Unexpectedly Facile Hydrolysis of Digoxin Esters. The Importance of Appropriate Controls in Lipase-Mediated Hydrolysis

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Digoxin (**1**) is a steroidal glycoside produced by the plant family *Digitalis* which is commonly used in the management of various cardiac arrythmias.[1] Bioactivity is dependent on the intact lactone ring and the triose oligosaccharide at the 3-position of the steroid ring. Due to the toxicity of digoxin at higher concentrations, it is necessary to measure serum concentrations of the drug to maintain proper dosing.[2] A simple and practical method is via immunoassays, which employ derivatives of digoxin for the construction of immunological reagents and as cross reactants. There is thus a need for derivatives of digoxin which contain the intact structural features of the steroid backbone, the lactone ring, and a modified carbohydrate required for proper antibody reactivity.

We required *some* digoxin derivatives with an extended carboxylic acid on the modified terminal digitoxose **4a–e** for the development of immunological reagents. Treatment of digoxin with sodium periodate cleaves the terminal digitoxose to produce the dialdehyde **2**,[3,4] which is reported to undergo reductive amination to form 3′-oxaperhydroazepine derivatives **3**.[3] We thus prepared the dialdehyde **2**,[4] and reacted it with a series of amino acids in order to synthesize the required digoxin acid derivatives. However, these reactions produced the deglycosolated product (digoxigenin bis-digitoxoside, **5**) rather than the desired acids![5]

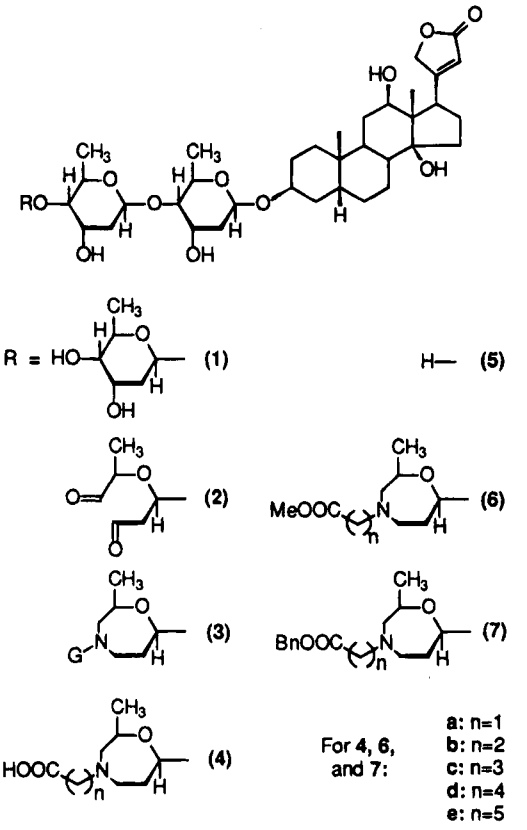
Enzymes have been effectively used for regioselective transformations where traditional organic synthesis fails.[6] We have previously reported the synthetic utility of lipase for enzymatic hydrolysis of ester derivatives of rapamycin, a molecule which is sensitive to hydrolysis, elimination, reduction, and oxidation under mild conditions.[6] The digoxin molecule presents similar challenges. Hoping for a selective lipase-mediated hydrolysis, we thus decided to prepare a series of digoxin esters. Dialdehyde **2** was reacted with a series of methyl amino ester hydrochlorides in the presence of sodium cyanoborohydride to produce methyl esters **6a–e**, while the benzyl esters **7a–e** were prepared using the benzyl amino ester *p*-toluenesulfonates.

During the preparation of the benzyl ester **7b**, facile transesterification was observed. This fact reinforced the need to perform appropriate control experiments during lipase hydrolysis. To determine the extent of hydrolysis, each of the esters was subjected to aqueous buffers (pH 6.0, 7.0, and 8.0), with and without lipase. Incubations were conducted for 24 h at room temperature. The course of hydrolysis was monitored by HPLC. The results are presented in Table 1. Only benzyl ester **7e** hydrolyzed in the presence of lipase. Methyl and benzyl esters with an $n = 2$ or 3 linker hydrolyzed without the enzyme catalyst, and the presence of lipase did not effect the rate of hydrolysis.

