Selective Acylation of A-Ring Precursors of Vitamin D Using Enzymes in Organic Solvents

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Whereas Chromobacterium viscosum lipase (CVL) catalyzes selectively the acylation of the C-5 hydroxyl of the three stereoisomeric vitamin D A-ring precursors 2a, 3a and 3b, only the C-3 hydroxyl of the fourth stereoisomer 2b is acylated under the same conditions in organic solvent. In a convenient application, the racemic vitamin D A-ring precursor 4, possessing only the C-5 hydroxyl, was resolved using suitable conditions identified from the studies of 2 and 3.

Introduction

The metabolism of vitamin D₃ (1a) involves C-25 hydroxylation in the liver to produce 25-hydroxyvitamin D₃ (1b) followed by C-1 hydroxylation in the kidney to give 1α,25-dihydroxyvitamin D₃ (1c, 1α,25-(OH)₂D₃), the hormonally active form of vitamin D. In addition to its long known calcemic action, a number of biological activities including those related to cancer, skin diseases, immunomodulatory effects, and Alzheimer's disease have been linked to this hormone. Accordingly, the synthesis of new analogues has drawn increasing attention from the standpoint of developing chemotherapeutically useful drugs. Substantial effort has also been directed toward developing cogent structure–function relationships to develop an improved paradigm for more effectively designing target analogues. For example, a number of A-ring carbinol diastereoisomers of 1α,25-(OH)₂D₃ (1e) recently synthesized in our laboratory proved highly informative in probing the structural demands of the nuclear vitamin D receptor, which mediates the genomic effects of vitamin D. It has also been determined that the C-1 epimer of 1α,25-(OH)₂D₃ is a potent inhibitor of the nongenomic calcium transport actions of 1α,25-(OH)₂D₃ (1e), and it has been found that the C-3 epimer is a natural metabolite of this same hormone. In addition, the synthesis of isotopically labeled derivatives of vitamin D₃ and its metabolites has provided convenient research tools for evaluation of biosynthetic pathways as well as chemical processes (e.g., [1,7]-sigmatropic hydrogen shifts).

A general approach leading to the synthesis of vitamin D analogues involves coupling of an appropriate CD-ring fragment and A-ring synthons such as enynes 2, 3, and 4 which have been derived from (S)- or (R)-carvone (5) or hydroxy ketol (6) (Chart 1). Recently, there emerged a need in our laboratory for preparing A-ring synths such as 2 and 3 modified selectively at their carbinol centers, as well as for preparing large amounts of racemic and enantiomerically pure A-ring enynol 4. Accordingly, we began an evaluation of enzymatic procedures for effecting these transformations. Lipases and proteases have been used effectively in regioselective acylation of natural products with various hydroxyl groups and in the resolution of racemic pharmaceutical compounds. Here we report a systematic study of the enzyme-catalyzed acylation in organic solvents of the four A-ring diastereoisomers 2a, 2b, 3a, and 3b and the novel finding that the cis stereoisomer 2b is uniquely acylated at its allylic (C-3) hydroxyl position, just the reverse of the nonallylic (C-5) acylation observed for 2a, 3a, and 3b. This acyla-
Even after long reaction times.

For acylation (Scheme 1). For the initial studies vinyl cylindracea lipase (CCL), Pseudomonas cepacia lipase papain gave only trace amounts of products instead of C-5.

In the case of the protease subtilisin no reaction occurred to determine which enzyme gives the best regioselectivity. The reaction procedure has also been extended to an application to the resolution of the related racemic synthons (±)-4.

**Results and Discussion**

### Acylation of 2a

The studies of enzymatic acylation were first focused on the A-ring fragment 2a, which possesses the natural carbonyl stereochemistry (3S,5R)₆,11 Initial experiments concerned screening lipases [Candida cylindracea lipase (CCL), Pseudomonas cepacia lipase (PSL), Chromobacterium viscosum lipase (CCL), porcine pancreatic lipase (PPL), and Candida antarctica lipase (CAL)] and proteases [subtilisin and papain] as catalysts to determine which enzyme gives the best regioselectivity for acylation (Scheme 1). For the initial studies vinyl acetate was used as the acylating agent and solvent, and the results are summarized in Table 1.

