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A mild, efficient and regioselective enzymatic procedure for 5′-*O*-benzoylation of 2′-deoxynucleosides

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Abstract—Lipase from *Candida antarctica* B catalyzes the selective monobenzoylation at the 5′-hydroxyl group of 2′-deoxynucleosides using vinyl benzoate as acyl transfer reagent in quantitative yields. The industrial suitability of this process via the reclaim and reuse of enzyme and vinyl benzoate has been demonstrated.

Antisense oligonucleotides constitute a promising class of chemotherapeutic agents, which enable selective inhibition of gene expression.[1] As modified oligonucleotides have become a major field of investigation for chemists, methods for their suitable protection/deprotection for the synthesis of nucleoside monomers have become equally important. Benzoylation remains one of the most frequently used methods for the protection of hydroxyl and amino functions in nucleoside and nucleotide chemistry.[2] The selective manipulation of hydroxyl groups over amino groups of nucleobases is an important reaction in oligonucleotide synthesis. The classical method of benzoylation of the hydroxyl group in nucleosides with benzoyl chloride or benzoic anhydride provides nonselective reactions. Other mild benzoylating reagents such as benzotetrazole,[3] benzotriazole,[4] and benzoyl cyanide[5] are reported for this purpose. However, lower selectivity has been observed toward the acylation of different hydroxyl functions. Caddick et al.[6] reported the selective protection of primary hydroxyl groups using microwave irradiation. The method worked with simple diols containing a primary and a secondary hydroxyl group, but was unsuccessful in complex molecules such as glucopyranosides. Recently, the application of ionic liquids (IL) in nucleoside chemistry has been investigated.[7] Although IL are an excellent reaction medium compared to conventional solvents used in nucleoside chemistry, no selectivity has been observed and the acylation reaction yielded a mixture of products.

The selective benzoylation of the 5′-hydroxyl group of thymidine has been reported by Mitsunobu et al.[8] This reaction required the use of hexamethylphosphoric triamide, which is toxic, and the by-product triphenylphosphine oxide can be difficult to remove from the products. An alternative synthesis was proposed by Ishido and co-workers, and involved the careful dropwise addition (several hours) of a dilute solution of benzoyl chloride in pyridine to 2′-deoxynucleosides.[9] Later, Sindona[10] reported the regioselective acylation of 2′-deoxynucleosides with carboxylic acids after activation with *N*,*N*-bis-(2-oxo-oxazolidin-3-yl)phosphorodiamidic chloride, a noncommercial reagent. Moderate yields of the 5′-*O*-benzoyl derivatives were obtained after column chromatography.

Enzyme-catalyzed esterification is an alternative to classical synthetic methods that can overcome these problems. The potential of enzymes in organic synthesis is well recognized, in particular when the substrates possess several functional groups of similar reactivity.[11] The chiral nature of enzymes allow selective acylation and/or deacylation processes on a variety of molecules avoiding additional chemical protection/deprotection steps. Recently, Santaniello and co-workers[12] reported the selective benzoylation of diols catalyzed by a suitable lipase.

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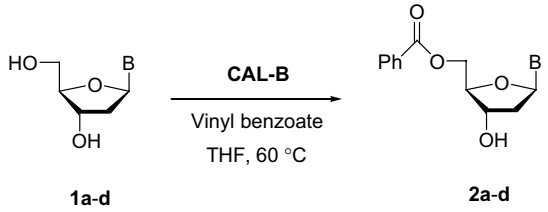
In our ongoing research related to the preparation of appropriate building blocks for solution phase oligonucleotide synthesis, we have developed the synthesis of 3′-and 5′-*O*-levulinyl nucleoside derivatives by enzymatic hydrolysis or acylation processes.[13] Previously, we have described the selective enzymatic acylation of thymidine and 2′-deoxyadenosine with oxime esters.[14]

The application of acetone oxime benzoate as an acylating agent for other nucleosides furnished moderate yields with slow reaction rates. Herein, we report a mild and efficient procedure for the selective benzoylation of 2′-deoxynucleosides through direct enzymatic acylation with vinyl benzoate, a commercially available reagent.

