Synlett K. Seio et al. Letter

A Systematic Study of the Synthesis of 2′-Deoxynucleosides by Mitsunobu Reaction

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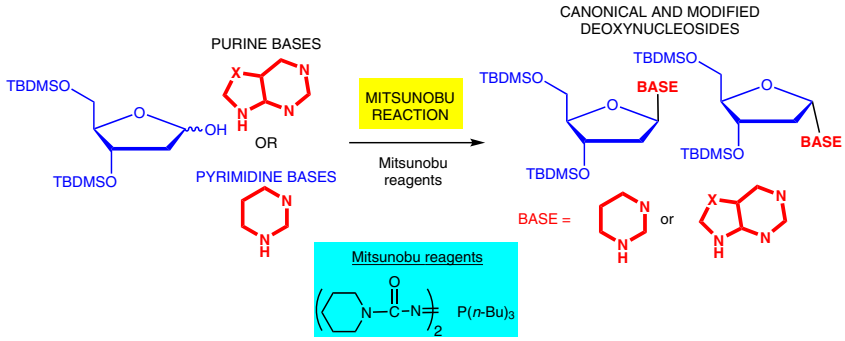


Received: 30.03.2017
Accepted after revision: 09.05.2017
Published online: 07.06.2017
DOI: 10.1055/s-0036-1588445; Art ID: st-2017-u0217-l

Abstract The Mitsunobu reaction has emerged as an important alternative for the preparation of synthetic 2′-deoxynucleosides, which have various biological and biotechnological applications. In this work, the Mitsunobu-based synthesis of 2′-deoxynucleosides was systematically studied. The effect of phosphine, azodicarbonyl reagent, and solvent on the product yield and α/β ratio was investigated, and the highest yield and β-selectivity were obtained using $(n\text{-Bu})_3$P and 1,1′-(azodicarbonyl)dipiperidine in DMF. The reaction was successfully applied to various nucleobase analogues.

Key words nucleoside synthesis, Mitsunobu reaction, N-glycosidation, stereoselectivity, solvent effects

Synthetic 2′-deoxynucleosides have been widely used as pharmaceutical agents[1] because of their various biological activities such as antiviral, antifungal, and anticancer properties. More recently, they have also found application as precursors for the synthesis of functional nucleic acids such as fluorescent nucleic acid probes[2] and chemically modified genes.[3] In addition to β-nucleosides, which have the same C1′ configuration of standard deoxynucleosides, the α-isomers are also useful for biotechnological applications such as the synthesis of triplex-forming oligonucleotides.[4] Therefore, methods for the preparation of deoxynucleosides are important for the development of nucleoside- and oligonucleotide-based technologies. Deoxynucleosides are typically synthesized by N-glycosylation of a nucleobase with a 2′-deoxyribose donor, which is activated for nucleophilic attack on the anomeric position. The most widely used 2′-deoxyribose donor is α-1-chloro-3,5-di-*O*-toluoyl-deoxyribose,[4] which reacts with nucleobases activated by an inorganic base,[5,2b] such as NaH, or persilylation[6] to obtain the desired nucleosides. However, this method is not applicable to nucleobases that are not activated by persilylation or are sensitive to basic conditions. Thus the Mitsunobu reaction has emerged as a promising alternative for the synthesis of nucleosides. For example, Szarek[7] reported the condensation of aldo- or ketohexoses with 6-chloropurine, and Chang[8] described the preparation of 2′-*C*-methyl-nucleoside analogues by Mitsunobu reaction. In addition, Hocek and co-workers[9] reported a systematic study of the Mitsunobu-based synthesis of ribonucleosides.[8] More recently, we have reported the synthesis of fully protected 7-deaza-6-*O*-(diphenylcarbamoyl)-7-iodo-2-*N*-acetyl-2′-deoxyguanosine[2a] (**1β** in Figure 1) by a Mitsunobu reaction between 3,5-*O*-bis(*tert*-butyldimethylsilyl)-2-deoxyribose (**2**)[10] and 7-deaza-6-*O*-(diphenylcarbamoyl)-7-iodo-2-*N*-acetyl-guanine.[10] Compound **1β** was devised as a synthon of 7-deaza-7-iodo-2′-deoxyguanosine having higher solubility and reactivity in palladium-catalyzed cross-coupling reactions for further functionalization.[2a] In our previous work, the Mitsunobu reaction proved to be the best method for the incorporation of the nucleobase containing the base-labile diphenylcarbamoyl group.