Table 1. Hydrolysis (%) of Digoxin Esters with and without Lipase

	control[a]			lipase[b]		
compound	pH 6.0	pH 7.0	pH 8.0	pH 6.0	pH 7.0	pH 8.0
6a	nr	nr	nr	nr	nr	nr
6b	24	42	47	20	37	46
6c	7	11	16	5	11	15
6d	nr	nr	nr	nr	nr	nr
6e	nr	nr	nr	nr	nr	nr
7a	nr	nr	nr	nr	nr	nr
7b	27	40	43	30	44	44
7c	4	7	10	7	16	19
7d	nr	nr	nr	nr	nr	nr
7e	nr	nr	nr	55	95	>99

[a] Buffer only. [b] Buffer containing lipase. nr = no reaction.



It is interesting that only **7e**, the benzyl ester most distant from the digitoxose moiety, was hydrolyzed employing lipase. This was quite intriguing, since the corresponding methyl ester **6e** is not a lipase substrate and since our previous work has shown that methyl esters are at least comparable to benzyl esters as lipase substrates.[6] This phenomenon was further evidenced by a competitive hydrolysis study between methyl ester **6e** and benzyl ester **7e**. The two esters were combined in the presence of lipase, and the hydrolysis was monitored by HPLC. As shown in Figure 1, methyl ester **6e** was observed to be unreactive in the presence of lipase, while benzyl ester **7e** was hydrolyzed. The rate of hydrolysis of benzyl ester **7e** was slightly enhanced at pH 8.0 (higher pH lead to lactone decomposition). When benzyl ester

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(6) Adamczyk, M., Gebler, J. C., and Mattingly, P. G. *Tetrahedron Lett.* **1994**, *35*, 1019, and references therein.

0022-3263/95/1960-3557$09.00/0

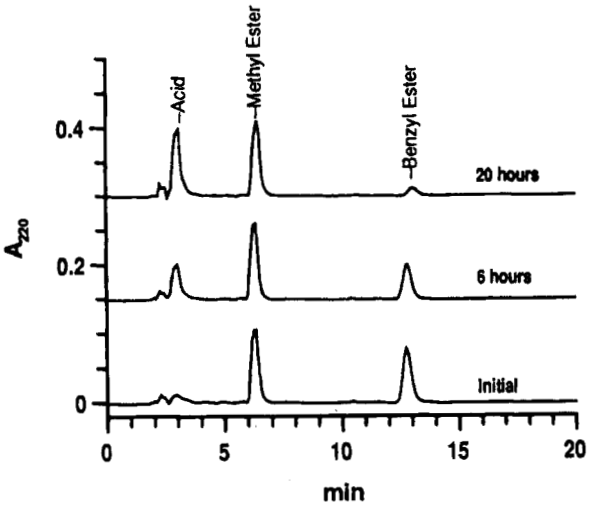

Figure 1. HPLC chromatograms for competitive hydrolysis of methyl ester **6e** and benzyl ester **7e**.

7e was placed in lipase free buffer, no ester cleavage was observed even after 1 week of incubation.

The fact that lipase-mediated hydrolysis is limited to benzyl ester **7e** demonstrates the selectivity of lipase for hydrophobic substrates. It appears that the hydrophilic nature of the carbohydrate, which is located too closely to the ester groups of compounds with shorter linking groups, prevents approach of the active site of the lipase. *Both* the increased hydrophobic nature of the benzyl group and the placement of the benzyl ester further away from the sugars results in creation of a preferred substrate for enzymatic hydrolysis.

It is also very interesting that esters with an $n = 2$ or 3 linker hydrolyse without requiring an enzyme catalyst under very mild conditions. Since other members of the series of methyl and benzyl esters do not exhibit this behavior, we conclude that this phenomenon is an intramolecular one, specific for the two linker lengths described. Since the oxaperhydroazepine nitrogen would be protonated at the pH range tested, selective ester hydrolysis could be initiated by intramolecular proton transfer from a neighboring imminium ion.

The hydrolyses of esters **7b** and **7c** and the lipase hydrolysis of **7e** were scaled up to afford useful quantities of immunological reagents, resulting in isolated yields of the corresponding acids **4b**, **4c**, and **4e** of ≥70%. The yields of materials produced are sufficient for the construction of immunogens and tracers required for complete development of an immunoassay capable of monitoring digoxin.