Of the enzymes studied, CVL gives the best result. It catalyzes acylation of the C-5-(R) hydroxyl group with excellent regioselectivity and in good yield. CCL also catalyzes acetylation of the C-5-(R) hydroxyl group with excellent regioselectivity and in good yield. CCL also possesses the natural carbinol stereochemistry.

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**Scheme 1**

**Table 1. Reaction of 2a with Vinyl Acetate 7a Catalyzed by Enzymes**

<table>
<thead>
<tr>
<th>enzyme</th>
<th>t (h)</th>
<th>convn (%)</th>
<th>8a (%)</th>
<th>9a (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL</td>
<td>115</td>
<td>82</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>PSL</td>
<td>115</td>
<td>85</td>
<td>47</td>
<td>53</td>
</tr>
<tr>
<td>CVL</td>
<td>96</td>
<td>72</td>
<td>&gt;95</td>
<td>&lt;5</td>
</tr>
<tr>
<td>PPL</td>
<td>&lt;10</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>papain</td>
<td>&lt;10</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>CAL</td>
<td>91</td>
<td>56</td>
<td>37</td>
<td>63</td>
</tr>
<tr>
<td>subtilisin</td>
<td>71</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* These processes were carried out at room temperature. † Calculated by 1H-NMR analysis. 

**Table 2. Reaction of 2a with Vinyl Esters 7a-e Catalyzed by CVL**

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>solvent</th>
<th>t (h)</th>
<th>convn (%)</th>
<th>8a (%)</th>
<th>9a (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Me</td>
<td>none</td>
<td>24</td>
<td>100</td>
<td>93(90)</td>
<td>7</td>
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<tr>
<td>b</td>
<td>Et</td>
<td>none</td>
<td>40</td>
<td>100</td>
<td>87(90)</td>
<td>13</td>
</tr>
<tr>
<td>c</td>
<td>Pr</td>
<td>none</td>
<td>96</td>
<td>100</td>
<td>84(85)</td>
<td>16</td>
</tr>
<tr>
<td>d</td>
<td>Ph</td>
<td>THF</td>
<td>96</td>
<td>100</td>
<td>85(70)</td>
<td>15</td>
</tr>
<tr>
<td>e</td>
<td>Ph</td>
<td>THF</td>
<td>113</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* These processes were carried out at 45 °C. † Calculated by 1H-NMR analysis. ‡ Values in parentheses represent isolated yields.

To more fully investigate the reaction conditions for this enzymatic acetylation, a variety of solvents were tested with two goals in mind. The first was to find the best enzyme–solvent system for acetylation of the C-5-(R) hydroxyl group. The second goal was to find conditions, if possible, for the regioselective acylation of the (3S)-hydroxyl group. Of the enzyme–solvent systems studied, CVL in THF gave the highest regioselectivity toward the C-5-(R) hydroxyl (8a), but the use of vinyl acetate as solvent afforded better results. CAL shows greater selectivity (74% of 8a, 26% of 9a) toward position 5 when the solvent is benzene. However, the opposite regioselectivity (39% of 8a, 61% of 9a) somewhat favoring the C-3 hydroxyl group is achieved when isopropyl ether is used as the solvent, but conditions for selective acylation at C-3 could not be defined.

To evaluate other acylating agents and take advantage of the observation that CVL shows excellent acetylation selectivity and yield with vinyl acetate as solvent, the reaction of 2a with other vinyl esters was studied. As shown in Table 2, the reactions were run at 45 °C using a ratio of vinyl ester to diol of ~10:1. The vinyl ester was used as the solvent whenever possible, but THF was added as solvent in those cases where the high boiling point of the acylating agent rendered cumbersome its removal from the product mixture. Acylation occurred selectively at the C-5-(R) hydroxyl except when vinyl benzoate was used wherein esterification did not take place even after heating at 45 °C for several days.

In summary, the results indicate that CVL will allow regioselective incorporation of a variety of acyl moieties at the C-5-(R) hydroxyl of 2a. While these results indicate that vinyl esters are generally suitable, acid anhydrides gave poor selectivity and afforded low conversion. Selective acylation at C-3 of 2a could not be effectively achieved.

