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Highly Regio- and Enantio-selective Deacylation of Carbocyclic 3',5'-Di-O-acyloxetanocins by Lipases[1]

Nobuya Katagiri,* Yoshihiro Morishita, and Masahiko Yamaguchi*

Faculty of Pharmaceutical Sciences, Tohoku University, Aobayama, Aoba-ku, Sendai 980-8578, Japan

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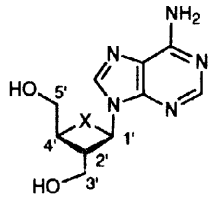
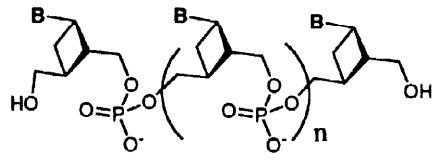
Abstract: Carbocyclic (-)-3',5'-di-O-acyloxetanocin A and T were hydrolysed by lipase MY to give the corresponding carbocyclic (-)-3'-O-acyloxetanocins with high regioselectivity. Carbocyclic (±)-3',5'-di-O-acetyloxetanocin A was deacetylated by lipase MY to form the corresponding (±)-3'-O-acetyloxetanocin A with high regioselectivity but with low enantioselectivity whereas the compound when treated with lipase type XIII gave carbocyclic (-)-5'-O-acetyloxetanocin A with high regio- and enantio-selectivity.

Oxetanocin A (**1**), isolated from the culture filtrate of *Bacillus megaterium*, shows antiviral, antitumor, and antibacterial activities.[2] Recently, the carbocyclic analogue (**2**) of **1** has attracted much attention as entities with potential antibacterial and antiviral activities.[3] We have been interested in the structures and functions of oligonucleotides containing carbocyclic oxetanocins from the viewpoint of antisense strategy,[4] and have examined the selective protection of the two primary hydroxy groups requisite for the synthesis of the oligonucleotides. In this communication, we report a highly regio- and enantio-selective deacylation of carbocyclic 3',5'-di-O-acyloxetanocins by lipases,[5] which provides not only an effective protection for one of the two hydroxy groups of carbocyclic oxetanocins but also a method for the kinetical resolution of racemic carbocyclic oxetanocins.



oxetanocin A (**1**): X=O
carbocyclic oxetanocin A (**2**): X=CH_2



oligonucleotides
B=thymine, cytosine, adenine, guanine

First, hydrolysis of carbocyclic 3',5'-di-O-acetyloxetanocin A [(-)-**3a**][6] was examined using various lipases in 10% phosphate buffer (pH 7.0)-acetone at 36 °C. The results are shown in Table 1. Four kinds of lipases (lipase MY, type I, VII, and XIII)[8] were used for the deacetylation of (-)-**3a**. Among them, lipase MY is the most effective for the hydrolysis. Thus, treatment of (-)-**3a** with lipase MY for 5 days gave 3'-O-acetylated compound (-)-**4a**[9] in 40% yield, concomitant with recovery (58%) of the starting material [(-)-**3a**] and trace amounts of the regio-isomer [(+)-**5a**][10] and (-)-**2**.[11] Prolonged reaction time (7 and 21 days) resulted in the improvement of the yield of (-)-**4a**, 51 and 70%, respectively. Though in this case the starting compound was recovered, the yields of (+)-**5a** and (-)-**2** were quite low. Interestingly, the di-O-benzoylated compound [(-)-**3b**][6] was hydrolysed more readily by lipase MY (for 6 days) to give the 3'-O-benzoylated product [(-)-**4b**][12] in 87% yield, together with a trace amount of (-)-**2**. This is the first instance to show that an O-benzoylated compound is more susceptible than the corresponding O-acetyl derivative to the lipase-catalysed hydrolysis.

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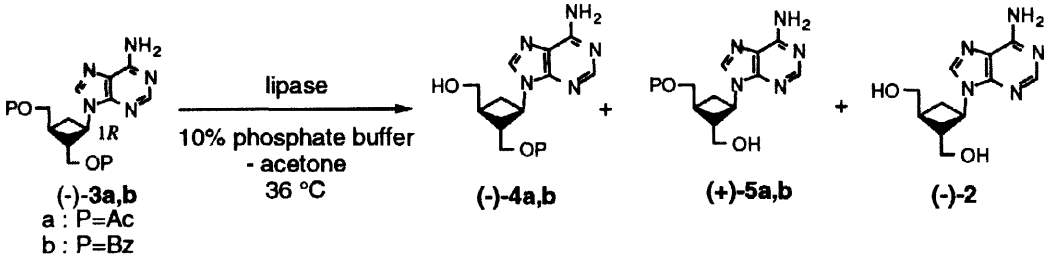
Table 1. Enzymatic Regioselective Deacylation of Carbocyclic Diacylated Oxetanocin-A [(-)-3a,b]