In this work, we have described the unexpectedly facile hydrolysis of certain digoxin derived esters. These compounds were obtained in good yield on a scale suitable for the preparation of immunological reagents. Our work also emphasizes the importance of performing appropriate control experiments while exploring the use of enzymes in the synthesis of organic compounds. Moreover, we have demonstrated an additional example[6] of a selective deprotection of a benzyl ester in the presence of other sensitive, hydrolyzable functionality, the equivalent of benzyl ester reduction. This chemoenzymatic hydrolysis further illustrates the substrate specificity of lipase in situations where complex molecular topology precludes the use of typical organic reactions.

Experimental Section

All reagents were purchased from Aldrich Chemical Co., Inc., and were used without further purification, except for buffers, which were purchased from Fisher Chemical. Solvents (HPLC grade) and silica gel were obtained from E. Merck Science and were used without further purification. ^{1}H NMR and ^{13}C NMR were recorded at 300 and 75 MHz, respectively. Electrospray mass spectrometry (ESMS) was performed on a Perkin-Elmer Siex API III. The lipase (*Pseudomonas sp.*) used was Amano LPL-80. HPLC was used to ascertain purity of new compounds. HPLC was performed on a Phenomenex Primesphere 5 μm C_{18} 250 mm × 3.2 mm column (column temperature of 55 °C), employing a photodiode array detector (λ_{max} for digoxin derivatives is ~220 nm) and elution at a flowrate of 0.5 mL/min. Acids were analyzed using mobile phase A (35% 50 mM NH_4OAc: 65% MeOH); esters utilized mobile phase B (25% 50 mM NH_4OAc: 75% MeOH). All isolated compounds were obtained at >98% purity.

General Procedure for the Synthesis of Oxaperhydroazepines 6a–e. Digoxin dialdehyde was prepared according to a literature procedure.[4] A portion of the isolated aldehyde (100 mg, 129 μmol) was dissolved in 1.0 mL of anhydrous MeOH. β-Alanine methyl ester hydrochloride (16 mg, 116 μmol) was added, and the solution was stirred for 5 min. Sodium cyanoborohydride (16 mg, 255 μmol) was added, and the reaction was stirred for 12 h. The reaction mixture was concentrated and chromatographed directly on silica gel, eluting with 5% MeOH/$CHCl_3$, to provide 65 mg (66%) of oxaperhydroazepine **6b** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.94 (s, 1H), 4.91–4.83 (m, 5H), 4.23 (br, 1H), 4.15 (d, 1H, J = 5.3 Hz), 4.02 (br, 1H), 3.81–3.69 (m, 3H), 3.68 (s, 3H), 3.40–3.30 (m, 2H), 3.22 (dd, 1H, J = 9.7 and 2.9 Hz), 3.16 (dd, 1H, J = 9.4 and 2.9 Hz), 3.03–2.99 (m, 2H), 2.82 (t, 1H, J = 6.6 Hz), 2.78–2.60 (m, 4H), 2.45 (t, 1H, J = 7.0 Hz), 2.43–2.34 (m, 2H), 2.16–2.10 (m, 3H), 1.93–1.26 (m, 24H), 1.22 (d, 3H, J = 6.2 Hz), 1.21 (d, 3H, J = 6.1 Hz), 1.14 (d, 3H, J = 6.2), 0.93 (s, 3H), 0.80 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.7, 172.9, 117.5, 104.1, 98.2, 95.4, 85.8, 82.5, 81.6, 77.2, 76.9, 75.1, 74.1, 73.8, 72.5, 68.4, 68.1, 66.4, 65.3, 55.6, 54.2, 51.7, 50.8, 45.6, 41.4, 37.1, 36.8, 36.3, 36.0, 35.0, 33.2, 32.8, 32.6, 30.3, 30.2, 29.8, 27.4, 26.6, 26.5, 23.6, 21.7, 19.5, 18.2, 9.0; HRMS calcd for $C_{45}H_{72}NO_{14}$ 850.4953, found 850.4942; HPLC (mobile phase A) retention time 11.2 min.

