signals in the ^1H NMR spectrum of the reaction mixture.

Rates of Alkylation of 4-(*p*-Nitrobenzyl)pyridine. A modification of the method of Schasteen and Reed[23] was used: the compounds (5 mg) were dissolved in a solution of 2.5 mM sodium acetate, 0.1 M ADA (pH 6.5), or 0.1 M *N*-ethylmorpholine acetate (pH 9.0) buffer (4.0 mL) and 5% 4-(*p*-nitrobenzyl)pyridine in acetone (1.0 mL). Aliquots (40 μL) were removed at various times and added to a triethylamine/acetone/water mixture (1:1:2, 1.0 mL). A_{600} values were read. At the end of the experiments, the mixture was heated at 100 °C for 20 min and then cooled; the A_{600} value of the sample was treated as the reaction end point. The percent of product formed was determined by

$$[(A_{600})_f - (A_{600})_i]/(A_{600})_f \times 100$$

In Vitro Preparation of *S*-[2-(N^7-Guanyl)ethyl]glutathione. The method used for the preparation of the major EDB adduct has been previously described.[15,19] Briefly, the stereospecifically labeled [1,2-$^2H_2$]-EDBs (10 mM) were incubated individually with liver cytosol prepared from phenobarbital-treated rats (3 mg of protein/mL), herring sperm or calf thymus DNA (3 mg/mL), and GSH (3 mM) in 0.1 M Tris-HCl buffer (pH 7.7) containing 15 mM sodium citrate and 1 mM EDTA (total volume 1000 mL). After the reaction proceeded for 2 h at 37 °C, it was stopped by the addition of a sodium dodecyl sulfate solution to a final concentration of 1% (w/v). The mixture was sequentially washed with equal volumes of a phenol/$CHCl_3$/isoamyl alcohol mixture (25:24:1, v/v/v), ethyl acetate, and finally ether. The DNA was precipitated with cold ethanol (2.5 volumes). Following isolation and resuspension of the DNA in 0.1 M potassium phosphate buffer (pH 7.0, 500 mL), the adducts were released from the DNA by heating for 30 min at 100 °C. After the solution was cooled, DNA was precipitated by the addition of cold ethanol (2.5 volumes) and centrifuged for 30 min at 10^4g. The supernatant was concentrated, and the adducts were purified by the reversed-phase C18 and aminopropyl ion-exchange HPLC systems described for the purification of synthetic compounds (see above). These compounds were then analyzed by one- and two-dimensional NMR techniques to determine the stereochemical orientation of the deuterium atoms. The spectra are displayed in Figure 5A,B.

NMR Spectroscopy. Samples of the purified adducts were prepared in ^2H_2O. The chemical shifts were determined externally by a ^2H_2O solution of DSS or assigning the $^2H^1HO$ signal to 4.8. One-dimensional proton NMR spectra were acquired by using 16 384 data points with a 30° pulse. The spectral resolution was increased by Gaussian multiplication prior to Fourier transformation. The two-dimensional COSY spectra were acquired through the use of the standard COSY software provided by the manufacturer. A 90-τ-45 pulse sequence with 1024 data points in F_2 and 512 in F_1 and magnitude calculations were employed. The spectral window and transmitter frequency were chosen to observe the coupled signals with optimal data point resolution. Prior to the transformations, sine-bell multiplication was used in both dimensions. The data were zero-filled once prior to the F_1 transformation and then symmetrized before it was plotted.

The NOE difference spectra were obtained by collecting 16 scans at low-power on-resonance selective irradiation at all times except during acquisition periods, followed by 16 scans at off-resonance irradiation. After a total of 2560 scans had been recorded, the free induction decay (FID) of the off-resonance data was subtracted from that of the on-resonance data. The resulting FID was Fourier transformed to yield the difference spectrum. The magnitude of the NOE was determined by integration of the signals in the difference spectrum.

Enzymochemical Regioselective Oxidation of Steroids without Oxidoreductases

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Abstract: Two hydrolytic, commercially available enzymes (out of eight tested), *Chromobacterium viscosum* lipase and *Bacillus subtilis* protease (subtilisin), have been found to esterify the model dihydroxy steroid 5α-androstane-3β,17β-diol (**1**) in dry acetone. These enzymes acylate the two hydroxyl groups in **1** with striking—and opposite—regioselectivities: while lipase reacts exclusively with OH in the C-3 position, subtilisin displays a marked preference for the C-17 hydroxyl (chemical reactivities of these two hydroxyl groups in **1** are comparable). The reactivity of various other hydroxy steroids with lipase and subtilisin was examined, and the structural requirements for these enzymatic reactions were elucidated. Several of these enzyme-catalyzed acylations in acetone were scaled up, and 0.7-1.0-g quantities of pure 3β or 17β monobutyryl steroids were obtained with good yields. In the case of the enzymatically prepared 3β-ester of 5α-pregnane-3β,20β-diol, the remaining free hydroxyl group was chemically oxidized, followed by alkaline deacylation at the C-3 position, to afford 5α-pregnan-3β-ol-20-one with a 63% overall isolated yield. Such an enzymochemical process provides an attractive alternative to the currently utilized enzymatic oxidations of steroids catalyzed by hydroxysteroid dehydrogenases.

