION OF CYANOETHYL, TBDMS, AND BENZOYL GROUPS IN ONE STEP AT HIGH PRESSURE

Masanori Kataoka,[1]* Chiharu Fukui,[1] Akiko Mimoto,[1] Hideaki Kuge,[2] Koichi Honke,[2] and Hiyoshizo Kotsuki[3]*

[1]Oceanography Section, Science Research Center, Kochi University, Akebono- cho, Kochi 780-8520, Japan. [2]Department of Biochemistry, Kochi University Medical School, Nankoku, Kochi 783-8505, Japan. [3]Laboratory of Natural Products Chemistry, Faculty of Science, Kochi University, Akebono-cho, Kochi 780-8520, Japan. e-mail: m.kataoka@kochi-u.ac.jp, kotsuki@kochi-u.ac.jp

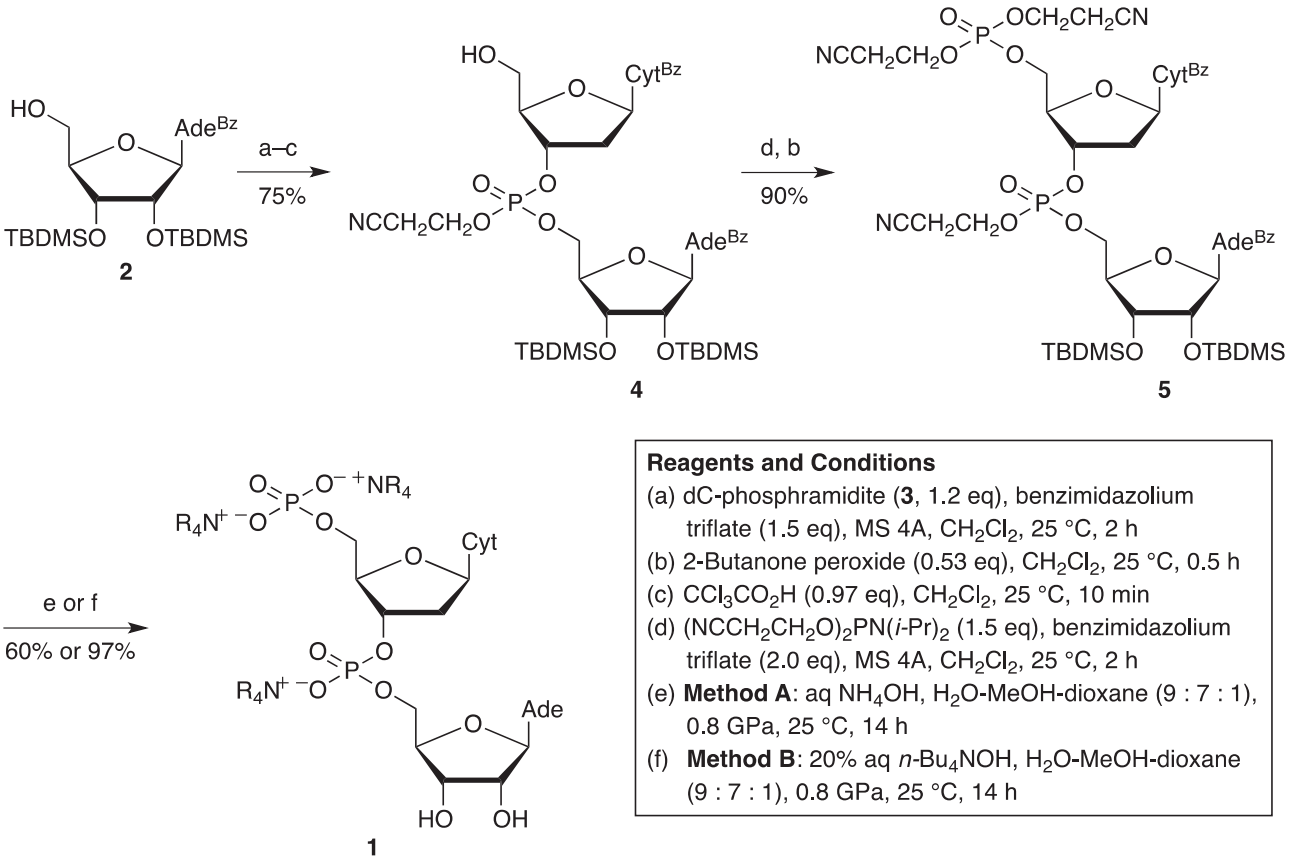
Abstract – A new convenient method for the preparative-scale synthesis of the dinucleotide pdCpA was developed. This method takes advantage of the complete deprotection of cyanoethyl, TBDMS, and benzoyl groups in one step under high-pressure conditions in the final stage of the synthetic sequence.

