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Effect of molecular sieves in the liquid-phase synthesis of nucleotides via the phosphoramidite method

Yoshihiro Hayakawa,* Akiyoshi Hirata, Jun-ichiro Sugimoto, Rie Kawai, Akira Sakakura and Masanori Kataoka

Laboratory of Bioorganic Chemistry, Graduate School of Human Informatics, Nagoya University, Chikusa, Nagoya 464-8601, Japan

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Abstract—It is demonstrated that the reaction of a nucleoside phosphoramidite and a nucleoside aided by a suitable promoter in the presence of molecular sieves 3A or 4A in a liquid phase is efficiently performed by the use of stoichiometric amounts of the reactants to give the desired coupling product in an excellent yield. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

In recent years, a variety of small-size nucleotides and their related compounds with biologically attractive activities and properties have been discovered. They include adenylyl(2′–5′)adenylyl(2′–5′)adenosine (2–5A core) and related derivatives,[1] cyclic bis(3′→5′)diguanylic acid,[2] cytidine-5′-monophosphono-*N*-acetylneuraminic acid (CMP-Neu5Ac),[3] aminoacylamido-AMP,[4] phosmidosine,[5] agrocin 84,[6] and dinogunellin.[7] Much research has been devoted to the biological activities of these compounds. In order to perform wide and detailed investigation, it is essential to obtain a sufficient amount of these substrates. In other words, it is important to develop an efficient synthetic method that provides a large amount of nucleotides and their related compounds. Large-scale synthesis of short-length nucleotide derivatives, such as those described here, are generally carried out in a solution phase. In large-scale, liquid-phase synthesis, the use of reactants in excess is a serious economical disadvantage. In addition, even when the internucleotide-bond formation reaction is carried out quantitatively, reactants used in excess result in the formation of by-products that must then be removed by troublesome operations. It is therefore important that an unnecessarily excess amount of reactants is avoided. However, existing methods for the construction of the internucleotide linkage generally employ excess amounts of reactants in order to obtain the target product in a high yield. For example, the standard phosphoramidite method[8] normally uses 2–4 equiv. of a phosphoramidite and a promoter toward a nucleoside.[9] When the internucleotide-bond formation is carried out using stoichiometric amounts of a phosphoramidite, a nucleoside, and a promoter, the yield of the target product is rather lowered. A reason for the low yield may be that moisture contained in the reaction solvent and reactants themselves decompose the phosphoramidite or/and its activated species. Accordingly, we examined the reaction of a nucleoside phosphoramidite and a nucleoside in the presence of molecular sieves (MS) 3A or 4A as the moisture scavenger and realized the high-yield condensation using stoichiometric amounts of the reactants and a promoter.[10]

2. Results and discussion

First, we carried out the condensation of a deoxyribonucleoside phosphoramidite and a nucleoside in the presence of MS and, as a control experiment, in the absence of MS. The reaction (30 min) of the nucleoside 3′-(allyl phosphoramidite) **4** and the **5′**-*O*-unsubstituted nucleoside **11** using 1*H*-tetrazole as the promoter in the presence of MS 3A, where **4**, **11**, and the promoter are used in 5.0 mmol each in a 0.1 M acetonitrile solution, and the subsequent oxidation by means of 2-butanone peroxide[11] or *tert*-butyl hydroperoxide (TBHP)[12] gave the dinucleoside phosphate **15** in a 99% yield [calculated on the basis of weight and the purity (^{31}P NMR assay) of the isolated product]. In contrast, the preparation without MS 3A gave **15** in an only 85% yield. In this case, the undesired formation of the *H*-phosphonate (ca. 15% yield), which results from hydrolysis of **4** and/or its activated species, was observed. Cyanoethyl phosphoramidites are also usable as the nucleoside phosphoramidite. The reaction using the cyanoethyl phosphoramidite generally requires a longer time for the completion. For example, 1*H*-tetrazole-promoted condensation of **8** and **11** by the use of stoichiometric amounts of reactants was completed in 60 min to give, after the oxidation, **19** in a

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* Corresponding author. Tel.: +81-52-789-4848; fax: +81-52-789-5646; e-mail: yoshi@info.human.nagoya-u.ac.jp