Site-specific conversion of identical functional groups in steroids, which is of enormous importance for the pharmaceutical industry, has always been a formidable task.[1] In particular, chemical oxidation of OH groups in polyhydroxy steroids seldom affords absolute specificity.[2] Therefore, most practical transformations of this type have been carried out microbiologically.[3] To avoid traditional problems associated with fermentations, a promising recent trend in the preparative oxidation of hydroxyl groups in steroids has been the use of isolated enzymes, hydroxysteroid dehydrogenases, as catalysts.[4] From the standpoint of organic chemists, however, this approach has the drawback that these enzymes require expensive cofactors and are very costly and many are not commercially available.

It occurred to us that perhaps instead of directly exploiting the keen regioselectivity of oxidoreductases at the oxidation step (as

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Table I. Preparative Regioselective Acylation of Dihydroxy Steroids with Trifluoroethyl Butyrate Catalyzed by Lipase and Subtilisin in Anhydrous Acetone[a]

starting material	enzyme	conversion,[b] % (reactn time)	isol yield, g (% theor)	product[c]
1	lipase	96 (23 h)	1.03 (83)	3β-(monobutyryl ester) of **1**
1	subtilisin	79 (7 days)	0.75 (60)	17β-(monobutyryl ester) of **1**
7	lipase	93 (40 h)	1.05 (84)	3β-(monobutyryl ester) of **7**
7	subtilisin	85 (7 days)	0.78 (63)	17β-(monobutyryl ester) of **7**
14	lipase	98 (8 h)	1.04 (85)	3β-(monobutyryl ester) of **14**

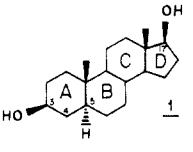
[a] In all cases, 1 g of the starting material was dissolved in 100 mL of anhydrous acetone, followed by addition of 5 mol equiv of TFEB, with another 10 mol equiv of TFEB being added incrementally throughout the reaction. Then 100 mg/mL *C. viscosum* lipase or 10 mg/mL subtilisin was added as a powder (both enzymes were pH adjusted—see the Experimental Section), and the suspensions[14] were shaken on a temperature-controlled orbit shaker at 45 °C and 250 rpm for the periods of time shown in the third column of the Table. When the reaction was stopped, the enzyme was removed by filtration, the solvent was evaporated, and the residue was washed and then purified by silica gel column chromatography. [b] Estimated by gas chromatography as a ratio of the area of the peak corresponding to the product ester to the sum of this parameter and the area of the peak corresponding to the substrate ether. In no case was any appreciable conversion detected without the enzyme. [c] Determined by elemental analysis and ^{13}C NMR as described in the Experimental Section.

with hydroxysteroid dehydrogenases[4]), one could take advantage of the positional specificity of other enzymes at the prior step of protection of OH groups. For example, if it is possible to enzymatically acylate a given hydroxyl group in a steroid molecule at will, then the remaining one(s) can be readily oxidized chemically, followed by deacylation (where the last two steps need no regioselectivity).

We have recently discovered that when water is replaced by anhydrous organic solvents as the reaction media of enzymatic reactions,[5] various hydrolases (lipases and proteases) acquire the ability to acylate glycols[6] and sugars and related compounds[7-9] nearly quantitatively and with remarkable positional selectivity. In the present study, we extended these findings to hydroxy steroids: certain hydrolytic enzymes easily discriminate between secondary OH groups in the A ring and the D ring (or the side chain) of steroids and, depending on the enzyme used, esterify a specific hydroxyl group with striking regioselectivity (and stereoselectivity). This site-specific protection was the key (and the only enzymatic) step employed in the regioselective, enzymochemical oxidation of a dihydroxy steroid.

Results and Discussion

The minimal dihydroxy steroid structure possible, 5α-androstane-3β,17β-diol (**1**), was selected as the initial model compound in our investigation. We set out to ascertain (i)



1

whether it is possible to enzymatically esterify **1**; (ii) if so, whether this esterification could be regioselective; and (iii) if so, whether different enzymes would exhibit distinct positional selectivities. To that end, we employed the methodology depicted in eq 1, where

$$\text{HO-steroid-OH} + R_1COOR_2 \rightarrow \text{R}_1\text{COO-steroid-OH} + R_2OH \quad (1)$$

HO-steroid-OH is **1**, R_1COOH is an aliphatic carboxylic acid, and R_2OH is an appropriate alcohol. That is, **1** was used as a nucleophile in a transesterification reaction with an activated ester catalyzed by a hydrolytic enzyme. As pointed out previously,[7-10] this process requires an organic solvent as the reaction medium because in aqueous solution water will replace the dihydroxy steroid as a nucleophile, resulting in hydrolysis rather than transesterification. Furthermore, dry organic solvents are required to avoid hydrolysis of the activated ester R_1COOR_2.

