Regioselective Acylation of 2′-Deoxynucleosides Through an Enzymatic Reaction with Oxime Esters[1]

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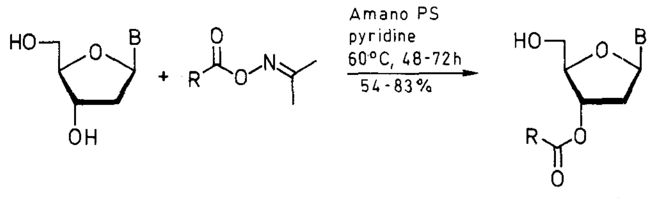
3′-*O*-Acyl derivatives of 2′-deoxynucleosides are obtained in good yield through an enzymatic reaction using oxime esters as acyl transfer agents.

Regioselective acylation of polyhydroxy compounds is a fundamental and difficult task in organic chemistry. Recently, the acylation of carbohydrates using enzymes in organic solvents has been well documented.[2] However, few examples are known of the regioselective acylation of nucleosides. The acylation of adenosine and uridine with *Subtilisin* protease in dimethylformamide[3] has been described, and Nozaki et al. have studied the regioselective acylation of 2′-deoxypyrimidine nucleosides[4] and the enzymatic hydrolysis of diacylated nucleosides.[5]

Oxime esters have been used as irreversible acyl transfer agents in enzymatic transesterification reactions.[6] We have demonstrated the utility of these imino compounds in the regioselective acylation of carbohydrates,[7] in which the acylation occurs at the primary hydroxyl group. We report here a versatile method for the regioselective acylation of 2′-deoxynucleosides on the secondary alcohol group, instead of the more chemically reactive primary alcohol group (Scheme).

Since nucleosides **1** are poorly soluble in hydrophobic organic solvents, we used pyridine as the reaction medium and lipase Amano PS as the catalyst. Other solvents were not as effective as pyridine, under these conditions, for this regioselective acylation. For example, when tetrahydrofuran or 1,4-dioxane were used, the reaction underwent loss of regioselectivity at room temperature. Moreover, this reaction did not take place between starting nucleosides and **2** in the absence of enzyme even if strong conditions were used.



1a B = Thymine
b B = Adenine

2a-f

3a-j

2	R	3	B	R
a	Me	**a**	Thymine	Me
b	Pr	**b**	Thymine	Pr
c	n-C_7H_{15}	**c**	Adenine	Pr
d	n-C_9H_{19}	**d**	Thymine	n-C_7H_{15}
e	1-propenyl	**e**	Thymine	n-C_9H_{19}
f	Ph	**f**	Adenine	n-C_9H_{19}
		g	Thymine	1-propenyl
		h	Adenine	1-propenyl
		i	Thymine	Ph
		j	Adenine	Ph

Scheme

In contrast to other enzymatic acylation reactions, only 3′-*O*-acylated 2′-deoxynucleosides **3** and starting material **1** were recovered in all instances. Reactions were monitored by TLC and the structure of compounds **3** was determined on the basis of their spectral data. For example, ^{13}C NMR spectra for compounds **3** show a shift on C-3′ of ca. 4 ppm. downfield with respect to the same carbon atom in the starting nucleosides. In addition, H-3′ shows a shift of ca. 1.5 ppm downfield with respect to nucleosides **1 a,b**. Complete ^1H and ^{13}C NMR spectral data are given in Tables 2 and 3, respectively.[8]

Table 1. Regioselective Acylation of Thymidine and 2′-Deoxyadenosine

Product	Reaction Time (h)	Yield[a] (%)	Molecular Formula[b]	mp[c] (°C)	IR[d] ν (cm^{-1})	$[\alpha]_D^{25}$ (*c*, solvent)[e]	MS (70 eV)[f] *m/z* (%)
3a[11]	48	71	$C_{12}H_{16}N_2O_6$	174–175	1710	+1.1 (0.68, DMSO)	284 (M^+, 1), 43 (100)
3b	48	74	$C_{14}H_{20}N_2O_6$	147–148	1719	+1.9 (0.52, DMSO)	312 (M^+, 1), 43 (100)
3c	48	68	$C_{14}H_{19}N_5O_4$	175–176	1730	−30.2 (0.52, DMSO)	321 (M^+, 2), 135 (100)
3d	48	82	$C_{18}H_{28}N_2O_6$	108–109	1741	−11.5 (0.52, $CHCl_3$)	368 (M^+, 1), 99 (100)
3e	48	83	$C_{20}H_{32}N_2O_6$	111–112	1740	−10.4 (0.54, $CHCl_3$)	396 (M^+, 1), 99 (100)
3f	48	75	$C_{20}H_{31}N_5O_4$	169–170	1743	−45.0 (0.50, $CHCl_3$)	405 (M^+, 1), 135 (100)
3g	72	65	$C_{14}H_{18}N_2O_6$	179–180	1718	−21.3 (0.45, DMSO)	310 (M^+, 1), 69 (100)
3h	72	58	$C_{14}H_{17}N_5O_4$	223–224 (dec)	1712	−42.1 (0.48, DMSO)	319 (M^+, 1), 69 (100)
3i	72	59	$C_{17}H_{18}N_2O_6$	184–185	1724	−30.4 (0.50, DMSO)	221 (M^+ − 125, 17), 105 (100), 77 (70)
3j	72	54	$C_{17}H_{17}N_5O_4$	253–255 (dec)	1712	−58.7 (0.46, DMSO)	250 (M^+ − 105, 2), 105 (100), 77 (86)