We selected *Candida antarctica* lipase B[15] (CAL-B), an immobilized enzyme, due to its well-demonstrated selectivity in the transesterification of the 5′-hydroxyl group and ability to be recycled.[13,14] Therefore, thymidine (**1a**) was dissolved in dry THF under nitrogen and treated with vinyl benzoate and CAL-B. The reaction was almost complete in 71 h at 60 °C (Scheme 1). Regioselective acylation of the 5′-OH group of **1a** was observed furnishing 5′-*O*-benzoylthymidine (**2a**),[8] (entry 1, Table 1) with small (13%) amounts of the starting material. The same process was repeated with nucleosides **1b**–**d**. The CAL-B maintained the regioselectivity although low conversions were achieved for cytidine and guanosine derivatives (entries 2–4, Table 1). The benzoylation of the 3′-OH group or the amino group on the base moiety was not observed indicating the exclusive selectivity toward the 5′-position. No acylation occurred under identical reaction conditions when CAL-B was left out. All reactions were carried out with 3 equiv of vinyl benzoate, a substrate/enzyme ratio of 1:1 (w/w), and at 0.2 M concentration. In order to reach conversions close to 100%, optimum ratios of acylating agent and enzyme were established. Thus, acylation of **1a** with 5 equiv of vinyl benzoate furnished **2a** in quantitative yield (isolated yield 98%, entry 6, Table 1). Importantly, pure **2a** was isolated via precipitation without a need for column chromatographic purification. The excess of vinyl benzoate was recaptured by solvent evaporation under vacuum from the mother liquor and recycled to minimize the waste.



1a-d **2a-d**

Scheme 1. a: B = T; b: B = C^{Bz}; c: B = A^{Bz}; d: B = G^{ibu} (ibu = isobutyryl).

To demonstrate the suitability of the reaction for industrial applications, both the acylating agent and the

Table 1. Regioselective enzymatic acylation of 2′-deoxynucleosides **1** using vinyl benzoate (VB) with CAL-B at 60 °C

Entry	Substrate	VB (eq)	Ratio (**1**:CAL-B)[a]	Conc. (M)	*t* (h)	**1** (%)[b]	**2** (%)[b,c]
1	**1a**	3	1:1	0.2	71	13	87 (62)
2	**1b**	3	1:1	0.2	98	74	26
3	**1c**	3	1:1	0.2	40	2	98 (93)
4	**1d**	3	1:1	0.2	96	76	24
5	**1a**[d]	3	1:1	0.2	118	1	99 (92)
6	**1a**[d]	5	1:1	0.2	72	ND	>99 (98)
7	**1a**[d]	5	1:1[e]	0.2	144	ND	>99 (96)
8	**1a**	5[f]	1:1	0.2	72	1	99 (95)
9	**1b**[d]	10	1:1	0.1	40	ND	>99 (95)
10	**1c**[d]	3	1:1	0.2	23	1	99 (95)
11	**1c**[d]	3	1:1[g]	0.2	72	1	99 (93)
12	**1c**[d]	3	1:1[h]	0.2	121	3	97 (90)
13	**1d**[d]	5	1:1	0.1	120	51	49
14	**1d**[d]	10	1:1	0.1	215	ND	99 (95)
15	**1d**[d]	10	1:2	0.1	95	ND	>99 (89)
16	**1a**[i]	5	1:1	0.2	63	ND	>99 (98)
17	**1b**[i]	10	1:1	0.1	68	2	98 (96)
18	**1c**[i]	3	1:1	0.2	32	ND	>99 (97)
19	**1d**[i]	10	1:2	0.1	89	1	99
20	**1c**[i]	3	1:1	0.2	30	ND	>99 (97)

[a] Ratio w/w.
[b] Based on ^{1}H NMR and HPLC signal integration.
[c] Percentages of isolated yields are given in parenthesis.
[d] 1 g scale of starting nucleoside.
[e] Recycled enzyme from entry 6.
[f] Recycled vinyl benzoate from entry 6. Starting nucleoside 0.3 g.
[g] First run with the recycled enzyme from entry 10.
[h] Second consecutive run with the recycled enzyme from entries 10 and 11.
[i] Entries 16–20 are large-scale experiments. Entries 16–19 are 5 g scale of starting nucleoside. Entry 20 is 25 g of starting nucleoside; ND = not detected under the experimental conditions.

enzyme were reused for subsequent reactions. The recycled CAL-B maintained total selectivity toward acylation of the 5′-OH with the exception of the longer reaction rate (entry 7, Table 1). On the other hand, benzoylation of **1a** with recycled vinyl benzoate gave identical results compared to the use of fresh acylating agent (entry 8, Table 1).