The specific experimental protocol of our inquiry was as follows: 5 mg/mL **1** and 5 mol equiv (0.09 M) of 2,2,2-trichloroethyl butyrate (this activated ester had been successfully used by us in previously reported enzymatic transesterifications[7-10]) were dissolved in dry acetone.[11] Then one of the commercially available and relatively inexpensive enzymes listed below was added to the reaction mixture in a powdered form[13]—porcine pancreatic lipase, *Candida cylindracea* lipase, *Mucor* lipase, *Aspergillus niger* lipase, *Chromobacterium viscosum* lipase, wheat germ lipase, subtilisin (protease from *Bacillus subtilis*), and papain (protease from papaya latex). The suspensions[14] were shaken at 250 rpm and 45 °C, and the progress of the reactions was monitored via gas chromatography by following the formation of the steroid product. Out of the eight lipases and proteases tested, only *C. viscosum* lipase and subtilisin showed an appreciable degree of conversion within 24 h. Therefore, these two enzymes were further explored as catalysts for the above reaction.

To optimize the transesterification process for both enzymes, the initial rates of the enzymatic reaction between **1** and various activated esters were measured as functions of the latter. In the case of subtilisin, trichloroethyl butyrate was much more reactive than either trichloroethyl acetate or trichloroethyl octanoate. The reactivity of the butyrate ester was increased by another 4.5-fold when the trichloroethyl moiety was replaced by the more electrophilic trifluoroethyl group. In the case of the lipase, 2,2,2-trifluoroethyl butyrate (TFEB) was 9 times more reactive than the trichloroethyl ester. Therefore, TFEB was employed in all subsequent experiments as R_1COOR_2 in eq 1.

Having established that lipase from *C. viscosum* (hereafter referred to as "lipase") and subtilisin are capable of acylating **1** in dry acetone, the next step was to produce enough material to identify the products of these reactions. Consequently, both reactions were scaled up to 1 g (3.45 mmol) of **1** in 100 mL of the solvent. In the case of lipase as a catalyst (for experimental conditions and details, see the first entry in Table I), this yielded 1.03 g of the product, which was pure by TLC, GC, and ^{13}C NMR; elemental analysis showed that it was a monobutyrate ester

(4) Riva, S.; Bovara, R.; Pasta, P.; Carrea, G. *J. Org. Chem.* **1986**, *51*, 2902–2906. Carrea, G. In *Biocatalysis in Organic Media*; Laane, C., Tramper, J., Lilly, M. D., Eds.; Elsevier: Amsterdam, 1987; pp 157–169. Carrea, G.; Cremonesi, P. *Methods Enzymol.* **1987**, *136*, 150–157.
(5) Klibanov, A. M. *CHEMTECH* **1986**, *16*, 354–359.
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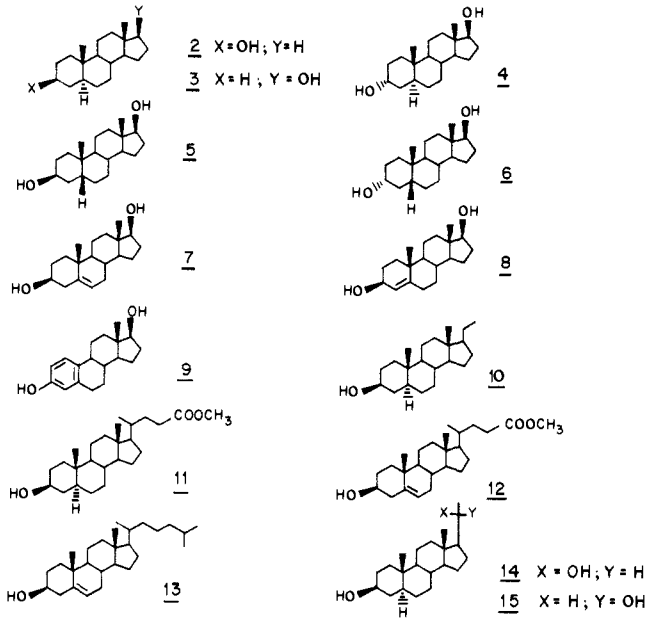
(11) Acetone is a satisfactory solvent for **1** (and other steroids) and a suitable reaction medium for lipase-[10,12] and protease-catalyzed[9] reactions.
(12) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192–3196.
(13) With the exception of subtilisin, all hydrolases were crude preparations (in order to reduce costs), and their concentrations were 100 mg/mL. In the case of subtilisin, a 20 mg/mL concentration was used.
(14) All enzymes are insoluble in acetone: Singer, S. J. *Adv. Protein Chem.* **1962**, *17*, 1–68.

of **1**. Inspection of the ^{13}C NMR spectrum of the product revealed that it was exclusively the C-3 monoester. This conclusion was confirmed by examining the lipase-catalyzed esterification of the corresponding monohydroxy analogues of **1**: while the initial rates with **1** and 5α-androstan-3β-ol (**2**) were comparable (3.30 and



3.85 μmol/h, respectively, under the conditions described above for analytical experiments), 5α-androstan-17β-ol (**3**) was completely unreactive. Thus, lipase exhibits absolute regioselectivity in the acylation of **1** by reacting only with the C-3 hydroxyl group of the steroid.

