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Scaleable and Efficient Synthesis of 2′-Deoxy-2′-*N*-phthaloyl Nucleoside Phosphoramidites for Oligonucleotide Synthesis

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Abstract—2′-Deoxy-2′-*N*-phthaloyl nucleosides were prepared from arabino nucleosides by triflate displacement with phthalimide in the presence of DBU. The corresponding phosphoramidites suitable for automated oligonucleotide synthesis were also synthesized. The scalability of described procedures was demonstrated on a 100-g scale preparation of 2′-deoxy-2′-amino-C phosphoramidite.

Structural modifications of oligonucleotides are becoming increasingly important as their possible clinical applications emerge.[1–5] As support for a 'New Ribozyme Motifs' program that is under development in our laboratory we were interested in creating a quick and scalable synthesis of 2′-amino nucleoside phosphoramidites. We have previously shown[6] that phthaloyl protection of the 2′-aminogroup during oligonucleotide synthesis is preferable as compared to trifluoroacetyl or Fmoc group. We also reported[6] the synthesis of 2′-*N*-phthaloyluridine phosphoramidite starting from 2′-aminouridine using Nefkins' method.[7] However, this procedure requires 2′-aminonucleosides as starting materials, which are not commercially available.

The first preparation of 2′-aminouridine was first described by Verheyden et al.[8] in 1971 by lithium azide opening of 2,2′-*O*-anhydrouridine in 50% yield followed by catalytic reduction to the corresponding amine. Several reports elaborating this approach with minor modifications have been published. A rather interesting approach utilizing intramolecular cyclization of 3′-*O*-trichloroacetimidate of 2,2′-*O*-anhydrouridine, followed by acid hydrolysis has been recently published[9] as an alternative to use of azide ion. Methods for the synthesis of the 2′-aminopurine nucleosides use the same general strategy, namely, introduction of 2′-azido group with subsequent reduction. In turn, 2′-azidopurine nucleosides were prepared by glycosylation with 2′-azido-2′deoxy ribose derivatives,[10] transglycosylation with 2′-amino-2′-deoxyuridine,[11] opening of 8,2-cyclopurine nucleosides with azide ion,[12,13] and displacement of the corresponding 2′-arabino triflates with azide ion.[14]

We were interested in a large scale procedure that is universal for purine and pyrimidine nucleosides which involves fewer amounts of chemical steps and which avoids the use of hazardous chemicals as lithium azide. Earlier we reported on the preparation of 2′-*O*-amino ribonucleosides and their phosphoramidites utilizing displacement of 2′-*O*-triflyl group in the corresponding arabino nucleosides with *N*-hydroxyphthalimide.[15] We decided to apply the similar approach, but using phthalimide or its derivatives.

The present communication describes syntheses of 2′-amino uridine, adenosine and cytidine and their corresponding 2′-deoxy-2′-*N*-phthaloyl phosphoramidites.

Arabinonucleosides **1** (Fig. 1, X = Ura, or X = N4Ac-Cyt[16]), **9** (Fig. 2) were protected with the tetraisopropyldisiloxyl group and then treated with trifluromethanesulfonic anhydride (ara-U) or trifluoromethanesulfonic chloride (ara-A or ara-C^{Ac}) to give arabino derivatives **3** or **11**. Inversion of configuration by substitution with phthalimide in the presence of DBU provided ribonucleosides **4**, or **12** respectively in 65–70% yield.[17]