For the enzymatic acylation of *N*-benzoyl-2′-deoxycytidine (**1b**), 10 equiv of vinyl benzoate, which increases the conversion rate, and more dilute conditions (0.1 M instead of 0.2 M) to favor the solubility of the starting nucleoside, were used. Under these conditions, exclusive formation of 5′-*O*-benzoylated derivative **2b**[16] was observed after 40 h (entry 9, Table 1). *N*-Benzoyl-2′-deoxyadenosine (**1c**) was found to be more reactive and only 3 equiv of the vinyl ester were necessary to drive the reaction to completion and furnish **2c**[9] in quantitative yield (99%, entry 10, Table 1). As entries 11 and 12 show, subsequent reactions can be successfully carried out with the recycled enzyme. In the case of *N*-isobutyryl-2′-deoxyguanosine (**1d**), a 0.1 M concentration was used to increase the poor solubility of the starting material in THF. The best results were obtained when the reaction was carried out with 10 equiv of vinyl benzoate with a substrate/enzyme ratio of 1:2 (w/w), (entries 13–15, Table 1). Thus, *N*-isobutyryl-5′-*O*-benzoyl-2′-deoxyguanosine (**2d**)[9] was isolated in 89% yield. It is noteworthy that the excellent yields and purity of **2a**–**d** were obtained from **1a**–**d** via a common precipitation protocol.[17]

Using the CAL-B benzoylation protocol described herein, we attempted to benzoylate the 3′-OH group of the 5′-*O*-protected **2c**. Interestingly, **2c** failed to undergo a second benzoylation reaction. Additionally, lipase from *Pseudomonas cepacia* (PSL-C),[18] which has demonstrated high selectivity in the transesterification of the 3′-hydroxyl group,[13,14] was used as a biocatalyst without success. In both reactions the starting material was recovered unchanged. Based on these experimental results, we postulate that the secondary hydroxyl group is not accessible for both CAL-B or PSL-C to install a second benzoyl group in the nucleoside, perhaps due to the steric hindrance created by the primary benzoyl group in the vicinity. This would explain our inability to find 3′,5′-bis-benzoylated products during the synthesis of **2**.

The screening of other acylating agents such as acetone oxime benzoate gave lower yields and benzoic anhydride led to nonselective acylation. In place of THF, toluene was tried as an alternative, cheaper and safer solvent. In the case of **1d**, a substantial amount of starting material was recovered due to the poor solubility in toluene. For **1a** and **1c** the reaction took much longer in toluene while maintaining the regioselectivity observed in THF.

Next, the large-scale acylation of nucleosides **1a**–**d** was studied (entries 16–20, Table 1). Experiments were carried out on 5 and 25 g scales of the starting material. Excellent results were obtained with CAL-B catalyzing the acylation process with total selectivity furnishing the 5′-*O*-benzoylated derivatives in quantitative yields.

In conclusion, we have developed an efficient and practical green alternative for the syntheses of 5′-*O*-benzoyl-2′-deoxynucleosides **2a**–**d** by selective acylation of the parent nucleosides catalyzed by *Candida antarctica* lipase B with vinyl benzoate. The use of THF as a solvent avoids the tedious work-up of the reaction with other solvents traditionally used in nucleoside chemistry such as pyridine or DMF. The easy scalability of the process and the fact that both the acylating agent and enzyme can be reclaimed and reused after each reaction makes the new process atom efficient[19] and very attractive for industrial applications. The protected monomers **2a**–**d** described in this study are important raw materials for the synthesis of therapeutically useful oligonucleotides[20] and modified nucleosides.[21]

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