Similar experiments were conducted with subtilisin (the second entry in Table I). Following crystallization, the product obtained was pure by TLC, GC, and ^{13}C NMR, and it was found to be a monobutyryl ester of **1** by elemental analysis. Examination of the ^{13}C NMR spectrum of the product showed that it was acylated only at the C-17 position. This conclusion was supported by experiments with the monohydroxy analogues of **1**: the initial rates of subtilisin-catalyzed esterification of **1–3** were 0.63, 0.07, and 0.47 μmol/h, respectively. Thus, subtilisin displays a marked preference for the C-17 OH group in **1**.

For comparison, we studied the nonenzymatic acylation of monohydroxy analogues of **1** with butyryl chloride. In this case, the rates of esterification of C-3 and C-17 hydroxyl groups were comparable: 4.29 and 2.66 μmol/h for **2** and **3**, respectively (at 5 mg/mL monohydroxy steroid, 0.1 M butyryl chloride, 45 °C). Similar reactivities of the two OH groups in **1** and its analogues in various chemical esterification reactions have been also reported in the literature.[15] Therefore, while chemical acylations do not significantly discriminate between C-3 and C-17 hydroxyls in **1**, their enzymatic counterparts can easily distinguish between them. Furthermore, depending on whether lipase or subtilisin is used, the OH group in either the A or D ring can be esterified.[16] It is interesting to note that even the specific enzyme β-hydroxysteroid dehydrogenase is unable to appreciably distinguish between, and oxidizes both, C-3 and C-17 hydroxyls in **1** and related compounds.[17]

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(16) It should be pointed out that lipase preinactivated by the active-site-specific reagent diethyl *p*-nitrophenyl phosphate (Maylie, M. F.; Charles, M.; Sarda, L.; Desnuelle, P. *Biochim. Biophys. Acta* **1969**, *178*, 196–198) and subtilisin preinactivated by the active-site-specific reagent phenylmethylsulfonyl fluoride (Fahrney, D. E.; Gold, A. M. *J. Am. Chem. Soc.* **1963**, *85*, 997–1000) were completely inactive in the transesterification reaction between **1** and TFEB in acetone. These observations rule out the artifactual origin of the process and demonstrate the necessity of a competent active site for the enzymatic reactions.

Table II. Reactivities of Various Hydroxy Steroids in the Acylation with Trifluoroethyl Butyrate Catalyzed by Lipase and Subtilisin in Anhydrous Acetone

steroid	compd	initial rate,[a] μmol/h lipase	subtilisin
5α-androstane-3β,17β-diol	**1**	3.30	0.63
5α-androstane-3α,17β-diol	**4**	0	0.53
5β-androstane-3β,17β-diol	**5**	0	0.41
5β-androstane-3α,17β-diol	6	0	0.63
5-androstene-3β,17β-diol	**7**	1.72	0.67
4-androstene-3β,17β-diol	**8**	0	0.32
17β-estradiol	**9**	0	0.63
5α-pregnan-3β-ol	**10**	6.26	0.06
3β-hydroxy-5α-cholanic acid methyl ester	**11**	8.32	0
3β-hydroxy-5-cholenic acid methyl ester	**12**	2.75	0
5-cholesten-3β-ol (cholesterol)	**13**	3.00	0
5α-pregnane-3β,20β-diol	**14**	4.52	0.10
5α-pregnane-3β,20α-diol	**15**	4.34	0.55

[a] Conditions: 15 mM steroids, 75 mM TFEB, 100 mg/mL *C. viscosum* lipase, 20 mg/mL subtilisin (both enzymes were pH adjusted—see the Experimental Section), 45 °C. Suspensions[14] in 1 mL of anhydrous acetone were shaken at 250 rpm. Every 15 min, 5-μL aliquots were withdrawn and assayed for the product (whose specific peak area was assumed to be approximately the same as that for the starting material) by gas chromatography. At least three time points were taken for the initial rate determination. No appreciable reaction was detected in the absence of the enzyme or in the presence of irreversibly inactivated lipase and subtilisin.[16]

To ascertain the structural requirements of lipase and subtilisin in the acylation reaction, we tested more than a dozen analogues of **1** as nucleophiles. The results obtained, depicted in Table II, afford several important conclusions. Comparison of the reactivities of **1** and **4–6** toward lipase indicates that the substrate must have the A/B ring fusion in the trans configuration and the C-3 hydroxyl group in the equatorial (β) position. The sensitivity of the enzyme to the immediate environment of the steroid's C-3 OH group is supported by the finding that the introduction of a double bond in the B ring is tolerated, while in the A ring it is not (compounds **7** and **8**, respectively). The phenolic group in **9**, as expected, was unreactive. The compounds with the altered side chain (**10–15**) were very reactive with lipase as long as the C-3 hydroxyl and C-5 hydrogen remained in β and α configurations, respectively.