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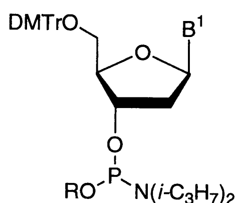
Table 1. Synthesis of dinucleoside phosphates

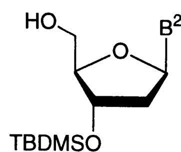
Amidite	Nucleoside	Promoter	Reaction time (min)	Product	Yield (%)[a]
1	**11**	*N*-PhIMT[b]	5	**12**	100, 99[c]
2	**9**	BIT	1	**13**	89
2	**9**	*N*-MeBIT	1	**13**	90
3	**9**	BIT	5	**14**	91
3	**9**	*N*-MeBIT	5	**14**	93
4	**11**	BIT	1	**15**	95, 91[c]
4	**11**	*N*-MeBIT	1	**15**	99, 96[c]
4	**11**	1*H*-tetrazole	30	**15**	99
4	**11**	BrDCI	1	**15**	94
4	**11**	PyTFB	5	**15**	95
5	**10**	BIT	3	**16**	83
5	**10**	*N*-MeBIT	3	**16**	80
6	**11**	*N*-PhIMT[b]	3	**17**	88
7	**11**	BIT	10	**18**	92
7	**11**	*N*-MeBIT	10	**18**	90
8	**11**	BIT	3	**19**	95
8	**11**	*N*-MeBIT	3	**19**	90
8	**11**	1*H*-tetrazole	60	**19**	93
20	**24**	BIT	15	**27**	87
20	**24**	*N*-MeBIT	10	**27**	91
20	**24**	ETT	90	**27**	90
21	**24**	*N*-PhIMT[b]	15	**28**	96
21	**26**	BIT	15	**29**	91
21	**26**	*N*-MeBIT	15	**29**	88
22	**25**	BIT	15	**30**	93
22	**25**	*N*-MeBIT	15	**30**	91
23	**26**	BIT	15	**31**	93
23	**26**	*N*-MeBIT	15	**31**	93

The preparation was carried out on a 0.1–5.0 mmol scale.
[a] Determined by ^{31}P NMR analysis of the crude product unless otherwise noted.
[b] These results have been reported in Ref. 10.
[c] Yield of the product isolated by chromatography. Purity of the isolated product is generally >98%.

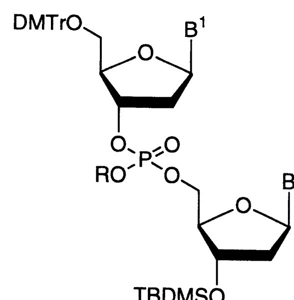
93% yield. In the synthesis using the O^6-unprotected guanosine derivative **7** or **10** as a building block, there are cases that the desired product is obtained in a somewhat low yield. In such cases, the *H*-phosphonate and the phosphoramidate, which arise from hydrolysis and oxidation, respectively, of **7** or **10**, were obtained as by-products. While, any derivatives with the guanine-O^6-modified structure was not detected. This fact that the guanine-O^6-modification was not caused is an advantage of the present method using a stoichiometric amount of reactants. As the promoter, a variety of compounds can be used, which include 2-(bromo)-4,5-(dicyano)imidazole (BrDCI),[13] pyridinium tetrafluoroborate (PyTFB),[14] 5-(ethylthio)-1*H*-tetrazole (ETT),[15] and azolium salts[10] such as *N*-(phenyl)imidazolium triflate (*N*-PhIMT), *N*-(*p*-acetylphenyl)imidazolium triflate (*N*-AcPhIMT), benzimidazolium triflate (BIT),[16] and *N*-(methyl)benzimidazolium triflate (*N*-MeBIT). These promoters have higher reactivity than 1*H*-tetrazole to complete the condensation more rapidly. For example, *N*-PhIMT accomplished the reaction of **4** and **11** in 1 min and the subsequent 2-butanone peroxide oxidation provided **15** in a 99% isolated yield. In this case, in the absence of MS 3A, the yield of the coupling product (^{31}P NMR analysis)



