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Letter

Selective Acetalization in Pyridine: A Sustainable 5′-*O*-(2-Methoxypropyl) Protecting Group in the Synthesis of Nucleic Acid Analogs

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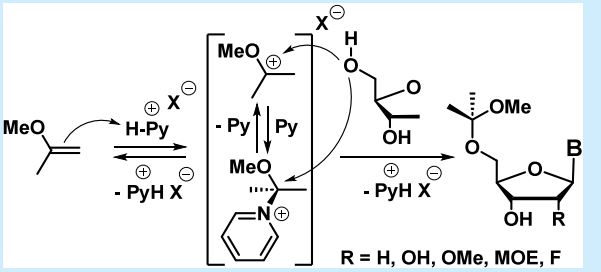
ABSTRACT: A mixture of 2-methoxypropene and an acid catalyst in pyridine results in an efficient 5′-*O*-(methoxyisopropyl) (MIP) acetalization of nucleosides, including 2′-deoxy, 2′-OH, 2′-*O*-methyl, 2′-*O*-methoxyethyl (MOE) and 2′-F-variants, in 44−77% isolated yields. For the reaction mechanism, we propose a pyridinium 2-methoxyprop-2-yl preassociation complex, which improves regioselectivity for the primary (5′-OH) over secondary (2′-OH and 3′-OH) hydroxy groups. The developed protocol makes the 5′-*O*-MIP-acetal an attractive protecting group for the sustainable synthesis of nucleosides and oligonucleotides in solution.



Acetals are important acid-labile protecting groups in nucleic acid chemistry. For example, 2′,3′-*O*,*O*-isopropylidene and 2′-*O*-tetrahydropyranyl (THP) protected ribonucleosides are common key intermediates for various nucleic acid products.[1,2] Recently, acetone acetals (2-methoxyisopropyl = MIP and 2-isopropoxyprop-2-yl = IIP) have received interest as alternative protecting groups[3] for the 5′-*O*-(4,4′-dimethoxytrityl) (DMTr) group in liquid-phase oligonucleotide synthesis (LPOS).[4−8] The facile irreversible acid-catalyzed removal of the 5′-*O*-(2-alkoxypropyl) group and the released volatile byproducts acetone and methanol or isopropanol can reduce depurination and facilitate purification and isolation of growing oligonucleotide products in LPOS-compatible workups: extraction,[9−11] precipitation,[12−16] and organic solvent nanofiltration (OSN).[17−19] This effort has culminated in sustainable oligonucleotide synthesis.[20]

The current choice of preparation of 5′-*O*-(2-alkoxyprop-2-yl) protected nucleoside building blocks consists of, however, a multistep synthesis via 3′-*O*-silyl protected intermediates,[4−8] which is both time and reagent consuming. Careful stoichiometric control of reagents can increase regioselectivity of the acetalization,[21] but the direct acid-catalyzed reaction between nucleosides and 2-alkoxypropene or 2,2-dialkoxypropane often result in a complex mixture of products, including 5′-*O*-, 3′-*O*-, and bis-3′,5′-*O*-(2-alkoxyprop-2-yl) protected nucleosides and cyclic acetal-bridged dinucleosides (Scheme 1). To overcome this challenge, herein, we present an efficient and regioselective acetalization in pyridine expanding opportunities for the acetal-based protecting group manipulation of nucleosides and carbohydrates[22] and offer 5′-*O*-MIP-protected nucleosides as ideal building blocks for LPOS.[23−27]