Conversely, in the case of subtilisin, changes in the A or B ring did not dramatically affect the reactivity of the steroid (compounds **1** and **4–9**). Very low reaction rates of steroids **10–13** are consistent with our previous conclusion that subtilisin strongly prefers the C-17 or side-chain hydroxyl group over that in the C-3 position. Finally, a much higher reactivity of **15** than **14** shows that subtilisin is sensitive to the stereochemistry of the nucleophile.

Therefore, both lipase and subtilisin require the substrate's hydroxyl group and its immediate surrounding to be in a certain orientation, while they are virtually indifferent to the rest of the steroid structure. To establish whether the former phenomenon is due to chemical or purely enzymatic factors, we examined the reactions of butyryl chloride with **2** and its 3α analogue. The initial rates of the two reactions were similar (4.29. and 6.38 μmol/h, respectively, at 5 mg/mL steroids, 0.1 M butyryl chloride, and 45 °C). Thus, in contrast to the enzymatic reaction, chemical reactivities of 3β- and 3α-hydroxyl groups are comparable. Therefore, the differences in reaction rates seen in Table II are due to the substrate specificity of these enzymes in organic solvents pointing to a unique way of binding of the steroid molecule in the enzyme active center.

Enzymatic esterification of some of the steroids in Table II was scaled up, and the data obtained are presented in Table I. One can see that lipase-catalyzed acylation of steroids is facile, efficient, and affords approximately 1 g of pure 3β-monoester products with

(17) Talalay, P. *The Enzymes* **1963**, *7*, 179–202.

83–85% isolated yields. In the case of subtilisin, the process is considerably slower, but it is still of a preparative value—pure 17β-monoesters were produced with 60% isolated yields.

To directly illustrate the feasibility of an enzymochemical oxidation of a steroid, we carried out the lipase-catalyzed esterification of **14**; afterward, the C-20 hydroxyl group in the resultant 3β-(monobutyryl ester) was oxidized with pyridinium chlorochromate[18] in methylene chloride and the C-3 hydroxyl group was then deprotected by a standard alkaline methanol treatment. Consequently, 0.63 g (63% overall isolated yield) of pure 5α-pregnan-3β-ol-20-one (allopregnanolone) was prepared.

In closing, we have demonstrated herein that *C. viscosum* lipase and subtilisin readily distinguish between C-3 and C-17 (or C-20) hydroxyl moieties in steroids[19] and in doing so exhibit opposite regioselectivities. This profound positional selectivity is particularly striking when considering that the substrates and reaction medium used in this study are entirely unnatural for both enzymes. Hence, by screening other (primarily microbial) hydrolases, one should be able to find enzymes capable of site-specific acylation of any desirable hydroxyl group in a steroid molecule. Needless to say, the scope of chemical transformations of such enzymatically protected steroids is not limited to the oxidations exemplified in the present study.

Experimental Section

Materials. Lipases (EC 3.1.1.3) were obtained as follows: porcine pancreatic, *C. cylindracea*, and wheat germ from Sigma Chemical Co. (St. Louis, MO); *A. niger* and *Mucor sp.* from Amano International Enzyme Co. (Troy, VA); and *C. viscosum* (lipase CV) from FinnSugar Biochemicals (Elk Grove Village, IL). Their specific activities were 13, 700, 9, 30, 10, and 120 units/mg of solid. Both subtilisin (EC 3.4.21.14) and crude papain (EC 3.4.22.2) were purchased from Sigma and had specific activities of 12.2 and 2.9 units/mg of solid. Porcine pancreatic lipase was dried under vacuum prior to use.[8] Lipases from *C. viscosum*[8] and subtilisin[9] were each dissolved in a minimal amount of water and adjusted to pH 7.0 and 7.9, respectively, and the solutions were freeze-dried. This pH adjustment substantially increases catalytic activity of both enzymes in organic media.[8,9] The remaining enzymes were used directly as supplied by the manufacturer without any pretreatment, for pH adjustment was found to have no significant effect on their activities in organic solvents.

Steroids were obtained as follows: **1–7** and **13–15** from Sigma; 5α-androstan-3α-ol and **8–12** from Steraloids, Inc. (Wilton, NH).