To the best of our knowledge, there has not yet been a systematic study of the Mitsunobu synthesis of 2′-deoxynucleosides. Thus, in this Letter, the use of the Mitsunobu reaction for the synthesis of deoxynucleosides was systematically investigated.

At first, the reaction between N^3-benzoylthymine (**3**) and **2** in the presence of Ph_3P and diisopropyl azodicarboxylate (DIAD) was studied in various solvents (Table 1, entries 1–5). When THF was used, a mixture of thymidine derivatives **4α** and **4β** was obtained in 50% yield and in a ratio of 47:53, respectively, as determined by ^{1}H NMR spectroscopy (Table 1, entry 1), revealing a slight β-selectivity. On the other hand, in acetonitrile (Table 1, entry 2) and dichloromethane (Table 1, entry 3), the yield was 42% and 57%, respectively, and the α-anomer selectivity increased,

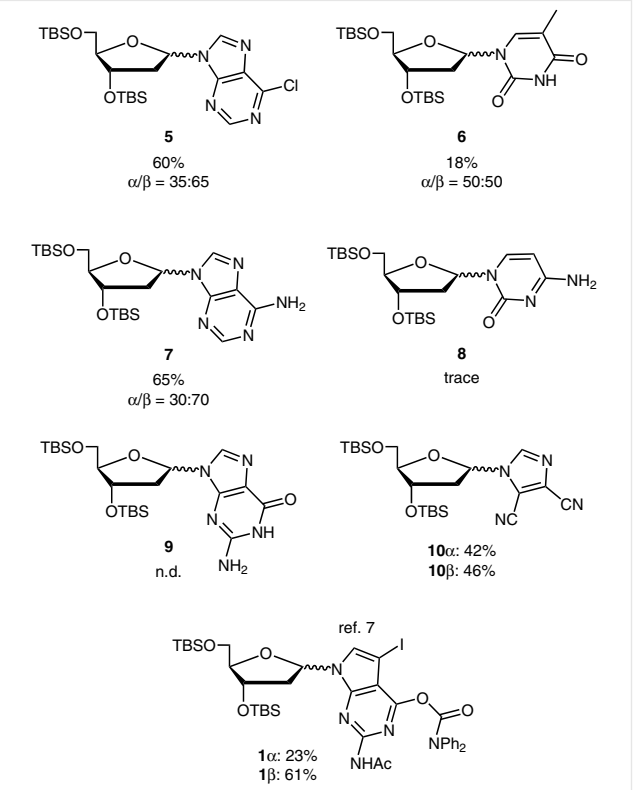


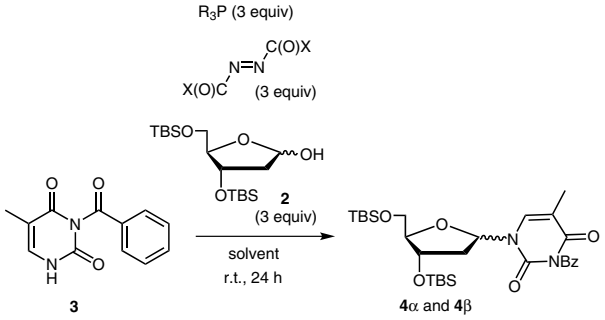
Figure 1 Application of the Mitsunobu reaction to the synthesis of various deoxynucleosides

leading to a **4α**/**4β** ratio of 59:41 and 61:39, respectively. Interestingly, when the amide-type solvents DMF (Table 1, entry 4) and DMA (Table 1, entry 5) were used, the yield increased to 79% and 62%, and the ratio of **4α**/**4β** was 30:70 and 47:53, respectively. These results show that the α/β selectivity was dependent on the solvent used, and DMF was the best solvent in terms of both yield and β-selectivity.