The incorporation of unnatural amino acids into proteins at predetermined sites has been extensively used to investigate protein structures and functions,[1] for the design of glycoprotein substrates that can participate in several important biological processes,[2] and for the discovery of new drug candidates.[3] In general, such cell-free protein syntheses commence with the chemical aminoacylation of pdCpA (**1**)[4] and the subsequent condensation with an abbreviated suppressor tRNA under catalysis with T4 RNA ligase (Scheme 1).[1d]
Despite this enormous utility in molecular engineering science, there is still a strong demand for improved product yields and greater purity.[5] Some of the major problems that remain to be overcome in this regard include the difficulty of obtaining sufficient amounts of the starting pdCpA (**1**) and the need for special techniques for its purification as well as sample regulation.
In our extensive efforts to develop a new synthetic method for a variety of nucleotides,[6] it became necessary to prepare large quantities of the dinucleotide pdCpA (**1**). Here we describe a new and convenient method for the synthesis of this key compound using a high-pressure-promoted deprotection strategy.



Scheme 1

Our synthetic procedure is shown in Scheme 2. Condensation of bis-TBDMS- and *N*-Bz-protected adenosine (**2**, 1.8 g)[7] with 1.2 eq of *N*-Bz-protected 2'-deoxycytidinephosphoramidite (**3**) in the presence of 1.5 eq of benzimidazolium triflate[8] and MS 4A in dichloromethane followed by oxidation with 0.53 eq of 2-butanone peroxide[9] at 25 °C for 0.5 h gave, after careful acidification by exposure to trichloroacetic acid at ambient temperature, phosphotriester **4** (2.4 g) in 75% overall yield. Next, **4** was converted to the



Reagents and Conditions

(a) dC-phosphramidite (**3**, 1.2 eq), benzimidazolium triflate (1.5 eq), MS 4A, CH_2Cl_2, 25 °C, 2 h
(b) 2-Butanone peroxide (0.53 eq), CH_2Cl_2, 25 °C, 0.5 h
(c) CCl_3CO_2H (0.97 eq), CH_2Cl_2, 25 °C, 10 min
(d) $(NCCH_2CH_2O)_2PN(i\text{-}Pr)_2$ (1.5 eq), benzimidazolium triflate (2.0 eq), MS 4A, CH_2Cl_2, 25 °C, 2 h
(e) **Method A**: aq NH_4OH, H_2O-MeOH-dioxane (9 : 7 : 1), 0.8 GPa, 25 °C, 14 h
(f) **Method B**: 20% aq *n*-Bu_4NOH, H_2O-MeOH-dioxane (9 : 7 : 1), 0.8 GPa, 25 °C, 14 h

Scheme 2

corresponding 5'-phosphorylated compound **5** in 90% yield by conventional phosphorylation (1.9 g): *i.e.*, treatment with 1.5 eq of bis(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite in the presence of 2.0 eq of benzimidazolium triflate[8] followed by oxidation with 0.53 eq of 2-butanone peroxide[9] at 25 °C for 0.5 h. Finally, deprotection of all of the cyanoethyl, TBDMS, and benzoyl groups was first attempted under normal conditions using ammonium hydroxide.[5c] In this case, however, the TBDMS group remained undeprotected and subsequent desilylation was required to complete this synthetic sequence.