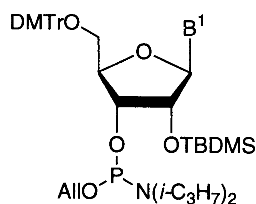
1, B^1 = Ade^{AOC}; R = All
2, B^1 = Cyt^{AOC}; R = All
3, B^1 = $Gua^{All,AOC}$; R = All
4, B^1 = Thy; R = All
5, B^1 = Ade^{Bz}; R = $NCCH_2CH_2$
6, B^1 = Cyt^{Ac}; R = $NCCH_2CH_2$
7, B^1 = Gua^{ibu}; R = $NCCH_2CH_2$
8, B^1 = Thy; R = $NCCH_2CH_2$

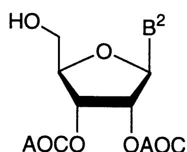


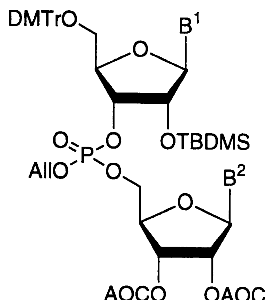
9, B^2 = Ade^{AOC}
10, B^2 = Gua^{ibu}
11, B^2 = Thy



12, B^1 = Ade^{AOC}; B^2 = Thy; R = All
13, B^1 = Cyt^{AOC}; B^2 = Ade^{AOC}; R = All
14, B^1 = $Gua^{All,AOC}$; B^2 = Ade^{AOC}; R = All
15, B^1 = Thy; B^2 = Thy; R = All
16, B^1 = Ade^{Bz}; B^2 = Gua^{ibu}; R = $NCCH_2CH_2$
17, B^1 = Cyt^{Ac}; B^2 = Thy; R = $NCCH_2CH_2$
18, B^1 = Gua^{ibu}; B^2 = Thy; R = $NCCH_2CH_2$
19, B^1 = Thy; B^2 = Thy; R = $NCCH_2CH_2$







was 79% and the undesired *H*-phosphonate was formed in a 21% yield. The present procedure is also effective for the condensation of a ribonucleoside 3′-phosphoramidite and a 5′-*O*-free ribonucleoside in stoichiometric use of the reactants and a promoter. In this case, a longer time, usually 10–15 min, is required for completing the condensation. Several examples of the preparation of dinucleoside phosphates via the present method are listed in Table 1. In all cases described above, the use of MS 4A in place of MS 3A as the moisture scavenger gave similar results.

3. Conclusion

We developed a highly efficient phosphoramidite method for the liquid-phase synthesis of short-length nucleotides, which provides the desired product in a high yield by the use of stoichiometric amounts of reactants and a promoter in the presence of MS 3A or 4A. Compared with existing methods requiring an excess of reactants and the promoter, the present approach is advantageous in regard to its relatively low cost and operational simplicity of product isolation. As previously reported,[10] this strategy was applied to the synthesis of CMP-Neu5Ac and 2–5A core.

4. Experimental

4.1. Materials

N-(Phenyl)imidazole (Aldrich), benzimidazole (Nacalai Tesque), *N*-(methyl)benzimidazole (Wako), trifluoromethanesulfonic acid (Central Glass), N^6-(benzoyl)-5′-*O*-(*p*′-dimethoxytrityl)-2′-deoxyadenosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**5**) (Glen Research), N^4-(acetyl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxycytidine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**6**) (Glen Research), N^2-(isobutyryl)-5′-*O*-(*p*,*p*′-dimethoxytrityl)-2′-deoxyguanosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**7**) (Glen Research),5′-*O*-(*p*,*p*′-dimethoxytrityl)thymidine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (**8**) (Glen Research), 2-butanone peroxide as a 55% solution in dimethyl phthalate (Kishida) were commercially supplied. The following compounds and a solution are reported in literature or prepared by reported methods. Azolium salts: *N*-(phenyl)imidazolium triflate,[10] benzimidazolium triflate,[16] and *N*-(methyl)benzimidazolium triflate;[10] protected 2′-deoxyribonucleoside 3′-phosphoramidites: **1**,[17,18] **2**,[17,18] **3**,[17,18] and **4**;[17] 3′-*O*-protected 2′-deoxyribonucleosides: **9**,[19] **10**,[20] and 11;[21] protected ribonucleoside 3′-phosphoramidites: **20**,[18] **21**,[22] **22**,[18] and **23**;[10] 2′,3′-*O*-protected ribonucleosides: **24**,[23] **25**,[23] and **26**;[23] a 1.0 M *tert*-butyl hydroperoxide/toluene solution.[12,24] Acetonitrile was distilled from CaH_2. Powdery molecular sieves 3A and 4A were used after drying the commercially supplied one (Nacalai tesque) at 200°C for 12 h. Nacalai tesque silica gel 60 (neutrality, 75 μm) was used for column chromatography.