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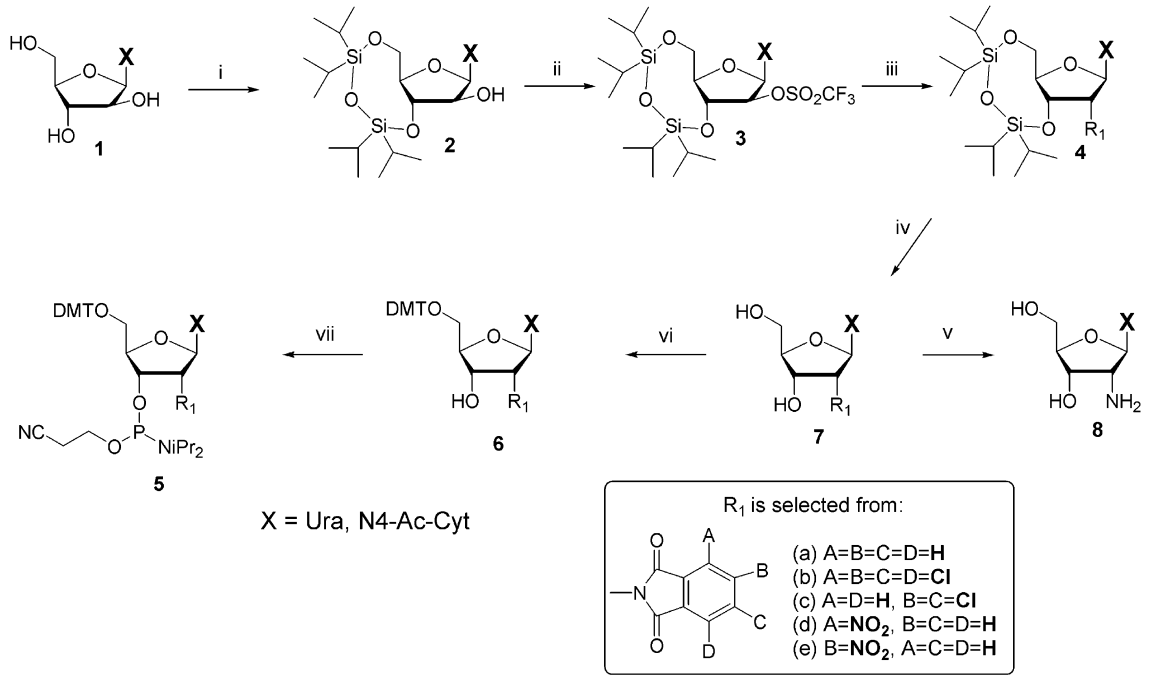

Figure 1. Synthesis of 2′-deoxy-2′-*N*-phthaloyl nucleosides and their phosphoramidites. Reagents and conditions: (i) TIPS-Cl/Pyr; (ii) $(CF_3SO_2)O$ or CF_3SO_2Cl, DMAP/CH_2Cl_2, 0 °C, 3 h; (iii) PhthNH, DBU/MeCN; (iv) $Et_3NH{\cdot}HF$/THF; (v) 40% aq $MeNH_2$; (vi) DMT-Cl/Pyr; (vii) 2-cyanoethyl *N*,*N*-diisopropyl chlorophosphoramidite.