To shorten this stepwise methodology, we anticipated that the application of high pressure would be useful, since it is known that deprotection / hydrolysis of this type can be effectively accelerated under high-pressure conditions.[10] Accordingly, when a mixture of **5** and excess aq ammonium hydroxide in a mixed solvent system composed of H_2O-MeOH-dioxane (9 : 7 : 1) was reacted at 0.8 GPa and 25 °C for 14 h, the desired ammonium salt of pdCpA (**1**, R = H) was obtained in 60% yield (**Method A**).[11, 12] To our delight, simultaneous desilylation of double TBDMS groups could be achieved even under such fluoride-free conditions.[13] Furthermore, the superiority of this technique was exemplified by the use of tetra-*n*-butylammonium hydroxide as a base,[14] and the corresponding tetra-*n*-butylammonium salt of pdCpA (**6**, R = *n*-Bu) was obtained in almost quantitative yield (**Method B**).[11, 12] This substance was sufficiently pure and could be used directly in further experiments to introduce unnatural amino acids to a ribose core. Notably, the latter improved strategy does not require a tedious counter cation exchange process,[15] and may be very useful for further elaboration in nucleotide chemistry.

In summary, we have developed a new convenient method for synthesis of the dinucleotide pdCpA (**1**) on a preparative scale. This method takes advantage of the simultaneous complete deprotection of cyanoethyl, TBDMS, and benzoyl groups under high-pressure conditions in the final stage. Further studies on protein engineering using the dinucleotide pdCpA (**1**) are now in progress in our laboratory.

ACKNOWLEDGEMENTS

We are grateful to Dr. K. Onodera and Mrs. M. Yuhi of Kochi University for MALDI-TOF/MS and HPLC measurements. This work was supported by Adaptable and Seamless Technology Transfer Program through target-driven R&D, JST (Japan Science and Technology Agency) and by the Program to Disseminate Tenure Tracking System of the MEXT (Japan).

REFERENCES AND NOTES

1. (a) T. G. Heckler, Y. Zama, T. Naka, and S. M. Hecht, *J. Biol. Chem*., 1983, **258**, 4492; (b) T. G. Heckler, L. H. Chang, Y. Zama, T. Naka, and S. M. Hecht, *Tetrahedron,* 1984, **40**, 87; (c) C. J. Noren, S. J. Anthoby-Cahill, M. C. Griffith, and P. G. Schultz, *Science,* 1989, **244**, 182; (d) T. G. Heckler, L. H. Chang, Y. Zama, T. Naka, M. S. Chorghade, and S. M. Hecht, *Biochemistry,* 1984, **23**, 1468; (e) G. Baldini, B. Martoglio, A. Schachenmann, C. Zugliani, and J. Brunner, *Biochemistry,* 1988, **27**, 7951; (f)

J. A. Ellman, D. Mendel, and P. G. Schultz, *Science,* 1992, **255**, 197; (g) V. W. Cornish, D. Mendel, and P. G. Schultz, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 621; (h) V. A. Karginov, S. V. Mamaev, H. An, M. D. Van Cleve, S. M. Hecht, G. A. Komatsoulis, and J. N. Abelson, *J. Am. Chem. Soc.,* 1997, **119**, 8166; (i) P. M. England, H. A. Lester, and D. A. Dougherty, *Biochemistry,* 1999, **38**, 14409; (j) S. M. Hecht, *Protein Engineering*, ed. by U. L. RajBhandary and C. Koehrer, Springer: New York, 2008; pp. 251–266; (k) L. Yakovleva, S. Chen, S. M. Hecht, and S. Shuman, *J. Biol. Chem*., 2008, **283**, 16093; (l) A. C. Forster, *Biotechnol. J*., 2012, **7**, 835.