**Acylation of the Stereoisomers of 2a: Its Enantiomer 3b and Cis Stereoisomers 3a and 2b.** Table 3 summarizes the results obtained for the acylation of the enantiomer of (3S,5R)-2a, namely (3R,5S)-3b shown in Scheme 2. For direct comparison to the data in Table 2 for 2a, CVL was also used and it was determined that the reaction with 3b also proceeds with quantitative...
regioselectivity toward the hydroxyl group at the C-5 position. The acylation reaction is however faster for 3b with vinyl acetate than its enantiomer 2a. Complete conversion of 3b with vinyl acetate at room temperature occurs in 7 h, while that of enantiomer 2a requires heating of the reaction mixture at 45 °C for 24 h (see entry a in Table 2 versus entry a in Table 3). The regioselectivities obtained with the different vinyl esters are somewhat higher than for 2a. Significantly, 3b reacts with vinyl benzoate at room temperature, which contrasts to the lack of reaction of enantiomer 2a under somewhat more forcing conditions (room temperature versus 45 °C).

Tables 4 and 5 and Schemes 4 and 5 summarize similar acylation studies of the cis stereoisomers (3S,5S)-3a and (3R,5R)-2b, respectively, which were synthesized as shown in Scheme 3. In the latter scheme, selective oxidation of the C-3 allylic alcohol of 2a and 3b using the Dess–Martin reagent[13] afforded ketones 12a and 12b, which were reduced in a conventional fashion to the requisite cis diols 2b and 3a, respectively. There was obtained ~3:1 cis to trans selectivity (separable by HPLC) in both cases.

As shown in Table 4, CVL-catalyzed acetylation of the cis diol 3a occurred with complete regioselectivity (100%).

Acylation of A-Ring Precursors of Vitamin D
isolated yields of 10.

Table 3. Reaction of 3b with Vinyl Esters 7a–e
Catalyzed by CVL<sup>a</sup>

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>solvent</th>
<th>t (h)</th>
<th>convn (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>&lt;sup&gt;10&lt;/sup&gt; (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>&lt;sup&gt;11&lt;/sup&gt; (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>a</td>
<td>Me</td>
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<td>7</td>
<td>100</td>
<td>94 (88)</td>
<td>6</td>
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<tr>
<td>b</td>
<td>Et</td>
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<td>7</td>
<td>100</td>
<td>94 (95)</td>
<td>6</td>
</tr>
<tr>
<td>c</td>
<td>Pr</td>
<td>none</td>
<td>5</td>
<td>100</td>
<td>95 (70)</td>
<td>5</td>
</tr>
<tr>
<td>d</td>
<td>ClCH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>THF</td>
<td>21</td>
<td>100</td>
<td>78 (71)</td>
<td>22</td>
</tr>
<tr>
<td>e</td>
<td>Ph</td>
<td>THF</td>
<td>119</td>
<td>100</td>
<td>92 (70)</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> These processes were carried out at room temperature. Calculated by 'H-NMR analysis. Values in parentheses represent isolated yields of 10.

Test

Table 4. Reaction of 3a with Vinyl Esters 7a,c–d
Catalyzed by CVL<sup>a</sup>

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>solvent</th>
<th>t (h)</th>
<th>convn (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>13 (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>14 (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>15 (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Me</td>
<td>none</td>
<td>48</td>
<td>100</td>
<td>1</td>
<td>95 (80)</td>
<td>4</td>
</tr>
<tr>
<td>c</td>
<td>Pr</td>
<td>none</td>
<td>144</td>
<td>100</td>
<td>&lt;2</td>
<td>&gt;98 (89)</td>
<td>0</td>
</tr>
<tr>
<td>d</td>
<td>ClCH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>THF</td>
<td>92</td>
<td>100</td>
<td>2.5</td>
<td>91 (85)</td>
<td>6.5</td>
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</tbody>
</table>

<sup>a</sup> These processes were carried out at room temperature. Calculated by 'H-NMR analysis. Values in parentheses represent isolated yields of 17.

Table 5. Reaction of 2b with Vinyl Esters 7a,c–d
Catalyzed by CVL<sup>a</sup>

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>solvent</th>
<th>t (h)</th>
<th>convn (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>16 (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>17 (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>18 (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Me</td>
<td>none</td>
<td>48</td>
<td>100</td>
<td>1</td>
<td>95 (80)</td>
<td>4</td>
</tr>
<tr>
<td>c</td>
<td>Pr</td>
<td>none</td>
<td>144</td>
<td>100</td>
<td>&lt;2</td>
<td>&gt;98 (89)</td>
<td>0</td>
</tr>
<tr>
<td>d</td>
<td>ClCH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>THF</td>
<td>92</td>
<td>100</td>
<td>2.5</td>
<td>91 (85)</td>
<td>6.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> These processes were carried out at room temperature. Calculated by 'H-NMR analysis. Values in parentheses represent isolated yields of 17.