We hypothesized that the required regioselectivity between the primary (5′-OH) and secondary (3′-OH) hydroxy groups in a nucleoside (**1**−**4**) could be achieved if the acetalization does not follow the standard acid-catalyzed mechanism. In the electrophilic addition between an alcohol and 2-methoxypropene, the first step of the reaction is protonation of the double bond, resulting in a stable oxocarbenium cation. Therefore, carbocation reacts with an alcohol that yields the acetal product, or it can react with a temporary nucleophile,[28] which then undergoes a leaving group-dependent SN1-replacement with an alcohol. By exposing 2-methoxypropene to an acid catalyst in the presence of pyridine (in the absence of alcohol), we could observe a 2-methoxy-prop-2-ylpyridinium (MIPPY) preassociation complex by NMR (^{1}H−^{15}N-HMBC spectrum shown in Figure 1, cf. additional NMR data in Figures S90−S109). The MIPPY complex could act as a potential intermediate of the acetalization and affect the regioselectivity. A similar preassociation complex has been suggested to contribute to selective removal of aldehyde acetals using triethylsilyltriflate-base combinations.[29]

The observations that protonation of 2-methoxypropene could occur under slightly basic conditions with the formation of a preassociation complex encouraged us to study reaction

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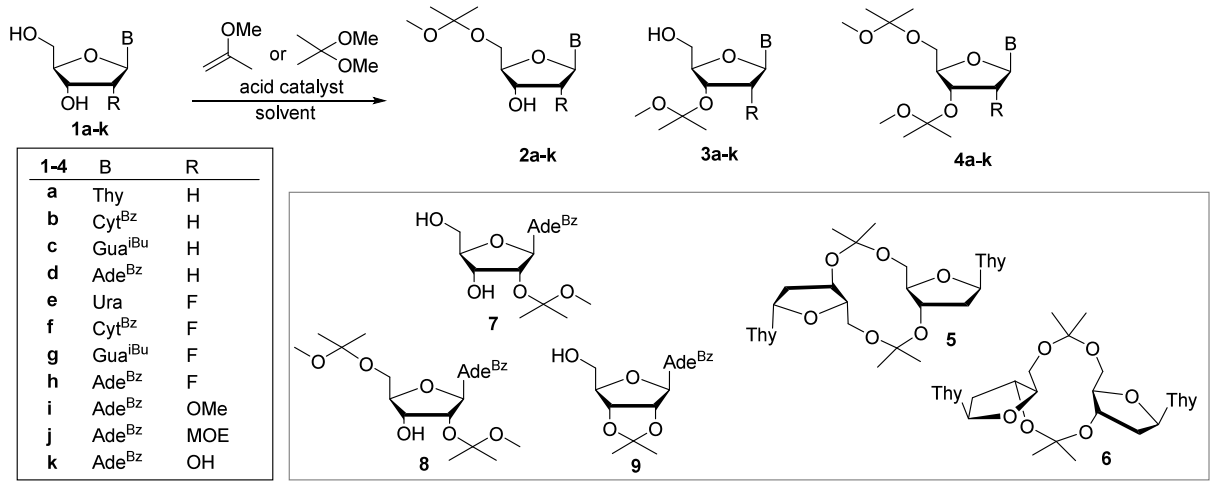
Scheme 1. Acetalization of Nucleosides with 2-Methoxypropene or 2,2-Dimethoxypropane



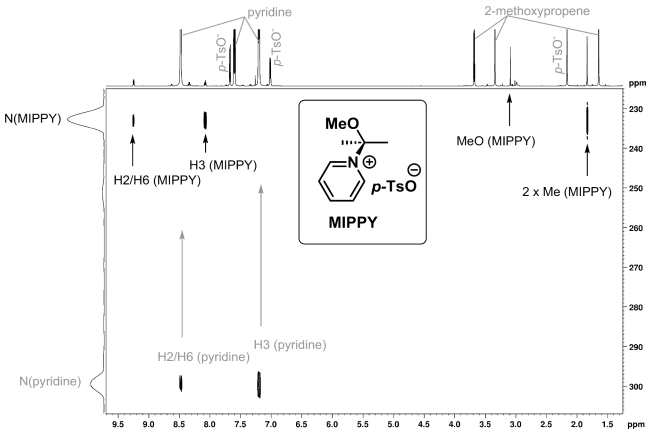


Figure 1. $^{1}H-^{15}N$-HMBC spectrum of the 2-methoxy-prop-2-ylpyridinium preassociation complex. Conditions: 2-methoxypropene, 0.31 mol L^{-1}; pyridine (4 equiv), $pyH^{+}pTsO^{-}$ (0.5 equiv), TMSCl (0.25 equiv. to remove residual water) in $CDCl_3$. Measured at 258 K.