Acetone (analytical grade) was used without further purification apart from drying by shaking with 3-Å molecular sieves (Linde), which were prewashed with acetone and dried. The solvent thus prepared (referred to as "dry") contained no more than 0.05% (w/w) water.[20]

2,2,2-Trichloroethyl esters were prepared as previously described.[7,10] 2,2,2-Trifluoroethyl butyrate (TFEB) was synthesized from butyryl chloride and 2,2,2-trifluoroethanol by a general methodology[21] and had the following characteristics: bp 106 °C; ^{1}H NMR ($CDCl_3$, TMS as the internal reference) δ 4.47 (2 H, q, J = 8.4 Hz), 2.40 (2 H, t, J = 7.4 Hz), 1.70 (2 H, m, J = 7.4 Hz), 0.97 (3 H, t, J = 7.4 Hz). Anal. Calcd for $C_6H_9F_3O_2$: C, 42.35; H, 5.29; F, 33.53. Found: C, 42.55; H, 5.18; F, 33.59. All other chemicals used in this work were purchased from commercial suppliers and were of the highest purity available.

Assays. All enzymatic transesterifications and chemical acylations were followed by gas chromatography (GC) with a 5-m HP1 capillary column coated with methylsilicone gum (Hewlett-Packard) (N_2 as carrier gas, 30 mL/min, detector and injector port temperatures 300 and 250 °C, respectively).

In addition to GC, the purity of products was tested by TLC with precoated silica gel IB-F sheets (Baker) and appropriate mixtures of chloroform and methanol. The spots were developed by spraying with concentrated H_2SO_4, followed by heating.

Optical rotations were measured at 589 nm (sodium line) at 25 °C in a Perkin-Elmer 253 B polarimeter.

All elemental analyses were performed by Robertson Laboratory, Inc. (Madison, NJ).

Structure Determinations. The positions of acylation in all enzymatically prepared dihydroxy steroid esters were established by ^{13}C NMR (Brucker WM 270 spectrometer) following the general strategy described in the literature.[22] The ^{13}C NMR spectra for all relevant nonacylated dihydroxy steroids (**1**, **7**, **14**) as well as allopregnanolone have been reported.[22c] O-Acylation of a given OH group (in C-3 and C-17 positions) in a steroid molecule results in a downfield shift of the peak corresponding to the carbon bonded to that hydroxyl and upfield shifts of the peaks corresponding to the two neighboring carbon atoms.[22a,b]

Preparative Enzymatic Acylations. The conditions of the syntheses are described in footnote *a* to Table I. Following removal of the enzyme, the solvent acetone was evaporated in a rotary evaporator (the product 2,2,2-trifluoroethanol evaporated as well). The remaining oily residue was dissolved in chloroform, washed with 5% aqueous $NaHCO_3$ and water, and dried with anhydrous $MgSO_4$, and the solvent was evaporated. The solid residue obtained was subjected to silica gel column chromatography, using chloroform–methanol (99:1) as the solvent (except that in the case of **14** the ratio of the solvents was 99.5:0.5). The product was subsequently isolated by evaporation of the solvent and then recrystallized from hexane. The isolated yields quoted in Table I were attained prior to recrystallization, for all the products prepared by using lipase were already pure by TLC, GC, and ^{13}C NMR at that point. In the case of subtilisin as a catalyst, the crystallization was required to obtain pure products.

Enzymatic Synthesis of 3β-(Monobutyryl ester) of 1. The product was a white crystalline solid: mp 90–92 °C; $[\alpha]^{25}_D$ –0.6° (*c* 0.5, $CHCl_3$); ^{13}C NMR (67.9 MHz, pyridine-d_5, TMS as the internal reference) δ 81.4, 73.5, 54.7, 51.4, 44.9, 43.6, 37.5, 37.1, 35.8, 34.5, 31.9, 31.0, 28.8, 28.0, 23.8, 21.2, 12.3, 11.9 (C17), (C3), (C9), (C14), (C5), (C13), (C12), (C1), (C8, C10), (C4), (C7), (C16), (C6), (C2), (C15), (C11), (C19), (C18). For the butyryl moiety: ^{13}C NMR δ 172.9, 36.7, 18.9, 13.8. Anal. Calcd for $C_{23}H_{38}O_3$: C, 76.24; H, 10.50. Found: C, 75.95; H, 10.37.

Enzymatic Synthesis of 17β-(Monobutyryl ester) of 1. The product was a white crystalline solid: mp 120–121 °C; $[\alpha]^{25}_D$ +4.2° (*c* 0.5, $CHCl_3$); ^{13}C NMR (67.9 MHz, pyridine-d_5, TMS as the internal reference) δ 82.6, 70.5, 54.6, 51.0, 45.3, 43.0, 39.3, 37.5, 37.4, 35.9, 35.5, 32.4, 31.9, 29.0, 28.0, 23.7, 21.1, 12.5, 12.4 (C17), (C3), (C9), (C14), (C15), (C13), (C4), (C1), (C12), (C10), (C8), (C2), (C7), (C6), (C16), (C15), (C11), (C19), (C18). For the butyryl moiety: ^{13}C NMR δ 173.1, 36.5, 18.9, 13.8. Anal. Calcd for $C_{23}H_{38}O_3$: C, 76.24; H, 10.50. Found: C, 75.96; H, 10.58.