(-)-3a,b
a : P=Ac
b : P=Bz

(-)-4a,b

(+)-5a,b

(-)-2

Entry	Lipase	P	Unit / mmol	Time	(-)-3	(-)-4	(+)-5	(-)-2
1	lipase MY	Ac	$8.7x10^4$	5 d	58	40	1	1
2	lipase MY	Ac	$8.7x10^4$	7 d	38	51	trace	-
3	lipase MY	Ac	$8.7x10^4$	21d	24	70	1	5
4	lipase Type I	Ac	$3.1x10^3$	17d	96	3	0	2
5	lipase Type VII	Ac	$3.2x10^5$	17d	89	10	0	2
6	lipase Type XIII	Ac	$1.1x10^4$	17d	88	8	0	4
7	lipase MY	Bz	$8.7x10^4$	6d	0	87	0	2

* The yields in entry 1, 4, 5, and 6 were determined by HPLC analyses (μBondapak C18; MeOH-H_2O=1:2). Entry 2, 3, and 7 were isolated yields.

Table 2. Deacylation of Carbocyclic Diacylated Oxetanocin-A [(-)-3a-d] by Lipase MY under Various Conditions

Entry	P	Solvents	(-)-3a-d	(-)-4a-d	(+)-5a-d	(-)-2
1	**a** Ac	acetone	63	35	0	2
2	**b** Bz	acetone	26	70	0	4
3	**c** hexanoyl	acetone	90	2	4	4
4	**d** cyclohexane-carbonyl	acetone	95	2	3	1
5	**b** Bz	1,4-dioxane	66	31	0.3	2
6	**b** Bz	pyridine	57	43	0	0.1
7	**b** Bz	ethanol	41	57	0.8	0.3
8	**b** Bz	acetonitrile	24	70	2	4

*Reaction conditions: (-)-**3a-d** , 0.03 mmol; lipase MY, $1.0x10^5$unit / mmol; solvent (4 ml) + 1/15M pH 7.0 phosphate buffer (0.4 ml), 36 °C, 137 h.

**Yields were determined by HPLC analyses (MeOH-H_2O=3:2 for b, 1:1 for c and d).

To shorten the reaction time, we investigated in more detail the deacylation by lipase MY (Table 2). We chose newly the dihexanoyl and dicyclohexanecarbonyl derivatives **3c,d**[6] as substrates. However, both compounds were more reluctant to the hydrolysis, compared with **3a,b**. Since we found **3b** to be the most susceptible to the hydrolysis, we then examined the hydrolysis of **3b** by lipase MY in various solvents. While 1,3-dioxane, pyridine, and ethanol were not effective for the hydrolysis, acetonitrile gave the same result as acetone.

In order to clarify the enantioselectivity of this lipase-mediated hydrolysis, deacetylation of (±)-**3a**[13] was also carried out using lipase MY and type XIII. When (±)-**3a** was treated with lipase MY for 25 days, 3'-

O-acetylated compound (±)-**4a** with high regioselectivity was obtained in 75% yield. However, the enantioselectivity was not observed. On the contrary, hydrolysis of (±)-**3a** for 3 days with lipase type XIII gave 5'-O-acetylated product (-)-**5a**[14] with 90% enantioselectivity in 48% chemical yield. In this reaction the 3'-O-acetylated product was not detected. Therefore, it is possible to use this method for the kinetical resolution of racemic carbocyclic oxetanocin derivatives.