parameters of the acetalization in different solvent/nucleophile environments in greater detail (Table 1). Thymidine (T, **1a**) was selected as a preliminary model diol. Reactions were performed in a 0.12 mmol scale (0.28 or 0.55 mol L^{-1} of **1a**) and monitored over 24 h by reversed phase HPLC (Figure 2). The reaction with 2-methoxypropene (2 equiv) in DMF in the presence of *p*-TsOH (0.1 equiv) at 25 °C (entry 1) led to a dynamic multicomponent mixture, consisting of cyclic isopropylidene-bridged dithymidines (**5** and **6**, existing as major products after 2 h, Figures S2 and S59−S62). Acetalization with 2-methoxypropene (1−4 equiv. 0.55−1.1 mol L^{-1}) in the presence of *p*-TsOH (0.1 equiv) in pyridine at 25 °C, in turn, resulted in a good regioselectivity between the primary (5′-OH) and secondary (3′-OH) hydroxy groups (**2a**:**3a**, ranging from 6:1 to 19:1, *n*/*n*) (entries 2−4). Formation of 5′,3′-*O*,*O*-bis-MIP-T (**4a**) but not **5** and **6** was also observed in each case. Decreasing temperature from 25 to 4 °C led to a slow reaction, but no marked improvement in selectivity was found (entry 5, a higher excess and concentration of 2-methoxypropene used). No marked difference in yields and regioselectivity of the acetalization was found when the *p*-TsOH·H_2O catalyst was replaced by pyridinium chloride, pyridinium tosylate, 2,6-lutidinium tosylate, or 2,4,6-collidinium tosylate (0.1 equiv each) (entries 7−9). Thus, the conjugate acid (HCl vs *p*-TsOH) or hydrate of *p*-TsOH did not interfere with the reaction. Polymer-bound pyridinium tosylate was also tested in various concentrations, but yields of 5′-*O*-acetalization remained modest (entry 10). In addition to pyridinium salts, tetrazole (0.1 equiv) as an acid catalyst (pK_a 4.9) was also examined. No product was observed in pyridine or in DMF (entries 11 and 12). DMAP (0.05 equiv) and *p*-methoxypyridine (0.1 equiv) as potential nucleophilic catalysts in pyridine did not affect the reaction (entries 13 and 14). Interestingly, reaction in 2,6-lutidine or in 2,4,6-collidine in the presence of their *p*-TsOH-salts (0.1 equiv) did not yield products (entries 15 and 16). This may be related to different pK_a values of the pyridine derivatives (2,4,6-collidine: 7.4; 2,6-lutidine: 6.7; pyridine: 5.3) but may also indicate that pyridine had a specific nucleophilic role in the reaction, referring to the hypothesized preassociation complex (similar complexation could not be observed with 2,6-lutidine or 2,4,6-collidine in corresponding NMR experiments, Figures S110−S119). The acid-catalyzed (*p*-TsOH·H_2O, 0.1 equiv) acetalization with 2-methoxypropene was performed also in DMF in the presence of pyridine, *p*-methoxypyridine (pK_a 6.5), 2,6-lutidine, and 2,4,6-collidine (2 equiv of each pyridine derivative). In the presence of pyridine, 5′-*O*-MIP-T (**2a**) was obtained in 28% yield, but no product was observed with other pyridine derivatives (entries 17−19).