[a] Calculated with respect to **1**, on pure isolatd products.
[b] Satisfactory microanalysis obtained with a Perkin-Elmer model 240 and a Carlo Erba model 1108 instruments: C, H, N ±0.3.
[c] Uncorrected, taken on a Büchi melting-point apparatus.
[d] KBr, partial. Recorded on a Perkin-Elmer 1720-XFT spectrometer.
[e] Measured on a Perkin-Elmer 241 polarimeter.
[f] Obtained on a Helwlett-Packard 5897 A spectrometer.

Table 2. ^{1}H NMR Spectral Data for Compounds **3a–j** δ (ppm).[a]

Product	Base Ring			Sugar Moiety						Acyl Moiety
	NH	Me	H6	H1′	(H2′, H2″)	H3′	H4′	(H5′, H5″)	OH	
3a	–	1.78(s)	7.75(s)	6.20(t)	2.24(m)	5.19(m)[b]	3.97(m)	3.62(m)	5.19(m)[b]	2.06 (3H, s)
3b	10.00(br)	1.82(s)	7.80(s)	6.25(t)	2.25(m)	5.29(m)[b]	4.00(m)	3.69(m)	5.29(m)[b]	0.98 (3H, t), 1.60 (2H, m), 2.40 (2H, t)
3d	8.60(br)	1.92(s)	7.50(s)	6.28(t)	2.35(m)[b]	5.35(m)	4.09(m)	3.90(m)	–	0.90 (3H, t), 1.30 (8H, m), 1.60 (2H, m), 2.35 (2H, t)[b]
3e	8.90(br)	1.95(s)	7.52(s)	6.28(t)	2.35(m)[b]	5.35(m)	4.08(m)	3.91(m)	–	0.90 (3H, t), 1.28 (12H, m), 1.61 (2H, m), 2.35 (2H, t)
3g	9.99(br)	1.80(s)	7.75(s)	6.20(t)	2.26(m)	5.28(m)	3.96(m)	3.62(m)	5.20(t)	1.90 (3H, dd), 5.95 (1H, m), 6.98 (1H, m)
3i	–	1.82(s)	7.77(s)	6.30(t)	2.44(m)	5.49(m)	4.14(m)	3.71(m)	5.25(br)	7.55 (2H, t), 7.67 (1H, m), 8.02 (2H, d)

Product	Base Ring			Sugar Moiety						Acyl Moiety
	NH_2	H2	H8	H1′	(H2′, H2″)	H3′	H4′	(H5′, H5″)	OH	
3c	7.40(br)	8.12(s)	8.46(s)	6.33(dd)	2.42(m)[c] 2.98(m)	5.39(m)	4.06(m)	3.60(m)	5.57(br)	0.91 (3H, t), 1.58 (2H, m), 2.36 (2H, t)
3f	5.82(br)	7.85(s)	8.30(s)	6.27(dd)	2.44(m) 3.20(m)	5.58(m)	4.25(m)	3.94(m)	6.54(br)	0.89 (3H, t), 1.30 (12H, m), 1.68 (2H, m), 2.38 (2H, t)
3h	7.35(br)	6.56(s)	6.76(s)	6.39(dd)	2.45(m)[c] 3.02(m)	5.46(m)[b]	4.11(m)	3.68(m)	5.46(m)[b]	1.90 (3H, dd), 5.98 (1H, m), 7.01 (1H, m)
3j	7.48(br)	8.28(s)	8.50(s)	6.59(dd)	2.75(m) 3.18(m)	5.75(m)	4.38(m)	3.82(m)	5.65(t)	7.69 (2H, t), 7.81 (1H, m), 8.18 (2H, d)

[a] Recorded on a Bruker AC 300 spectrometer with the following solvents: DMSO-d_6 for **3a–c** and **3g–j**; $CDCl_3$ for **3d–f**.