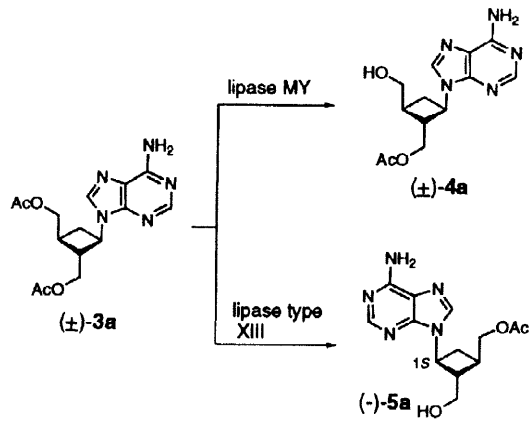
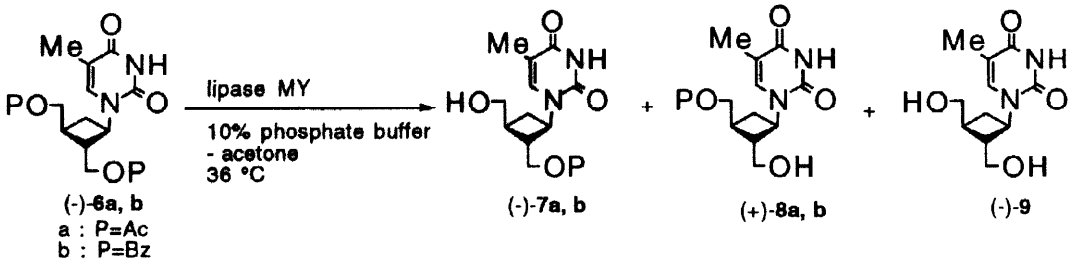


Table 3. Enzymatic Regioselective Deacylation of Diacylated Carbocyclic Oxetanocin-T [(-)-6a,b]



Entry	P	Unit / mmol	Time	(-)-6	(-)-7	(+)-8	(-)-9
1	Ac	8.7×10^{4}	16 d	88.9	10.5	0.6	0
2	Bz	8.7×10^{4}	19 d	40	60	0	-
3	Bz	8.7×10^{4}	21d	26	70	0	0

* The yield in entry 1 was determined by HPLC analysis (MeOH-H_2O=1:2). Entry 2 and 3 were isolated yields.

To design the complementary oligonucleotide to that of carbocyclic oxetanocin A, we examined next the deacylation of carbocyclic 3',5'-di-O-acylated oxetanocin T (-)-**6a,b** using lipase. Thymine derivatives (-)-**6a,b** were more resistant to the hydrolysis by lipase, compared with adenine derivatives (-)-**3a,b** (Table 3). Treatment of (-)-**6a**[6] with lipase MY for 16 days gave only a 10.5% yield of the 3'-O-acetylated product (-)-**7a** concomitant with the recovery of (-)-**6**. As in the case of (-)-**3b**, the dibenzoylated compound (-)-**6b**[6] was hydrolized more readily to give the monobenzoylated compound (-)-**7b**[15] in 70% yield. In both reactions, formation of the fully deacylated product (-)-**9** was not observed.

In conclusion, we have achieved the highly regio- and enantio-selective deacylation of carbocyclic 3',5'-di-O-acyloxetanocins. This methodology is versatile not only for the selective protection requisite for the synthesis of oligonucleotides containing carbocyclic oxetanocin moieties but also for the kinetical resolution of racemic carbocyclic oxetanocin derivatives. Work on the synthesis of oligonucleotides from (-)-**4b** and (-)-**7b** is in progress, and the results will be reported soon elswhere.