Test

Scheme 2

\[
\text{3b} + \text{7} \xrightleftharpoons[CVL, \text{Solvent, r.t.}]{10} \text{11}
\]

[a, R=Me; b, R=Et; c, R=Pr; d, R=CH<sub>2</sub>Cl; e, R=Ph]

Scheme 3

\[
\text{2a}, \text{R}=\text{OH}; \text{R'}=\text{H}; \text{3b}, \text{R}=\text{H}; \text{R'}=\text{OH}
\]

Scheme 4


As shown in Table 5, parallel acylation of the remaining stereoisomer cis diol 2b proved most interesting wherein C-3 hydroxylation now dominates, just the opposite of its three C-3,5 stereoisomers 2a, 3a, and 3b. The selectivity and rate was qualitatively only slightly lower than that for 3a. Vinyl butyrate, used as both the acylating reagent and solvent, exhibited complete regioselectivity toward the hydroxyl group at the C-3 position.
From the results of the acylation of the four stereoisomers 2a, 2b, 3a, and 3b, it is clear that a hydroxyl group with the C-5-(S) or β orientation isacylated considerably more rapidly than with the corresponding (R) or α orientation (cf. 3a > 2a; and also (R)→(+)-4 > (S)→(-)-4 as discussed below). It also seems apparent that a hydroxyl with the C-3-(R) or α orientation isacylated more easily than with the C-3-(S) or β orientation, but selective acylation at a C-5-(S) or β orientation is more important (cf. acylation at C-5 dominates in the case of 3b). This also rationalizes why the selectivities for C-5 acylation are greater for substrate 3a (Table 4) than for substrate 3b (Table 3), but the differences are not large. Only in the case of isomer 2b (3R,5R), which possesses the C-5-(R) configuration (which attenuates strongly C-5 acylation) and the C-3-(S) orientation, but selective acylation at a C-5-(S) or β orientation is more important (cf. acylation at C-5 dominates in the case of 3b). These results could be useful in gleaning information about the steric requirements of the enzyme active site.

Resolution of A-Ring Synthon (±)-4. Given the difference in reaction times observed for the enantiomers 2a and 3b as well as 2b and 3a, we examined the possibility that CVL could be useful in the kinetic resolution of racemic A-ring fragment 4. Recently, this laboratory reported that 4-hydroxy-cyclohexanone (6) could be transformed to optically pure (S)→(-)-4, via racemic 4 through initial formation of its (1S,1’S) and (1R,1’S) carbamates using (S)-napthyl isocyanate, followed by separation via HPLC and then deprotection of the (1S,1’S) carbamate. Using CVL, the resolution of (±)-4 affording the natural enynol (-)-isomer could be nicely effected in only one step (Scheme 6). The reaction was carried out at room temperature using CVL with vinyl acetate acting as both acylating agent and solvent. After 18 h, GC analysis revealing 52% conversion, the reaction was stopped and flash chromatography (silica gel, 20% EtOAe-hexanes) of the product mixture gave the enantioomerically pure (S)→(-)-4 in high yield (97%, ≥97% ee). The remaining acetate (99% yield) was treated with sodium methoxide to afford (R)→(+)-4 which had 94% ee. Thus, this route provides a convenient direct route to 4a possessing the natural C-5 configuration (corresponding to the steroidal 3β-hydroxyl) of vitamin D3.

Summary

Evaluation of the enzymatic acylation of the vitamin D A-ring enymes 2a, 2b, 3a, and 3b revealed that CVL is the best enzyme for effecting practical levels of regioselectivity, and the novel finding was made that only the cis diol 2b is acylated at the allylic C-3 hydroxyl, the other stereoisomers being acylated at C-5. Direct application of this method leads to convenient kinetic resolution of fragment (±)-4. An explanation for the origin of the selectivity exerted by CVL for the stereoisomeric series 2a, 2b, 3a, and 3b awaits further evaluation.