[b] Superimposed signals.

[c] Superimposed by DMSO-d_6 signals.

Table 3. ^{13}C NMR Spectral Data for Compounds **3a–j** δ (ppm).[a]

Product	Base Ring					Sugar Moiety					Acyl Moiety	
	C2	C4	C5	C6	Me	C1′	C2′	C3′	C4′	C5′	C=O	R
3a	152.18	166.76	112.11	137.85	12.11	84.99[b]	36.66	75.21	85.55[b]	61.81	174.10	20.98
3b	151.70	165.54	111.35	137.46	13.13	85.21[b]	37.59	75.68	85.74[b]	62.26	174.51	14.34, 18.94, 36.84
3d	150.58	164.32	110.88	136.31	12.16	85.06[c]	37.16	74.47	85.06[c]	62.01	173.39	13.73, 22.24, 24.46, 28.55, 28.71, 31.24, 33.86
3e	150.59	164.36	110.88	136.33	12.18	85.07[c]	37.16	74.50	85.07[c]	62.00	173.40	13.77, 22.88, 24.46, 28.77, 28.92[d], 29.08, 31.51, 33.86
3g	151.22	164.72[b]	110.75	136.87	12.87	84.66[b]	37.30	75.34	85.35[b]	61.92	166.15[b]	18.46, 122.40, 147.55
3i	150.72	163.90[b]	110.00	136.20	12.53	84.01[b]	36.85	75.88	84.76[b]	61.60	166.45[b]	129.04, 129.57,[d] 133.77

Product	Base Ring					Sugar Moiety					Acyl Moiety	
	C2	C4	C5	C6	C8	C1′	C2′	C3′	C4′	C5′	C=O	R
3c	152.66	149.10	119.56	156.43	139.92	84.40[b]	36.86	75.31	85.71[b]	62.03	172.66	13.62, 18.08, 35.60
3f	151.89	148.02	119.22	156.25	139.87	87.21[c]	38.12	75.59	87.21[c]	63.15	173.21	13.71, 22.62, 24.78, 28.46,[d] 28.74,[d] 31.81, 34.30
3h	153.30	147.79	122.62	156.70	140.75	85.23[b]	37.28	75.81	86.25[b]	62.82	166.36	18.60, 122.50, 147.80
3j	152.63	149.09	119.48	156.38	139.86	85.43[b]	36.81	76.83	85.51[b]	62.48	165.43	128.97, 129.53,[d] 133.80

[a] Recorded on a Bruker AC 300 spectrometer with the following solvents: D_2O for **3a**, DMSO-d_6 for **3b** and **3g–j** and $CDCl_3$ for **3d–f**.

[b] Tentative assignment.

[c] Superimposed signals.

[d] Double intensity.

This procedure is very versatile because acylation with various oxime esters saturated or unsaturated chains yields exclusively the 3′-*O*-acyl derivatives **3**. Also, *N*-acylation of 2′-deoxyadenosine is not observed. The results are summarized in Table 1.

The best yields are obtained with long chain oxime esters, due probably to their higher lipophilicity. On the other hand, if the results between the acylation of thymidine and adenosine are compared, the yields are slightly higher with thymidine, perhaps due to its greater solubility, even though total regioselectivity is observed in both cases. In addition, when unsaturated or aromatic oxime esters are used, longer reaction times are necessary than for saturated imino compounds **2**.

In conclusion, we describe here a novel enzymatic method for the preparation of 3′-*O*-acyl-2′-deoxynucleosides that is an improvement on other reported methods. The introduction of different groups into the nucleoside, the simplicity of the procedure and the medicinal importance of these compounds are noteworthy.

Oxime esters were prepared by reacting acetone oxime with the corresponding acyl chloride[9] or through oximolysis of acetonoxime with vinyl esters[10] and were purified by distillation under vacuum. Pyridine was dried and distilled over KOH and stored under nitrogen to avoid moisture. 2′-Deoxynucleosides, **1a, b**, were purchased from Aldrich and Amano PS lipase from Amano Pharmaceutical Co. TLC was carried out on silica gel 60 F_{254} (Merck) and flash chromatography was performed with Merck silica gel 60/230–400 mesh.

3′-*O*-Acylated 2′-Deoxynucleoside Derivatives 3a–j; General Procedure:

Amano PS lipase (1.5 g) and oxime ester **2** (5 mmol) were added to a solution of nucleoside **1** (4 mmol) in pyridine (15 mL) at 60 °C under N_2. After stirring at 60 °C for time given in Table 1 the enzyme was filtered off and washed with MeOH (2 × 10 mL). The solvents are distilled under reduced pressure and the residue subjected to flash chromatography with AcOEt/MeOH 100:1.

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