Enzymatic Synthesis of 3β-(Monobutyryl ester) of 7. The product was a white crystalline solid: mp 126–127 °C; $[\alpha]^{25}_D$ –53.4° (*c* 0.5, $CHCl_3$); ^{13}C NMR (67.9 MHz, acetone-d_6, TMS as the internal reference) δ 140.8, 122.9, 81.7, 74.0, 52.3, 51.3, 43.5, 38.9, 37.9, 37.5, 37.4, 32.8, 32.2, 30.9, 28.5, 24.1, 21.4, 19.7, 11.5 (C5), (C6), (C17), (C3), (C14), (C9), (C13), (C4), (C1), (C12), (C10), (C7), (C9), (C16), (C2), (C15), (C11), (C19), (C18). For the butyryl moiety: ^{13}C NMR δ 172.9, 36.8, 19.1, 13.9. Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.67; H, 10.00. Found: C, 76.46; H, 9.88.

Enzymatic Synthesis of 17β-(Monobutyryl ester) of 7. The product was a white crystalline solid: mp 111–112 °C; $[\alpha]^{25}_D$ –61.7° (*c* 0.5, $CHCl_3$); ^{13}C NMR (67.9 MHz, acetone-d_6, TMS as the internal reference) δ 142.4, 121.4, 82.9, 71.7, 51.9, 51.2, 43.3, 43.2, 38.2, 37.7, 37.4, 32.6, 32.5, 32.2, 28.3, 24.2, 21.3, 19.8, 12.3 (C5), (C6), (C17), (C3), (C14), (C9), (C4), (C13), (C1), (C12), (C10), (C7), (C2), (C8), (C16), (C15), (C11), (C19), (C18). For the butyryl moiety: ^{13}C NMR δ 173.4, 36.7, 19.2, 13.9. Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.67; H, 10.00. Found: C, 76.01; H, 9.64.

Enzymatic Synthesis of 3β-(Monobutyryl ester) of 14. The product was a white crystalline solid: mp 144–145 °C; $[\alpha]^{25}_D$ –7.6° (*c* 0.5, $CHCl_3$); ^{13}C NMR (67.9 MHz, pyridine-d_5, TMS as the internal reference) δ 73.5, 69.7, 59.1, 56.4, 54.7, 44.9, 43.1, 40.5, 37.0, 35.7, 34.6,

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32.4, 28.9, 28.0, 26.2, 24.9, 24.4, 21.5, 12.7, 12.3 (C3), (C20), (C17), (C14), (C9), (C5), (C13), (C12), (C1), (C8, C10), (C4), (C7), (C6), (C2), (C16), (C15), (C21), (C11), (C18), (C19). For the butyryl moiety: ^{13}C NMR δ 172.8, 36.7, 18.9, 13.7. Anal. Calcd for $C_{25}H_{42}O_3$: C, 76.92; H, 10.76. Found: C, 76.76; H, 10.63.

Preparation of 5α-Pregnan-3β-ol-20-one. An amorphous solid of 3β-(monobutyryl ester) of **14**, obtained as described above prior to silica gel column chromatography, was dissolved in 180 mL of CH_2Cl_2. Then 3 g of pyridinium chlorochromate[18] was added, and the suspension was stirred at room temperature for 1 h, followed by washing with 100 mL of both a saturated solution of NaCl in water and water. Afterward, the solution was dried with anhydrous $MgSO_4$ and the solvent evaporated in a rotary evaporator. The solid residue was dissolved in 50 mL of MeOH containing 5% KOH, and the solution was refluxed for 30 min. The solvent was subsequently evaporated, and the oily residue was dissolved in $CHCl_3$ and washed with both 0.1 N HCl and with water. Following drying and evaporation of the solvent, the remaining solid was subjected to silica gel column chromatography (chloroform–methanol (99:1) as the solvent). The white crystalline product formed was pure by GC, TLC, and ^{13}C NMR: mp 195–196 °C; $[\alpha]^{25}_D$ +94.4° (*c* 0.5, $CHCl_3$); ^{13}C NMR (67.9 MHz, $CDCl_3$, TMS as the internal reference) δ 209.7, 71.2, 63.8, 56.6, 54.2, 44.8, 44.2, 39.0, 38.1, 37.0, 35.5, 32.0, 31.5, 31.4, 28.6, 24.4, 22.8, 21.2, 13.4, 12.3 (C20), (C3), (C17), (C14), (C9), (C5), (C13), (C12), (C4), (C1), (C8), (C2), (C7, C10), (C21), (C6), (C15), (C16), (C11), (C18), (C19). Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.25; H, 10.69. Found: C, 79.30; H, 10.92. These melting points, $[\alpha]^{25}_D$, and ^{13}C NMR data were in agreement with those obtained for the authentic steroid purchased from Sigma.