2. N. E. Fahmi, S. Golovine, B. Wang, and S. M. Hecht, *Carbohydr. Res*., 2001, **330**, 149.
3. (a) Y. Yamagishi, I. Shoji, S. Miyagawa, T. Kawakami, T. Katoh, Y. Goto, and H. Suga, *Chem. Biol.,* 2011, **18**, 1562; (b) R. E. Watts and A. C. Forster, *Methods Mol. Biol*., 2012, **805**, 349; (c) Y. V. Guillen Schlippe, M. C. T. Hartman, K. Josephson, and J. W. Szostak, *J. Am. Chem. Soc.,* 2012, **134**, 10469.
4. S. A. Robertson, J. A. Ellman, and P. G. Schultz, *J. Am. Chem. Soc*., 1991, **113**, 2722.
5. (a) S. A. Robertson, C. J. Noren, S. J. Anthony-Cahill, M. C. Griffith, and P. G. Schultz, *Nucleic Acids Res.,* 1989, **17**, 9649; (b) T. Niinomi and M. Sisido, *Chem. Lett*., 1993, 1305; (c) M. Lodder, S. Golovine, A. L. Laikhter, V. A. Karginov, and S. M. Hecht, *J. Org. Chem.,* 1998, **63**, 794; (d) X.-F. Zhu and A. I. Scott, *Nucleosides, Nucleotides Nucleic Acids,* 2001, **20**, 197; (e) S. Chen, N. E. Fahmi, R. C. Nangreave, Y. Mehellou, and S. M. Hecht, *Bioorg. Med. Chem*., 2012, **20**, 2679; (f) M. Kwiatkowski, J. Wang, and A. C. Forster, *Bioconjugate Chem*., 2014, **25**, 2086.
6. Y. Hayakawa, T. Iwase, E. J. Nurminen, M. Tsukamoto, and M. Kataoka, *Tetrahedron,* 2005, **61**, 2203, and references cited therein.
7. In terms of solubility in organic solvents, we preferred to use the bis-silylated substrates.
8. (a) Y. Hayakawa, M. Kataoka, and R. Noyori, *J. Org. Chem.,* 1996, **61**, 7996; (b) S. Iwai, T. Mizukoshi, Y. Fujiwara, C. Masutani, F. Hanaoka, and Y. Hayakawa, *Nucleic Acids Res*., 1999, **27**, 2299; (c) Y. Hayakawa, R. Kawai, A. Hirata, J. Sugimoto, M. Kataoka, A. Sakakura, M. Hirose, and R. Noyori, *J. Am. Chem. Soc.,* 2001, **34**, 8165.
9. 2-Butanone peroxide = MeC(OOH)(Et)OOC(OOH)(Et)Me. This reagent is available from Sigma-Aldrich as a 2,2,4-trimethyl-1,3-pentanediol diisobutyrate solution. This was highly convenient for oxidizing the phosphitylated intermediates compared to the use of bis(trimethylsilyl) peroxide due to the ease of purification, and hence better yields were obtained: the latter reagent gave the product in around 40% yield (see Supporting Information): (a) Y. Hayakawa, M. Uchiyama, and R. Noyori, *Tetrahedron Lett*., 1986, **27**, 4191; (b) T. Kato and Y. Hayakawa, *Synlett,* 1999, 1796; (c) M. Kataoka, A. Hattori, S. Okino, M. Hyodo, M. Asano, R. Kawai, and Y. Hayakawa, *Org. Lett*., 2001, **3**, 815 & 2939.
10. For example, see: (a) Y. Yamamoto, T. Furuta, J. Matsuo, and T. Kurata, *J. Org. Chem.,* 1991, **56**, 5737;

(b) T. Furuta, J. Matsuo, T. Kurata, and Y. Yamamoto, *High Press. Res*., 1992, **11**, 93; (c) I. Matsuo, M. Wada, and Y. Ito, *Tetrahedron Lett*., 2002, **43**, 3273; (d) W. Kroszczynski, E. Olszewska, P. Salanski, and J. Jurczak, *Helv. Chim. Acta,* 2004, **87**, 1488; (e) J. Nakano, H. Ohta, and Y. Ito, *Bioorg. Med. Chem. Lett*., 2006, **16**, 928; (f) J. Nakano, A. Ishiwata, H. Ohta, and Y. Ito, *Carbohydr. Res*., 2007, **342**, 675.