Next, a more detailed NMR analysis of the acetalization was carried out (Figure 3). Thymidine was exposed to 2-methoxypropene (2 equiv) in the presence of *p*-TsOH·H_2O (0.1 equiv) in pyridine-d_5 at 25 °C in an NMR tube (0.55 mol L^{-1} of T). From the molar ratios of the products, relative formation rates of 85:15 for 5′-*O*-MIP-T (**2a**) and 3′-*O*-MIP-T (**3a**) were extracted from the beginning of the acetalization. The maximal accumulation of 5′-*O*-MIP-T (**2a**) was observed after 10 h (**2a**:**3a**, 12:1, *n*/*n*). Conversion of both 5′-*O*-MIP-T (**2a**) and 3′-*O*-MIP-T (**3a**) to 3′,5′-*O*,*O*-bis-MIP T (**4a**) was observed, when the acetalization progressed (cf. molar ratio of **2a**:**3a** during reaction in Figure S1). Hydrolysis of 2-methoxypropene to acetone and 2,2-dimethoxypropane can be seen in the reaction mixture.

The reversibility of the reaction and acidity of the catalyst can affect the regioselectivity of the acetalization. When 2-methoxypropene was replaced by 2,2-dimethoxypropane (1.1 mol L^{-1}, 2 equiv) in pyridine in the presence of *p*-TsOH·H_2O (0.1 equiv), a 15% conversion to 5′-*O*-MIP-T was observed.

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Table 1. Molar Ratios of the Products and Starting Materials under Variable Acetalization Conditions Monitored by RP-HPLC[a]

	reaction conditions				molar ratios of compounds/%				
entry	**1** (conc./mol L^{-1})	2-methoxypropene (conc./mol L^{-1})	acid catalyst (0.1 equiv) (+ additive)	solvent	**1**	**2**	**3**	**4**	**2**/**3**, *n*/*n*
1*	**a** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O	DMF	45	15	24	3	2:1
2	**a** (0.55)	1 eq (0.55)	*p*-TsOH·H_2O	Py	40	48	8	4	6:1
3	**a** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O	Py	1	51	3	45	17:1
4	**a** (0.28)	4 eq (1.1)	*p*-TsOH·H_2O	Py	1	56	3	40	19:1
5**	**a** (0.28)	8 eq (2.2)	*p*-TsOH·H_2O	Py	38	8	50	4	6:1
6	**a** (0.55)	2 eq (1.1)	PyH^+Cl^-	Py	5	60	6	29	10:1
7	**a** (0.55)	2 eq (1.1)	PyH^+*p*-TsO^-	Py	10	64	8	18	8:1
8	**a** (0.55)	2 eq (1.1)	$LutH^+$*p*-TsO^-	Py	4	63	6	27	11:1
9	**a** (0.55)	2 eq (1.1)	$CollH^+$*p*-TsO^-	Py	11	65	8	16	8:1
10	**a** (0.55)	2 eq (1.1)	PyH^+*p*-TsO^-(PS)	Py	84	13	3	0	4:1
11	**a** (0.55)	2 eq (1.1)	Tetrazole	Py	100	0	0	0	n.a.
12	**a** (0.55)	2 eq (1.1)	Tetrazole	DMF	100	0	0	0	n.a.
13	**a** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O + 0.05 eq DMAP	Py	11	65	8	16	8:1
14	**a** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O + 0.1 eq 4-MeOPy	Py	8	65	7	20	9:1
15	**a** (0.55)	2 eq (1.1)	$LutH^+TsO^-$	Lut	100	0	0	0	n/a
16	**a** (0.55)	2 eq (1.1)	$CollH^+TsO^-$	Coll	100	0	0	0	n.a.
17	**a** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O + 2 eq Py	DMF	66	28	5	1	6:1
18	**a** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O + 2 eq Lut	DMF	99	1	0	0	n.a.
19	**a** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O + 2 eq Coll	DMF	100	0	0	0	n.a.
20	**b** (0.28)	4 eq (1.1)	*p*-TsOH·H_2O	Py	6	64	7	23	9:1
21	**c** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O	Py	13	75	6	6	13:1
22	**d** (0.28)	4 eq (1.1)	*p*-TsOH·H_2O	Py	1	53	2	44	27:1
23	**e** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O	Py	5	83	3	9	28:1
24	**f** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O	Py	2	79	3	16	26:1
25	**g** (0.28)	4 eq (1.1)	*p*-TsOH·H_2O	Py	13	82	3	2	27:1
26	**h** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O	Py	0	90	0	10	1:0
27	**i** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O	Py	0	83	0	17	1:0
28	**j** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O	Py	0	85	3	12	28:1
29	**k** (0.55)	2 eq (1.1)	*p*-TsOH·H_2O	Py	3	62	4***	31****	n.a.