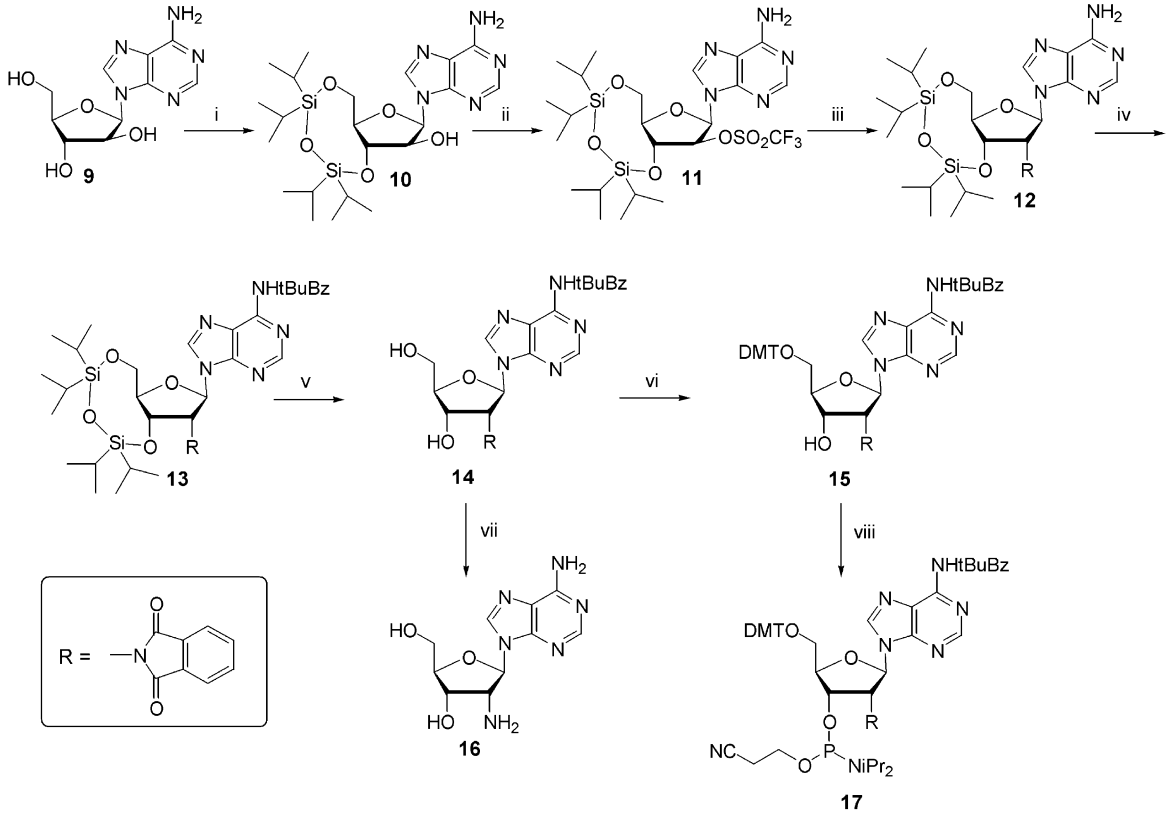

Figure 2. Synthesis of 2′-deoxy-2′-*N*-phthaloyl adenosine and its phosphoramidite. Reagents and conditions: (i) TIPS-Cl/Pyr; (ii) CF_3SO_2Cl, DMAP/CH_2Cl_2, 0 °C, 3 h; (iii) PhthNH, DBU/MeCN; (iv) *t*-BuBzCl/Pyr, then morpholine; (v) $Et_3N{\cdot}HF$/THF; (vi) DMT-Cl/Pyr; (vii) 40% aq methylamine; (viii) 2-cyanoethyl *N*,*N*-diisopropyl chlorophosphoramidite.

Table 1. Triflate displacement with substituted phthalimides

Compd	Reaction conditions	Elimination yield (%)	Yield of **4** from **2** (%)
4a	60 °C, 3 h, then rt. Overnight	10–20	60
4b	Rt, 20 h	10–20	56
4c	70–80 °C, 3 h	Traces	70
4d	Rt, 20 h	20	40
4e	Rt, 20 h	20	35

It is worth noting that substitutions with sodium or potassium salts of phthalimide as well as with DBU salt in the presence or absence of DBU produced no reaction.

The main side product on this step is the 2′-deoxy-1′,2′-didehydro-nucleoside,[18] which is formed in competing elimination reaction. This 'elimination product' can be easily separated by crystallization. We also investigated the effect of various substituents in the phenyl ring of phthalimide on triflate displacement reaction (Table 1). 2′-Arabino-triflate **3** (Fig. 1. X = N4-Ac-Cyt) was treated with 4,5-di-chloro-, 3,4,5,6-tetrachloro-, 3-nitro- or 4-nitro-phthalimides in the presence of DBU (1.2 equiv) to produce corresponding 2′-*N*-phthaloyl-cytidine derivatives **4b–e**. It is interesting to note that in case of tetrachlorophthalimide, the desired product was formed in 70% isolated yield and only traces of 'elimination product' were detected in this reaction.

The exocyclic amino group of adenosine derivative **12** (Fig. 2) was acylated with 2 equiv of *t*-butylbenzoyl chloride to provide compound **13** after morpholine hydrolysis.

Subsequent silyl deprotection ($Et_3N{\cdot}HF$, 3 h, rt) afforded 2′-deoxy-2′*N*-phthaloyl nucleosides **7** or **14** in 95% yield. Application of the standard procedures of dimethoxytritylation and phosphitylation to these compounds resulted in high yield (>90%) formation of the corresponding phosphoramidites **5**[19] and **17**.[19] 2′-Amino nucleosides **8** and **16** were obtained by further hydrolysis of derivatives **7** and **14** with 40% aq methylamine in nearly quantitative yields.

In conclusion, we have identified a straightforward universal synthetic path to 2′-deoxy-2′-amino-nucleosides and their phosphoramidites starting from arabinonucleosides. Described synthetic procedures can be easily scaled-up to a scale of 100 g and higher. In the case of 2′-amino-C, the described process includes only one flash chromatography step—purification of final phosphoramidite. All other intermediate products were successfully crystallized, allowing cost-effective preparations in 40–50% overall yields on a 100 g scale.