4.2. A general procedure for the preparation of dinucleoside phosphates

A heterogeneous mixture of a nucleoside phosphoramidite (5.0 mmol), a nucleoside (5.0 mmol), a promoter (5.0 mmol), and molecular sieves 3A or 4A (ca. 1 g) in acetonitrile (50 mL) was stirred at 25°C. The reaction was monitored by TLC. After completing the reaction, to the mixture was added a 6.7% 2-butanone peroxide/dimethyl phthalate-toluene solution (10 mL) or a 1.0 M TBHP/toluene solution (10 mL) and stirring was continued for an additional 5 min. [The 'product yield determined by ^{31}P NMR analysis' shown in Table 1 was obtained in this stage according to the following procedure. To the reaction mixture was added a suitable amount of triphenylphosphine oxide. An aliquot of the mixture was taken and subjected to the ^{31}P NMR measurement, in which the irradiation mode was set to NOE-Eliminated ^{1}H Complete Decoupling Measurement (NNE mode). Comparison of intensity of signals due to the dinucleoside phosphate due to triphenylphosphine oxide gave the yield of the target product.] The reaction mixture was diluted with ethyl acetate (200 mL) and the molecular sieves were removed by filtration. The filtrate was washed with an aqueous solution saturated with $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried and concentrated. The resulting crude material was dissolved in ethyl acetate (20 mL) and poured to a vigorously stirred petroleum ether (500 mL) to precipitate a target dinucleoside phosphate as colorless power in an almost quantitative amount.

4.3. Determination of structure and purity of the product

All dinucleoside phosphates listed in Table 1 are known.[10] Structure of the product was confirmed by comparison of its ^{1}H NMR, ^{31}P NMR, and TOF mass spectral data with those

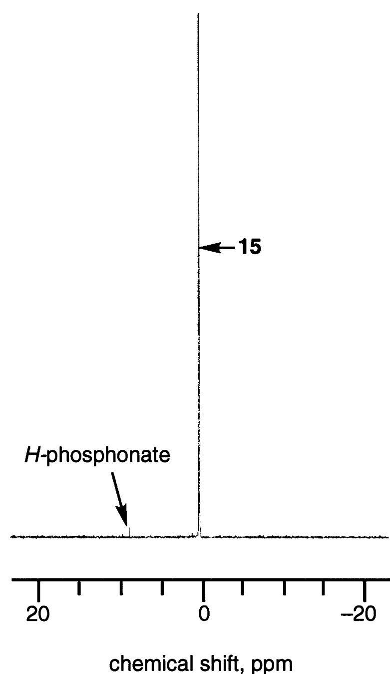


Figure 1. The ^{31}P NMR spectrum of the crude isolated product of **15** prepared by the method using 1*H*-tetrazole as a promoter.

of an authentic sample. Purity of the product obtained as above (the crude isolated product) was determined by the NNE-mode ^{31}P NMR analysis. For example, the ^{31}P NMR spectrum (Fig. 1) of **15** prepared by the procedure using 1*H*-tetrazole as a promoter indicated that the crude isolated product includes ca. 99% of the desired compound and ca. 1% of the undesired *H*-phosphonate arising from the starting phosphoramidite **4**. In some cases, the product was contaminated by not only the *H*-phosphonate but also a phosphoramidate resulting from oxidation of the unreacted phosphoramidite. These contaminants were removed by chromatography on a short silica gel column with a 1:10:10 methanol/ethyl acetate/hexane mixture as an eluent.

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