[a]In the reaction in entry 1, (*) cyclic isopropylidene-bridged dinucleosides **5** (12%) and **6** (1%) have been observed as byproducts. The reactions are performed at 25 °C, but for (**), the reaction in entry 5 is performed at 4 °C. In the reaction in entry 29, (***) sum (4%) of 2′-*O*-MIP- and 3′-*O*-MIP-A^{Bz} reported; (****) sum (31%) of 3′,5′-*O*,*O*-MIP- and 2′,5′-*O*,*O*-MIP-A^{Bz} reported. Py = pyridine, Lut = 2,6-lutidine, Coll = 2,4,6-collidine, 4-MeOPy = 4-methoxypyridine, and *p*-TsO^-(PS) = polystyrene-bound toluenesulfonic acid.

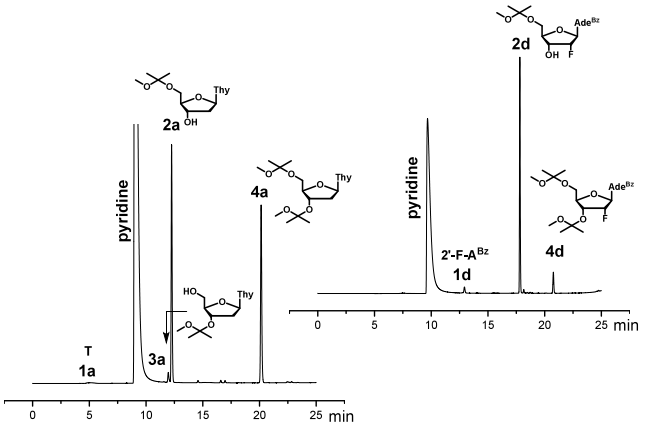

Figure 2. Examples of RP HPLC profiles of the acetalization. Reaction conditions: cf. entries 3 and 26 in Table 1.

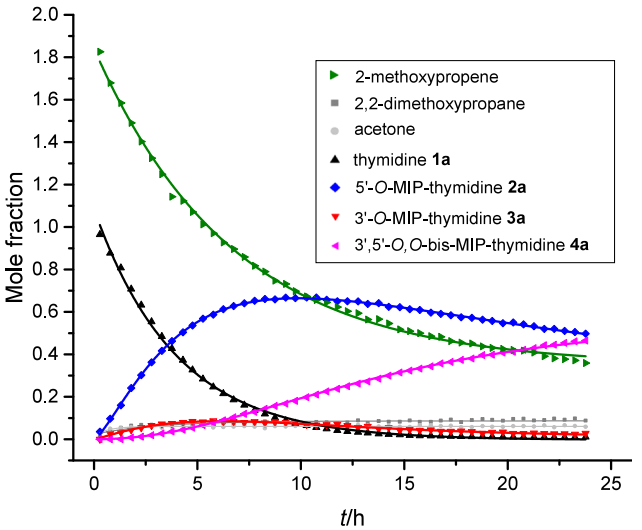

Figure 3. Time-dependent product distribution of acetalization of thymidine (0.55 mol L^{-1}) using 2 equiv of 2-methoxypropene (1.1 mol L^{-1}) and 0.1 equiv of *p*-TsOH·H_2O in pyridine-d_5 at 25 °C, monitored by ^1H NMR.