11. All high-pressure reactions were performed in a piston-cylinder type apparatus (Hikari Koatsu HR-15-B3).
12. **Method A:** To a solution of the protected pdCpA **5** (57 mg, 46 μmol) in MeOH (750 μL) and 1,4-dioxane (110 μL) was added 28% aq NH_4OH (900 μL), and the mixture was transferred to a Teflon reaction vessel and kept at 0.8 GPa and 25 °C for 14 h. After concentration, the crude product was subjected to reverse-phase short-column chromatography on 30 μm ODS (30 g) eluted with a mixture of double-distilled H_2O and MeCN (100 : 0 → 95 : 5) to provide the ammonium salt of pdCpA **1** as an amorphous solid (19 mg, 60% yield).

    Method B: To a solution of the protected pdCpA **5** (370 mg, 300 μmol) in MeOH (3.7 mL), 1,4-dioxane (540 μL), and double-distilled H_2O (1.8 mL) was added 20% aq *n*-Bu_4NOH (2.5 mL, 1.8 mmol), and the mixture was transferred to a Teflon reaction vessel and kept at 0.8 GPa and 25 °C for 14 h. After dilution by the addition of double-distilled H_2O, the mixture was subjected to gel filtration on Sephadex G-10 (480 mL, eluted with double-distilled H_2O / MeOH = 80 : 20) to give the tetra-*n*-butylammonium salt of pdCpA **1** (480 mg, 97% yield estimated by OD_{260}) as an amorphous solid.

    1 (as its tetra-*n*-butylammonium salt): FTIR (KBr) ν 1653, 1604, 1576, 1488, 1378 cm^{-1}; UV (MeOH) λ_{max} 260 nm (ε 23,800); ^1H NMR (500 MHz, CD_3OD) δ 8.62 (1H, s), 8.17 (1H, s), 8.10 (1H, d, *J* = 7.5 Hz), 6.41 (1H, dd, *J* = 5.4, 8.3 Hz), 6.11 (1H, d, *J* = 6.3 Hz), 5.94 (1H, d, *J* = 7.5 Hz), 4.98–4.92 (1H, m), 4.72 (1H, dd, *J* = 6.0, 6.9 Hz), 4.59–4.54 (1H, m), 4.32–4.28 (1H, m), 4.26–4.22 (1H, m), 4.13–3.97 (4H, m), 3.62 (1H, t, *J* = 6.9 Hz), 3.26–3.14 (24H, m), 2.65–2.56 (1H, m), 2.40 (1H, t, *J* = 7.2 Hz), 2.28–2.19 (1H, m), 1.69–1.59 (24H, m, TBA), 1.40 (24H, sextet, *J* = 7.5 Hz), 1.01 (36H, t, *J* = 7.5 Hz); ^{31}P NMR (202.5 MHz, CD_3OD) δ 4.28, –0.46; MALDI-TOF/MS Calcd for $C_{19}H_{27}N_8O_{13}P_2$ $[M + H]^+$ *m/z* 637.12, found *m/z* 637.52.
13. The fluoride-free deprotection of silyl groups under strongly basic conditions has been reported. For example, see: **KOH**: (a) L. L. H. de Fallois, J.-L. Décout, and M. Fontecave, *Tetrahedron Lett*., 1995, **36**, 9479; **LiOH**: (b) P. Wipf and S. Lim, *J. Am. Chem. Soc*., 1995, **117**, 558; (c) H. Liang, L. Hu, and E. J. Corey, *Org. Lett*., 2011, **13**, 4120; **NaOMe**: (d) P. A. Wender, F. Christopher Bi, M. A. Brodney, and F. Gosselin, *Org. Lett*., 2001, **3**, 2105.
14. At the end of the reaction, the mixture showed a pH of *ca.* 8.0 as a result of neutralization by the

deprotection of cyanoethyl and benzoyl groups.

15. (a) J. A. Ellman, D. Mendel, S. Anthony-Cahill, C. J. Noren, and P. G. Schultz, *Methods Enzymol*., 1991, **202**, 301; (b) M. J. Damha and K. K. Ogilvie, *Methods Mol. Biol*., 1993, **20**, 81.