Communications to the Editor

The Synthesis and Structure Determination from Powder Diffraction Data of $LaMo_5O_8$, a New Oxomolybdate Containing Mo_{10} Clusters

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The formation of metal clusters in mixed oxides in which molybdenum is present in a low formal oxidation state is now well established.[1] Examples include $Zn_2Mo_3O_8$, which contains triangular Mo_3 units,[2] $NaMo_4O_6$, which exhibits infinite chains of edge-sharing Mo_6 octahedra,[3] and $In_{11}Mo_{40}O_{62}$, with finite chains of four and five edge-sharing Mo_6 octahedra.[4] Structures containing more than one type of metal–metal bonded unit are also known, e.g., $La_3Mo_4SiO_{14}$.[5] In a careful survey of the system La–Mo–O by analytical electron microscopy[6] using a Jeol 2000FX TEMSCAN instrument, we found evidence for a new phase with a La:Mo ratio of 2:9. Oxygen analysis by X-ray emission spectroscopy[7] suggested the composition $La_2Mo_9O_{12}$, and a sample with this composition was prepared[8] for subsequent interrogation by powder diffraction methods.

Recent developments in both instrumental and computational aspects of powder diffractometry have made possible the precise determination of unknown structures from powder data. Examples include $FeAsO_4$,[9] determined from neutron data, $ZrKH(PO_4)_2$[10] and $AlPO_4$-12[11] from laboratory X-ray data, and ∝-$CrPO_4$,[12] I_2O_4,[13] and $MnPO_4 \cdot H_2O$[14] from synchrotron X-ray data. In the present work, high resolution synchrotron powder X-ray data were collected on the X13A beam line at the NSLS, Brookhaven National Laboratory, and neutron data were obtained on the diffractometer D2b at the ILL, Grenoble. The unit cell, a = 9.912 (1) Å, b = 9.093 (1) Å, c = 7.575 (1) Å and β = 109.05 (5)°, was determined by auto-indexing of the X-ray data[15] and con-

Table I. Neutron (N) and X-ray (X) Atomic Coordinates for $LaMo_5O_8$[a,b]

		x	*y*	*z*
La	N	0.0399 (8)	−0.001 (1)	0.255 (1)
	X	0.0420 (5)	0.0037 (7)	0.2609 (7)
Mo1	N	0.490 (1)	0.1161 (9)	0.610 (1)
	X	0.4863 (7)	0.1155 (9)	0.606 (1)
Mo2	N	0.5848 (8)	0.124 (1)	−0.004 (1)
	X	0.5845 (6)	0.1249 (7)	−0.0019 (9)
Mo3	N	0.3845 (9)	0.128 (1)	0.182 (1)
	X	0.3826 (7)	0.1269 (9)	0.1772 (9)
Mo4	N	0.2939 (9)	0.1271 (9)	0.808 (1)
	X	0.2970 (7)	0.1283 (9)	0.8083 (9)
Mo5	N	0.6805 (9)	0.122 (1)	0.387 (1)
	X	0.6769 (7)	0.119 (1)	0.3850 (8)
O1	N	0.848 (1)	−0.008 (2)	0.397 (1)
	X	0.844 (4)	−0.002 (7)	0.398 (5)
O2	N	0.934 (1)	0.227 (1)	−0.003 (2)
	X	0.932 (6)	0.230 (4)	0.01 (1)
O3	N	0.116 (1)	0.249 (1)	0.799 (2)
	X	0.118 (6)	0.253 (4)	0.80 (1)
O4	N	0.213 (1)	0.243 (1)	0.199 (2)
	X	0.215 (7)	0.255 (4)	0.21 (1)
O5	N	0.231 (1)	0.001 (1)	−0.008 (1)
	X	0.232 (4)	0.004 (4)	−0.003 (5)
O6	N	0.018 (2)	0.2484 (9)	0.399 (2)
	X	0.017 (8)	0.247 (4)	0.40 (1)
O7	N	0.826 (2)	0.241 (1)	0.602 (2)
	X	0.826 (7)	0.235 (4)	0.61 (1)
O8	N	0.329 (1)	0.004 (2)	0.382 (1)
	X	0.321 (4)	0.017 (8)	0.392 (5)

[a] Z = 4; space group $P2_1/a$. [b] R-factors: neutrons: R_I = 11.0%, R_p = 17.3%, R_{wp} = 19.9%, R_{exp} = 12.6%, χ^2 = 2.5; X-rays: R_I = 11.4%, R_p = 23.2%, R_{wp} = 27.0%, R_{exp} = 15.5%, χ^2 = 3.0.

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