Experimental Section

General. Subtilisin (type VIII bacterial), papain, porcine pancreatic lipase, PPL, (type II crude), and Candida cylindraceae lipase, CCL, (type VII crude), were obtained from Sigma Chemical Co. Pseudomonas cepacia lipase, PSL, was obtained from Amano Pharmaceutical Co. Chromobacterium viscosum lipase, CVL, was from Genzyme Co. Candida antarctica lipase, CAL SP 436L, was from Novo Nordisk Co. In all cases, crude product mixtures were subjected to 1H-NMR integration analysis (±3%) to determine percent conversion and relative percentages of esterified products as summarized in Tables 1–5.

Enzymatic Acetylation of (3S,5R)-2a in Vinyl Acetate. To a solution of 2a (10 mg, 0.066 mmol) in vinyl acetate (2.5 mL) was added one of the following enzymes: 300 mg of CCL, PPL, or papain; 10 mg of CCL, 60 mg of subtilisin; or 45 mg of CAL. The suspension was shaken at room temperature, and the progress of the reaction was followed by TLC until no further reaction was apparent. After removal of the enzyme by filtration and evaporation of the solvent and 1H-NMR analysis, the residual mixture was purified by HPLC (Rainin Microsorb, 1 × 25 cm, 5 μm silica gel column, 25% ethyl acetate/hexanes, 4 mL/min) to give monoacettes 8a and 9a. The results are summarized in Table 1. 8a: 1H-NMR δ 8.02 (s, 3H, C2-Me), 2.04 (s, 3H, Me), 2.20 (dd, 1H, H6, JHH = 17.1, JHH = 7.3 Hz), 6.51 (dd, 1H, Hs, JHH = 17.1, JHH = 4.6 Hz), 3.10 (s, 1H, sp-CH), 4.25 (m, 1H, Hs) and 5.14 (m, 1H, Hs). Additional data used for preparing Table 1 are presented in the supporting information section. In addition, an extended survey of these enzymes using various other solvents proved less effective: THF (PSL, CVL, CAL), benzene (PSL, CAL), pyridine (PSL, subtilisin), isopropyl ether (CAL), and dioxane (CAL).

Enzymatic Acetylation of 2a with Other Vinyl Esters. In a typical procedure, CVL (10 mg) was added to a solution of 2a (10 mg, 0.066 mmol) and vinyl ester (0.6 mmol) in THF (2.5 mL) or neat vinyl ester (2.5 mL) as solvent as summarized in Table 2. The suspension was shaken at 45 °C, and the progress of the reaction was followed by TLC. At 100% conversion the mixture was filtered and the solvent was removed under reduced pressure. After 1H-NMR analysis, the crude material was subjected to HPLC (Rainin Microsorb, 1 × 25 cm, 5 μm silica gel column, 25% etyl acetate/hexanes, 4 mL/min) to give monoacettes 8a and 9a. The results are summarized in Table 2. 8a: 1H-NMR δ 8.02 (s, 3H, C2-Me), 2.04 (s, 3H, Me), 2.20 (dd, 1H, H6, JHH = 17.1, JHH = 7.3 Hz), 6.51 (dd, 1H, Hs, JHH = 17.1, JHH = 4.6 Hz), 3.10 (s, 1H, sp-CH), 4.25 (m, 1H, Hs) and 5.14 (m, 1H, Hs). Additional data used for preparing Table 1 are presented in the supporting information section. In addition, an extended survey of these enzymes using various other solvents proved less effective: THF (PSL, CVL, CAL), benzene (PSL, CAL), pyridine (PSL, subtilisin), isopropyl ether (CAL), and dioxane (CAL).