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References and Notes

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6. Compounds [(-)-**3a-d**] and (-)-**6a,b** were prepared by the method previously described.[7] (-)-**3a**: mp 140-141 °C ($CHCl_3$-hexane), $[\alpha]_D^{26}$ -33.6 (*c*=1.0, $CHCl_3$). (-)-**3b**: mp 162 °C ($CHCl_3$-hexane), $[\alpha]_D^{23}$ -37.6 (*c* = 1.0, $CHCl_3$). (-)-**3c**: foam. $[\alpha]_D^{20}$ -27.6 (*c* = 1.0, $CHCl_3$). (-)-**3d**: 128-129 °C ($CHCl_3$-hexane), $[\alpha]_D^{21}$ -25.5 (*c* = 1.0, $CHCl_3$).(-)-**6a**: foam, $[\alpha]_D^{22}$ -22.6 (*c* = 1.0, $CHCl_3$). (-)-**6b**: mp 112-113 °C (AcOEt), $[\alpha]_D^{26}$ -23.0 (*c*=1.0, $CHCl_3$).
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8. Lipase type I, VII, and XIII were purchased from Sigma.
9. **4a**: mp 158-159 °C. IR($CHCl_3$): 3240, 1735 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz)δ:1.99 (3H,s), 2.25-2.35 (1H, m), 2.58 (1H, dt, *J*=11.7, 8.6 Hz), 2.71 (1H, dt, *J*=11.9, 9.2 Hz), 3.34 (1H, apparent tt, *J*=8.4, 5.5 Hz), 3.76 (1H, dd, *J*=11.0, 4.0 Hz), 3.80 (1H, dd, *J*=11.2, 4.0 Hz), 4.16 (1H, dd, *J*=11.6, 5.1 Hz), 4.28 (1H, dd, *J*=11.5, 6.2 Hz), 4.68 (1H, apparent q, *J*=9.0 Hz), 5.70 (2H, brs), 7.81 (1H, s), 8.32 (1H, s). $[\alpha]_D^{26}$ -29.4 (*c* = 1.00, $CHCl_3$).
10. **5a**: foam. IR($CHCl_3$): 3300, 1735 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz)δ: 2.08 (3H, s), 2.26-2.47 (1H, m), 2.26-2.47 (2H, m), 2.60-2.78 (2H, m), 3.76-3.86 (2H, m), 4.15 (1H, dd, *J*=11.3, 5.5 Hz), 4.22 (1H, dd, *J*=11.5, 5.2 Hz), 4.55 (1H, apparent q, *J*=9.1 Hz), 6.27 (2H, brs), 7.85 (1H, s), 8.31(1H, s). $[\alpha]_D^{26}$ +45.6 (*c* = 1.00, $CHCl_3$).
11. **2**: mp 163 °C (*lit.* mp 149-151 °C), $[\alpha]_D^{21}$ -15.6 (*c* = 1.00, H_2O)[*lit.* $[\alpha]_D^{22}$ -13.5 (*c* = 1.00, H_2O)]. Bisacchi, G. S.; Breitman, A.; Cianci, C. W.; Clark, J. M.; Field, A. K.; Hagen, M. E.; Hockstein, D. R.; Malley, M. F.; Mitt, T.; Slusarchyk, W. A.; Sundeen, J. E.; Terry, B. J.; Tuomari, A. V.; Weaver, E. R.; Young, M. G.; Zahler, R. *J. Med. Chem.* **1991**, *34*, 1415.
12. **4b**: IR($CHCl_3$): 3400, 1718 cm^{-1}. ^{1}H-NMR($CDCl_3$, 400 MHz)δ: 2.41 (1H, apparent qt, *J*=8.8, 4.4 Hz), 2.63 (1H, dt, *J*=11.5, 8.8 Hz), 2.72 (1H, dt, *J*=11.7, 9.3 Hz), 3.45 (1H, apparent tt, *J*=8.6, 5.7 Hz), 3.81 (1H, dd, *J*=11.2, 4.4 Hz), 3.86 (1H, dd, J=11.2, 4.0 Hz), 4.06 (1H, brs), 4.48 (1H, dd, *J*=11.7, 5.5 Hz), 4.52 (1H, dd, *J*=11.5, 6.1 Hz), 4.82 (1H, apparent q, *J*=8.7 Hz), 6.32 (2H, brs), 7.34-7.40 (2H, m), 7.48-7.54 (1H, m), 7.84-7.88 (2H, m), 7.92 (1H, s), 8.30 (1H, s). $[\alpha]_D^{24}$ -49.2 (*c* = 1.00, $CHCl_3$).
13. Compound (±)-**3a** was synthesized by the method previously reported. Katagiri, N.; Sato, H.; Kaneko, C. *Chem. Pharm. Bull.* **1990**, *38*, 288.
14. (-)-**5a**: $[\alpha]_D^{26}$ - 41.1 (*c* = 1.00, $CHCl_3$). The *e.e.* was determined by HPLC analysis using Chiralpak AS (hexane-EtOH = 1:1).
15. (-)-**7b**: colorless foam. IR($CHCl_3$): 3400, 1705, 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz)δ:1.90 (3H, s), 2.12-2.26 (2H, m), 2.38-2.48 (1H, m), 2.95-3.04 (1H, m), 3.71 (1H, dd, *J* = 11.0, 5.5 Hz), 3.78 (1H, dd, *J* = 10.6, 3.3 Hz), 4.43 (1H, dd, *J* = 11.6, 5.5 Hz), 4.47 (1H, dd, *J* = 12.6, 5.1 Hz), 4.66-4.75 (1H, m), 7.26 (1H, s), 7.38-7.48 (2H, m), 7.52-7.60 (1H, m), 7.88-8.02 (2H, m), 8.84-9.10 (1H, br). $[\alpha]_D^{27}$ -38.0 (*c* = 0.50, $CHCl_3$).