When isolated 5′-*O*-MIP-T (**2a**) was dissolved in pyridine in the presence of *p*-TsOH·H_2O (0.1 equiv) and methanol, slow degradation (16% over 24 h) to thymidine (**1a**) was observed. These observations demonstrate that protonation of acetal oxygens can, in fact, occur under the given conditions.

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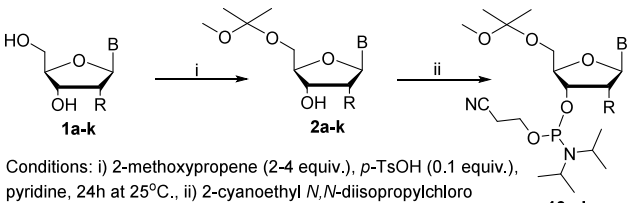
Hydrolysis products acetone and 2,2-dimethoxypropane can act as side reagents, affecting the regioselectivity.

The sugar puckering and potential electronic influence of the 2′-group of nucleosides can affect the product ratio.[30] Acetalization of N^4-benzoyl 2′-deoxyadenosine (dA^{Bz}, **1d**), N^4-benzoyl adenosine (A^{Bz}, **1k**), and 2′-*O*-modified variants, viz., N^4-benzoyl-2′-deoxy-2′-F (2′-F-A^{Bz}, **1h**), -2′-*O*-methyl (2′-OMe-A^{Bz}, **1i**), and -2′-*O*-methoxyethyl adenosine (2′-MOE-A^{Bz}, **1j**), was next examined due to their utility in therapeutically relevant oligonucleotides. For solubility reasons, a 0.28 mol L^{-1} initial concentration of dA^{Bz} (**1d**) was used, but otherwise, the reactions were performed in pyridine at 0.55 mol L^{-1} of the adenosine derivative and 1.1 mol L^{-1} 2-methoxypropene (4 equiv. compared to **1d** and 2 equiv. compared to **1h**–**k**) in the presence of *p*-TsOH·H_2O (0.1 equiv) (entries 22 and 26–29). After 24 h at 25 °C, the yields of 5′-*O*-acetalization varied from 53% to 90%, being the lowest (53%) with dA^{Bz} (**2d**) (entry 22) and the highest (90%) with 2′-F-A^{Bz} (**2h**) (entry 26). The high regioselectivity with **1h** and **1i** (**2**:**3**, 1:0, *n*/*n*) was notable. The higher yield (83–90%) of 5′-*O*-MIP-2′-modified adenosines (**2h**–**j**) in comparison to that of 5′-*O*-MIP-dA^{Bz} (**2d**) may be attributed to the favored *N*-conformation of ribose, which increases the reactivity of the 5′-OH vs 3′-OH groups.[30] Interestingly, 5′-*O*-MIP-A^{Bz} (**2k**) was obtained in 62% yield (entry 29). 3′-*O*-MIP (**3k**), 2′-*O*-MIP (**7**), and bis-MIP-A^{Bz} (**4k** and **8**), but not 2′,3′-*O*,*O*-isopropylidene A^{Bz} (**9**), were detected, despite the potential protonation of the methoxy oxygen of the 2′/3′-*O*-MIP group (**3k** and **7**) and subsequent nucleophilic attack of the neighboring OH group to the acetal carbon.

Acetalization of the other nucleobase variants of 2′-deoxyribonucleosides (dC^{Bz} and dG^{iBu}) and 2′-deoxy-2′-F-ribonucleosides (2′-F-U, 2′-F-dC^{Bz}, and 2′-F-dG^{iBu}) was examined with the same conditions (entries 20, 21, 23–25). In general, higher yields were obtained for 5′-*O*-MIP-2′-deoxy-2′-F-ribonucleotides **2e**–**h** (79–90%) than for 5′-*O*-MIP-2′-deoxyribonucleotides **2a**–**d** (58–75%). The base protecting groups did not influence the outcome of the acetalization reactions studied.