(14) Note that α (down) and β (up) have their usual definitions for all of the structures described in this paper except for 1 (i.e., vitamin D3 and its metabolites). Because vitamin D is typically drawn in its more stable nonsteroidal, 6+4-trans conformation, the α’s (up) and β’s (down) are reversed in orientation for its A ring only.
(15) Enantiomeric purities indicated as ee ≥ 97% (±3%) by NMR analysis signifies that only one enantiomer was detected even at high spectrum amplitude.
(16) (a) For all major products, full spectral data are given in the supporting information. Selected 1H-NMR signals are also presented in the Experimental Section. The purity of all major isomers or intermediates was estimated by a combination of HPLC and NMR analysis. The level of purity is indicated by the inclusion of copies of 1H-NMR spectra and 13C-NMR spectra in the supporting information.
(b) Minor acylation products, the proportions of which were used in the preparation of Tables 1–5, were analyzed as mixtures (using a combination of TLC, HPLC, and 1H-NMR analysis) together with the major products produced in the enzymatic reactions. Detailed data for minor products are also presented at the end of the supporting information section.
Acylation of A-Ring Precursors of Vitamin D

The procedure parallels that indicated above for obtaining the compounds summarized in Table 3, were for reactions conducted at room temperature. Compositions 18a-e and 17a-c were purified by HPLC (Rainin Microbore, 1 × 25 cm, 5 μm silica gel column, 0.2% TFA/0.1% acetic acid/hexanes, 4 mL/min) to give compounds 18a–d. The 1H-NMR data of 18a are given below. 18b: 1H NMR δ 1.13 (t, 3H, Me, JHH = 7.6 Hz), 2.03 (s, 3H, Me), 2.20 (dd, 1H, JHH = 17.1 Hz, JHM = 7.1 Hz), 2.31 (q, 2H, MeCH2, JHH = 7.6 Hz), 2.61 (dd, 1H, JHH = 17.2, JHM = 4.9 Hz), 3.09 (s, 1H, sp-CH), 4.26 (m, 1H, H5), and 5.16 (m, 1H, H4). 8c: 1H NMR δ 0.94 (t, 3H, Me, JHH = 7.4 Hz), 2.02 (s, 3H, Me), 2.19 (dd, 1H, JHH = 17.1 Hz, JHM = 4.9 Hz), 3.10 (1H, sp-CH), 4.25 (m, 1H, H5), and 5.15 (1H, H4). 8d: 1H NMR δ 2.03 (s, 3H, C2-Me), 2.26 (dd, 1H, JHH = 17.1 Hz, JHM = 7.1 Hz, JHH = 7.2 Hz), 2.26 (t, 3H, EtCH2, JHH = 7.4 Hz), 2.60 (dd, 1H, JHH = 17.1 Hz, JHM = 4.9 Hz), 3.11 (1H, sp-CH), 4.04 (s, 2H, CICH2), 4.30 (t, 1H, JHH = 4.1 Hz), and 5.25 (1H, H5). Additional data used for preparing Table 3 are presented in the supporting information section.16

Enzymatic Acylation of (3R,5S)-3b with Vinyl Esters.

The reaction mixture was stirred at room temperature for 1 h under argon, and then the resulting mixture was diluted with ether and washed with a 1:1 mixture (v/v) of saturated aqueous Na2S2O3 and NaHCO3 solution. The organic layer was then extracted with 100 to 200 °C at 4 °C/min. The retention times observed were 28.7 min for (−)-4 and 34.4 min for 19. The reaction mixture was filtered and the enzyme washed with methylene chloride. The solvent was removed, and the products were separated by flash column chromatography using silica gel (20% ethyl acetate/hexanes as eluent) to afford (S)-7 (94% ee according to 1H-NMR spectroscopy using 25 cm, 5 μm silica gel column, 0.2% TFA/0.1% acetic acid/hexanes, 4 mL/min).

Preparation of Cis-Isomers (3R,5R)-2b and (3S,5S)-3a.

Sodium borohydride (1.3 g, 34 mmol) in methanol (30 mL) was added to (3R,5R)-2b (302 mg, 2 mmol) in methanol (30 mL). After stirring for 30 min, the reaction mixture was extracted with ether three times with ether and then the combined organic extract was washed with Na2S2O3 and NaHCO3 solution. The organic layer was then dried (MgSO4) and evaporated to dryness. The residue was purified by flash chromatography on silica gel using 45% ethyl acetate/hexanes to afford after vacuum drying 310 mg (82%) of ketone 12a (or 12b): 1H NMR δ 1.99 (s, 3H, Me), 2.48–2.65 (dd, 1H, JHH = 16.3, JHM = 8.7 Hz + m, 1H, H4), 2.72–2.90 (dd, 1H, JHH = 15.8, JHM = 4.0 Hz + m, 1H, H4), 3.15 (s, 1H, sp-CH), and 4.31 (m, 1H, H5). When the same procedure was applied to the enantiomer 3b, essentially the same result was obtained except the reaction time had to be increased to 24 h (possibly due to decreased reactivity of oxidant).