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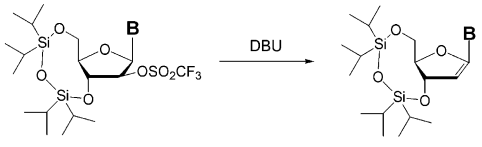
References and Notes

1. Agrawal, S. *Trends Biotech*. **1996**, *14*, 376.
2. Usman, N.; Blatt, L. M. *J. Clin. Inv*. **2000**, *106*, 1197. Cristoffersen, R. E.; Marr, J. J. *J. Med. Chem*. **1995**, *38*, 2023.
3. Crooke, S. T.; Bennet, C. F. *Annu. Rev. Pharmacol. Toxicol*. **1996**, *36*, 107.
4. Gold, L. *J. Biol. Chem*. **1995**, *270*, 13581.
5. Usman, N.; Stinchcomb, D. T. In *Catalytic RNA*; Eckstein, F.; Lilley, D. M. J., Eds.; Springer-Verlag: Heidelberg, 1996; Vol.10, pp 243–264.
6. Beigelman, L.; Karpeisky, A.; Matulic-Adamic, J.; Haeberli, P.; Sweedler, D.; Usman, N. *Nucleic Acids Res*. **1995**, *23*, 4434.
7. Nefkins, J. S. L.; Tesser, G. I.; Nivard, R. G. F. *Recl. Trav. Chim. Pays-Bas* **1960**, *79*, 688.
8. Verheuden, J. P. H.; Wagner, D.; Moffat, J. G. *J. Org. Chem*. **1971**, *36*, 250.
9. McGee, D. P.; Vaughunn-Settle, A.; Vargeese, C.; Zhai, Y. *J. Org. Chem*. **1996**, *61*, 781.
10. Hobbs, J. B.; Eckstein, F. *J. Org. Chem*. **1977**, *42*, 714.
11. Imazawa, M.; Eckstein, F. *J. Org. Chem*. **1979**, *44*, 2039.
12. Ikehara, M.; Maruyama, T.; Miki, H.; Takatsuka, Y. *Chem. Pharm. Bull*. **1977**, *25*, 754.
13. Ikehara, M.; Maruyama, T. *Chem. Pharm. Bull*. **1978**, *26*, 240.
14. Robins, M. J.; Hawrelak, S. D.; Hernandez, A. E.; Wnuk, S. F. *Nucleosides Nucleotides* **1992**, *11*, 821.
15. Karpeisky, A.; Gonzalez, C.; Burgin, A. B.; Beigelman, L. *Tetrahedron Lett*. **1998**, *39*, 1131.
16. Bhat, V.; Ugarkar, B. G.; Sayeed, V. A.; Grimm, K.; Kosora, N.; Domenico, P. A.; Stocker, E. *Nucleosides Nucleotides* **1989**, *8*, 179.
17. Triflation and displacement with phthalimide (typical procedure): To a solution of 5′,3′-tetraisopropyldisiloxyl-1-β-D-arabinofuranosyl-N4-acetylcytosine **2** (71.5 g, 135.5 mmol), DMAP (3 mmol) stirring at −10 °C under argon in anhydrous dichloromethane was added triflic chloride (1.2 mmol) dropwise via syringe. After stirring at 0 °C for three h, TLC (70% EtOAc) indicated complete reaction. Pyridine and DMAP were removed by washing with cold 1.5% acetic acid in water followed by aqueous sodium bicarbonate. The organic layer was dried over sodium sulfate, filtered, and the filtrate evaporated in vacuo. The triflate was used without further purification. To a solution of 5′,3′-tetraisopropyldisiloxyl-2′-*O*-triflyl-1-β-D-arabinofuranosyl-nucleoside and, phthalimide (1.2 mmol) stirring at 60 °C under argon in anhydrous acetonitrile was added the solution of DBU (1.2 mmol) in acetonitrile slowly via self-equalized separatory funnel. The reaction mixture was stirred at 60 °C for 3 h and then overnight at room temperature, at which time TLC (90% EtOAc/hexane) indicated complete reaction. The precipitated 'elimination product' was filtered off and washed with cold acetonitrile. Combined mother liquor and washings were concentrated to a minimal volume. The residue was diluted with dichloromethane and washed with saturated sodium bicarbonate solution. The organic layer was then dried over sodium sulfate, filtered, and dried in vacuo. The residue was dissolved in ethyl acetate and filtered through slicagel pad. The appropriate fractions were combined and evaporated to dryness. The resulted product was crystallized from toluene–hexane (1:2) to provide 53.4 g (60% on compound **3**) of 2′-deoxy-2′-*N*-phthaloyl-derivative **4a**.
18.



19. ^{31}P NMR ($CDCl_3$, δ ppm): **5a** (X = Ura): 151.395, 149.644; **5a** (X = Cyt^{Ac}): 152.114, 150.621; **17**: 151.509, 150.558.