Next, the 1,1′-(azodicarbonyl)dipiperidine (ADDP)-P(*n*-Bu)$_3$[11] reagent system was applied to our transformation. It has been reported that the nitrogen anion intermediate generated from ADDP is more basic than the corresponding anion generated from DIAD; thus, ADDP can activate nucleophiles with pK_a values >13, and is therefore expected to be useful for the reaction of a wider variety of synthetic nucleosides. As shown in Table 1, entry 6 the ADDP-P(*n*-Bu)$_3$ system in DMF gave the highest yield (95%) and high β-selectivity (**4α**/**4β** = 37:63). The reaction in DMA (Table 1, entry 7) gave the same yield, but no stereoselectivity was observed. On the other hand, when THF (Table 1, entry 8), 1,4-dioxane (Table 1, entry 9), acetonitrile (Table 1, entry 10), dichloromethane (Table 1, entry 11), toluene (Table 1, entry 12), or pyridine (Table 1, entry 13) was used, the reaction proceeded in lower yield and with α-selectivity.

In the following experiments, we investigated the effect of the phosphine structure. The reaction using Et$_2$PhP (Table 1, entry 14) produced **4α** and **4β** in almost the same yield and ratio as with *n*-Bu$_3$P (Table 1, entry 6). When a less basic phosphine, namely, MePh$_2$P (Table 1, entry 15) or Ph$_3$P (Table 1, entry 16), was used, the yield decreased but the α/β ratio did not change. In addition, Cy$_3$P (Table 1, entry 17) and (*o*-Tol)$_3$P (Table 1, entry 18) having large cone angles were not effective in this reaction. We can therefore conclude that the yield of the reaction was dependent on the nature of the phosphine whereas the α/β ratio was not affected.

Table 1 Effect of Phosphine, Azodicarbonyl Reagent, and Solvent on the Product Yield and α/β Ratio in the Mitsunobu-Based Deoxynucleoside Synthesis

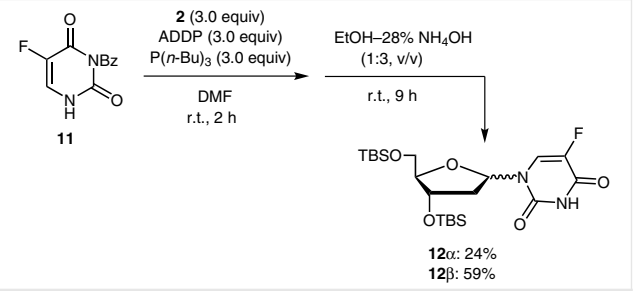


	Solvent	X	R^3	Yield (%)[a]	**4α**/**4β**[b]
1	THF	isopropyloxy	Ph_3	50	47:53
2	MeCN	isopropyloxy	Ph_3	42	59:41
3	CH_2Cl_2	isopropyloxy	Ph_3	57	61:39
4	DMF	isopropyloxy	Ph_3	79	30:70
5	DMA	isopropyloxy	Ph_3	62	47:53
6	DMF	piperidin-1-yl	*n*-Bu_3	95	37:63
7	DMA	piperidin-1-yl	*n*-Bu_3	95	50:50
8	THF	piperidin-1-yl	*n*-Bu_3	25	61:39
9	1,4-dioxane	piperidin-1-yl	*n*-Bu_3	6	66:34
10	MeCN	piperidin-1-yl	*n*-Bu_3	61	58:42
11	CH_2Cl_2	piperidin-1-yl	*n*-Bu_3	13	63:37
12	toluene	piperidin-1-yl	*n*-Bu_3	8	70:30
13	pyridine	piperidin-1-yl	*n*-Bu_3	9	54:46
14	DMF	piperidin-1-yl	Et_2Ph	94	38:62
15	DMF	piperidin-1-yl	$MePh_2$	87	32:68
16	DMF	piperidin-1-yl	Ph_3	34	32:68
17	DMF	piperidin-1-yl	Cy_3	n.d.[c]	–
18	DMF	piperidin-1-yl	*o*-Tol_3	n.d.[c]	–

[a] Isolated yield of the mixture of **4α** and **4β**.
[b] Determined by ^{1}H NMR analysis.
[c] n.d. = not detected.