Preparation of Cis-Isomers (3R,5R)-2b and (3S,5S)-3a with Vinyl Esters.

The procedure was the same as described for obtaining the data given in Table 2. The data obtained for 3a and 2b are summarized in Tables 4 and 5, respectively. Both 3a and 2b were reacted at room temperature. Compositions 13a–d and 17a–c were purified by HPLC (Rainin Microbore, 1 × 25 cm, 5 μm silica gel column, 20% TFA/0.1% acetic acid/hexanes, 4 mL/min). 13a: 1H NMR δ 1.98–2.17 (m, 8H, 2Me + H4), 2.33–2.54 (m, 2H, H2), 3.11 (1H, sp-CH), 4.09 (1H, m, H5), and 5.19 (1H, H4). 13c: 1H NMR δ 0.94 (t, 3H, Me, JHH = 7.4 Hz), 1.63 (m, 2H, MeCH2), 2.05 (s, 3H, Me), 2.27 (t, 2H, EtCH2, JHH = 7.5 Hz), 2.33–2.52 (m, 2H, H2), 3.10 (1H, sp-CH), 4.09 (1H, H5), and 5.21 (1H, H4). 13d: 1H NMR δ 0.26 (s, 3H, Me), 3.12 (1H, sp-CH), 4.20 (1H, CICH2), 4.70 (m, 1H, H5), 5.19 (5.0 Hz), 5.25 (1H, sp-CH), and 5.25 (1H, H5, 5.7 Hz). 17a: 1H NMR δ 1.90 (s, 3H, C2-Me), 2.09 (3H, Me), 2.17 (dd, 1H, JHH = 14.4, JHM = 5.8, JHM = 3.0 Hz), 3.15 (1H, sp-CH), 4.03 (m, 1H, H5), and 5.46 (1H, JHH = 5.7 Hz). 17c: 1H NMR δ 0.96 (t, 3H, Me, JHH = 7.4 Hz), 1.67 (m, 2H, MeCH2), 1.89 (s, 3H, C2-Me), 2.17 (dd, 1H, JHH = 13.2, JHM = 5.7, JHH = 3.0 Hz), 2.63 (2H, EtCH2, JHH = 7.4 Hz), 3.15 (1H, sp-CH), 4.04 (m, 1H, H5), and 5.47 (1H, JHH = 5.5 Hz). 17d: 1H NMR δ 1.91 (s, 3H, Me), 2.22 (dd, 1H, JHH = 13.4, JHM = 5.9, JHM = 3.1 Hz), 3.18 (1H, sp-CH), 4.06 (m, 1H, H5), 4.08 (2H, CICH2), and 5.53 (1H, JHH = 5.6 Hz). Additional data used for preparing Tables 4 and 5 are presented in the supporting information section.

(5R)- and (5S)-3-Ethynyl-5-hydroxy-2-methyl-2-cyclohexen-1-one (12a and 12b, respectively). To a mixture of the Dess-Martin periodinane reagent (1.5 g, 3.75 mmol) in dry acetonitrile (30 mL) was added 2a (380 mg, 2.5 mmol). The reaction mixture was stirred at room temperature for 1 h under argon, and then the resulting mixture was diluted with ether and washed with a 1:1 mixture (v/v) of saturated aqueous Na2S2O3 and NaHCO3 solution. The organic layer was then dried (MgSO4) and evaporated to dryness. The residue was purified by flash chromatography on silica gel using 45% ethyl acetate/hexanes to afford after vacuum drying 310 mg (82%) of ketone 12a (or 12b): 1H NMR δ 1.99 (s, 3H, Me), 2.48–2.65 (dd, 1H, JHH = 16.3, JHM = 8.7 Hz + m, 1H, H4), 2.72–2.90 (dd, 1H, JHH = 15.8, JHM = 4.0 Hz + m, 1H, H4), 3.15 (s, 1H, sp-CH), and 4.31 (m, 1H, H5). When the same procedure was applied to the enantiomer 3b, essentially the same result was obtained except the reaction time had to be increased to 24 h (possibly due to decreased reactivity of oxidant).

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Spectral and analytical data (23 pages). This material is contained on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.