After the small-scale studies, a gram-scale synthesis of various 5′-*O*-MIP-protected nucleosides was performed (Scheme 2). Accordingly, each of the aforementioned nucleosides were treated with 2-methoxypropene (2–4 equiv) in the presence of *p*-toluenesulfonic acid (0.1 equiv) in pyridine at 25 °C (**S12**–**S44**). The 5′-*O*-MIP products (**2a**–**k**), as well as 3′-*O*-MIP and 3′,5′-*O*,*O*-bis-MIP side products, were isolated and characterized by ^{1}H and ^{13}C NMR (Figures S17–S66) and HRMS spectroscopy. 5′-*O*-MIP-dA^{Bz}, 5′-*O*-MIP-dG^{iBu}, and 5′-*O*-MIP protected 2′-modified nucleosides **6c**–**k** were purified by silica gel column chromatography in 64–77% yields (Scheme 2). 5′-*O*-MIP-T (**2a**) and 5′-*O*-MIP-dC^{Bz} (**2b**) were purified by precipitation in a mixture of EtOAc and hexane (1:4, *v*/*v*) in 44% and 47% yields, respectively. When precipitation was used for purification, partial overacetalization of nucleosides to 3′,5′-*O*,*O*-bis-MIP-products (**4a** and **4b**) was preferred to consume the starting material (**1a** and **1b**); otherwise, they were precipitated with the desired 5′-*O*-MIP product (**2a** and **2b**) (Figure S3). The 5′-*O*-MIP protected nucleosides were also phosphorylated via a conventional protocol to give the corresponding 3′-*O*-phosphoramidites, useful building blocks for LPOS,[4−8] in 71–93% yields (Scheme 2, Figures S45–SS60).

Scheme 2. Synthesis of 5′-*O*-MIP-Protected Phosphoramidite Building Blocks of Nucleosides



Conditions: i) 2-methoxypropene (2-4 equiv.), *p*-TsOH (0.1 equiv.), pyridine, 24h at 25°C., ii) 2-cyanoethyl *N,N*-diisopropylchloro phosphoramidite (1.2 equiv.), Et_3N (1.4 equiv.), 3-5h at 25°C.

1,2,10	B	R	Isolated yields of **2a-k**/%	Isolated yields of **10a-j**/%
a	Thy	H	44	78
b	Cyt^{Bz}	H	47	93
c	Gua^{iBu}	H	75	86
d	Ade^{Bz}	H	72	83
e	Ura	F	76	85
f	Cyt^{Bz}	F	77	80
g	Gua^{iBu}	F	69	78
h	Ade^{Bz}	F	64	71
i	Ade^{Bz}	OMe	71	81
j	Ade^{Bz}	MOE	77	74
k	Ade^{Bz}	OH	50	n.a.

In conclusion, for the first time, a regioselective 5′-*O*-(2-methoxyprop-2-yl) (MIP) acetalization of nucleosides using 2-methoxypropene in pyridine in the presence of an acid catalyst is described. Pyridine (but not its methylated analogs 2,6-lutidine and 2,4,6-collidine) was observed to have an important role in the reaction, presumably via formation of the pyridinium 2-methoxyprop-2-yl preassociation complex, which improves regioselectivity of the acetalization reaction. This facile protection protocol is expected to allow large-scale production of 5′-*O*-MIP-protected nucleosides useful for liquid-phase oligonucleotide synthesis (LPOS). The demonstrated ability of regioselective 5′-*O*-MIP protection, combined with its traceless removal, resulting in volatile byproducts acetone and methanol, may also open new opportunities for synthetic design of novel nucleosides and carbohydrates in a broader area of complex chemistry. Further understanding of the reaction mechanism, acetalization with different sugar configurations, and further scalability of the reaction are planned as next steps leading to a more sustainable manufacturing of therapeutic oligonucleotides.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.5c02400.

Experimental details, HPLC chromatograms, and NMR spectra of the compounds presented in this article (PDF)

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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