6a: ^{1}H NMR ($CDCl_3$) δ 5.94 (s, 1H), 4.95–4.83 (m, 5H), 4.23 (m, 1H), 4.17 (m, 1H), 4.01 (br, 1H), 3.86–3.72 (m, 3H), 3.70 (s, 3H), 3.40–3.30 (m, 3H), 3.22 (dd, 1H, J = 9.7 and 3.0 Hz), 3.16 (dd, 1H, J = 9.4 and 2.9 Hz), 3.03–2.99 (br, 1H), 2.82 (m, 1H), 2.78–2.70 (m, 2H), 2.65 (dd, 1H, J = 12.9 and 9.4 Hz), 2.26–1.26 (m, 25H), 1.215 (d, 3H, J = 6.2 Hz), 1.21 (d, 3H, J = 6.1 Hz), 1.14 (d, 3H, J = 6.2), 0.92 (s, 3H), 0.79 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.6, 171.5, 117.5, 104.1, 98.2, 95.4, 85.8, 82.5, 81.5, 77.2, 75.1, 74.1, 73.7, 72.4, 68.4, 68.0, 66.4, 64.7, 59.9, 55.5, 51.6, 50.9, 45.6, 41.4, 37.1, 36.8, 36.3, 35.8, 35.0, 33.2, 32.5, 30.3, 30.1, 30.0, 29.8, 27.4, 26.6, 26.5, 23.5, 21.7, 19.5, 18.1, 8.9, HRMS calcd for $C_{44}H_{70}NO_{14}$ 836.4783, found 836.4796; HPLC (mobile phase A) retention time 10.6 min.

6c: ^{1}H NMR ($CDCl_3$) δ 5.92 (s, 1H), 4.93–4.83 (m, 5H), 4.23 (m, 1H), 4.15 (m, 1H), 4.00 (br, 1H), 3.81–3.73 (m, 3H), 3.66 (s, 3H), 3.40–3.30 (m, 2H), 3.22 (dd, 1H, J = 9.5 and 2.6 Hz), 3.16 (dd, 1H, J = 9.3 and 2.8 Hz), 3.06 (br, 1H), 2.78–2.54 (m, 3H), 2.45 (t, 1H, J = 7.0 Hz), 2.33 (m, 2H), 2.20–1.26 (m, 27H), 1.20 (d, 6H, J = 6.2 Hz), 1.13 (d, 3H, J = 6.1 Hz), 0.91 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.7, 174.0, 117.5, 104.1, 98.2, 95.4, 85.8, 82.5, 81.6, 77.2, 75.0, 74.0, 73.7, 72.4, 68.4, 68.1, 66.4, 66.3, 65.5, 55.5, 51.5, 51.0, 45.6, 41.4, 37.1, 36.7, 36.2, 36.0, 35.0, 33.2, 32.8, 31.7, 30.3, 30.2, 29.8, 27.4, 26.6, 26.4, 23.5, 22.6, 21.6, 19.5, 18.2, 18.1, 8.9; HRMS calcd for $C_{46}H_{74}NO_{14}$ 864.5109, found 864.5097; HPLC (mobile phase A) retention time 12.0 min.

6d: ^{1}H NMR ($CDCl_3$) δ 5.91 (s, 1H), 4.87–4.82 (m, 5H), 4.22 (m, 1H), 4.15 (m, 1H), 4.02 (br, 1H), 3.82–3.70 (m, 3H), 3.65 (s, 3H), 3.40–3.30 (m, 2H), 3.22 (dd, 1H, J = 9.7 and 2.7 Hz), 3.12 (dd, 1H, J = 9.6 and 2.9 Hz), 3.06 (br, 1H), 2.78–2.51 (m, 3H), 2.43 (t, 1H, J = 7.3 Hz), 2.31 (t, 1H, J = 7.3 Hz), 2.20–1.26 (m, 29H), 1.19 (d, 6H, J = 6.2 Hz), 1.12 (d, 3H, J = 6.2), 0.90 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.8, 174.0, 117.5, 104.0, 98.2, 95.4, 85.8, 82.5, 81.6, 77.2, 75.0, 73.9, 73.7, 72.4, 68.4, 68.0, 66.4, 66.3, 65.5, 55.6, 51.5, 51.0, 45.6, 41.3, 37.1, 36.7, 36.2, 35.9, 34.9, 33.8, 33.1, 32.5, 30.2, 30.1, 29.7, 27.4, 26.8, 26.5, 23.5, 22.7,

21.7, 19.5, 18.2, 9.0; HRMS calcd for $C_{47}H_{76}NO_{14}$ 878.5266, found 878.5251; HPLC (mobile phase A) retention time 12.3 min.