To test the applicability of the reaction using ADDP and *n*-Bu_3P in DMF, we synthesized various derivatives of standard deoxynucleosides, and the results are shown in Figure 1. The reaction of **2** and 6-chloropurine gave a 35:65 mixture of **5α** and **5β**[12] in 60% yield. The reaction with unprotected thymine gave **6**[13] in 18% yield with no stereoselectivity. Notably, a mixture of regioisomers in which the thymine and deoxyribose moieties were connected at different positions, such as N3, O2, and O4, was obtained, indicating that N1 was the most reactive but not the only nucleophilic position of thymine. On the other hand, the reaction of **2** with unprotected adenine gave a 30:70 mixture of **7α** and **7β**[14] in 65% yield, whereas the reaction with unprotected cytosine or guanine to give **8** or **9**, respectively, was not effective because of the low solubility of the nucleobases in DMF.

The developed procedure was also tested for the preparation of nonstandard deoxynucleosides, namely, previously reported **1**, **10** (Figure 1), and **12** (Scheme 1). Although the α and β isomers of above natural nucleoside derivatives could not be separated because of the similar physicochemical properties of them, the isomers of the below unnatural nucleosides could be separated by silica gel chromatography. The reaction of 4,5-dicyanoimidazole with **2** gave imidazole nucleoside **10**. Unlike in the case of standard deoxynucleoside derivatives, the isomers of **10** were separated by silica gel column chromatography to give **10α** and **10β** in 42% and 46% yield, respectively. As previously reported, the reaction with a protected 7-deazaguanine derivative gave **1α** and **1β** in 23% and 61%, respectively, after purification by silica gel column chromatography. The Mitsunobu reaction was also applicable to the synthesis of 5-fluorouridine derivative **12**. In this case, compound **2** was coupled with N^3-benzoyl-5-fluorouracil (**11**) to give the fully protected deoxynucleoside, which was treated with aqueous ammonia to afford **12α** and **12β** in 24% and 59% yield, respectively, after silica gel column chromatography.



Scheme 1 Preparation and separation of the α and β isomers of a 5-fluorodeoxyuridine derivative

A comparison between our deoxynucleoside synthesis and the Mitsunobu-based ribonucleoside synthesis reported by Hocek et al.[9] shows important differences in terms of results and conditions. First, in the ribonucleoside synthesis the best results were originally obtained using P(*n*-$Bu)_3$, DIAD, and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile.[9a] On the other hand, our reaction proceeded smoothly in the presence of P(*n*-$Bu)_3$ and ADDP in DMF without the need for a strong base such as DBU, because the zwitterion generated from the addition of ADDP and P(*n*-$Bu)_3$ is sufficiently basic to abstract the proton from the nucleobase. Moreover, recent study by Downey and co-workers[9b] reported the use of ADDP and P(*n*-$Bu)_3$ also gave selectively the β-anomers because of the formation of the 1′,2′-epoxyribose intermediate. In our deoxynucleoside synthesis, a mixture of α- and β-anomers was obtained in a ratio depending on the solvent and the nucleobase structure because of the lack of the 2′-hydroxy group which is necessary to form the epoxy intermediate. Thus, our method is useful for the preparation of separable α- and β-anomers, as in the case of compounds **1**, **10**, and **12**.

In this paper, we present a systematic study of the synthesis of deoxynucleosides by Mitsunobu reaction under mild conditions. The reaction generated both α and β isomers in a ratio dependent on the nucleobase structure and the solvent. In particular, the reactions in DMF (Table 1, entry 6) gave the highest yield and β-selectivity. This approach is expected to be applied to the synthesis of unnatural deoxynucleosides useful for the pharmaceutical and biological applications.

Funding Information

This work was supported by JSPS KAKENHI Grant Number 15H01062, 15K13738, and 26288075

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1588445.

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