6e: ^{1}H NMR ($CDCl_3$) δ 5.93 (s, 1H), 4.91–4.83 (m, 5H), 4.23 (m, 1H), 4.17 (m, 1H), 4.02 (br, 1H), 3.83–3.72 (m, 3H), 3.68 (s, 3H), 3.40–3.28 (m, 2H), 3.22 (dd, 1H, J = 9.7 and 2.9 Hz), 3.12 (dd, 1H, J = 9.4 and 2.9 Hz), 3.03 (br, 2H), 2.78–2.51 (m, 3H), 2.43 (t, 1H, J = 7.3 Hz), 2.31 (t, 1H, J = 7.3 Hz), 2.20–1.26 (m, 31H), 1.21 (d, 6H, J = 6.2 Hz), 1.15 (d, 3H, J = 6.3), 0.92 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.8, 174.1, 117.5, 104.1, 98.2, 95.4, 88.5, 85.8, 82.5, 81.6, 76.8, 75.0, 73.9, 73.7, 72.4, 68.4, 68.0, 66.4, 65.5, 58.7, 55.5, 51.5, 50.9, 45.6, 41.3, 37.1, 36.7, 36.2, 35.9, 34.9, 33.2, 32.5, 30.2, 30.1, 29.7, 29.6, 27.4, 26.9, 26.8, 26.4, 23.5, 21.6, 19.6, 18.1, 8.9; HRMS calcd for $C_{48}H_{78}NO_{14}$ 892.5422, found 892.5402; HPLC (mobile phase A) retention time 13.9 min.

General Procedure for the Synthesis of Oxaperhydroazepines 7a–e. 6-Aminocaproic acid benzyl ester *p*-toluenesulfonate was prepared according to a literature procedure.[7] Aminocaproic acid benzyl ester *p*-toluenesulfonate (90 mg, 0.23 mmol) was dissolved in 2.0 mL of MeOH, diisopropylethylamine (36 μL, 0.20 mmol) was added, and the solution was stirred for 5 min. A solution of digoxin dialdehyde (200 mg, 0.26 mmol) in 2.0 mL of MeOH was added, followed by sodium cyanoborohydride (16 mg, 255 μmol), and the reaction was stirred for 12 h. The reaction mixture was diluted with 8 mL of $CHCl_3$ and filtered through a plug of silica gel, eluting with 30% MeOH/$CHCl_3$. The eluate was concentrated and the residue repurified on silica gel, eluting with 5% MeOH/$CHCl_3$, to provide 133 mg (63%) of oxaperhydroazepine **7e** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.94 (s, 1H), 5.11 (s, 2H), 4.95–4.79 (m, 5H), 4.23 (m, 1H), 4.17 (m, 1H), 4.01 (br, 1H), 3.83–3.70 (m, 3H), 3.39–3.29 (m, 2H), 3.23 (dd, 1H, J = 9.3 and 2.8 Hz), 3.12 (dd, 1H, J = 9.4 and 2.9 Hz), 3.08 (br, 1H), 2.74–2.54 (m, 3H), 2.46–2.26 (m, 3H), 2.20–1.25 (m, 32H), 1.21 (d, 3H, J = 6.2 Hz), 1.20 (d, 3H, J = 6.2 Hz), 1.11 (d, 3H, J = 7.4 Hz), 0.91 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.8, 174.7, 128.5, 128.2, 117.5, 104.0, 98.1, 95.4, 85.9, 82.5, 81.6, 77.2, 77.1, 75.0, 73.8, 73.7, 72.4, 68.4, 68.0, 66.4, 66.3, 66.1, 65.5, 58.8, 55.5, 50.9, 45.6, 41.4, 37.1, 36.7, 36.2, 36.0, 35.0, 34.2, 33.2, 32.6, 30.3, 30.2, 29.8, 27.4, 26.8, 26.6, 26.4, 23.5, 21.7, 19.6, 18.2, 18.1, 9.0; HRMS calcd for $C_{54}H_{82}NO_{14}$ 968.5735, found 968.5729; HPLC (mobile phase B) retention time 12.1 min.

7a: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.93 (s, 1H), 5.14 (s, 2H), 4.90–4.83 (m, 5H), 4.23 (m, 1H), 4.17 (m, 1H), 4.02 (br, 1H), 3.85–3.70 (m, 3H), 3.39–3.29 (m, 2H), 3.23 (dd, 1H, J = 9.3 and 2.8 Hz), 3.12 (dd, 1H, J = 9.4 and 2.7 Hz), 3.10 (br, 1H), 2.90 (m, 1H), 2.79 (m, 2H), 2.66 (dd, 1H, J = 13.1 and 9.4 Hz), 2.20–1.25 (m, 26H), 1.22 (d, 3H, J = 6.2 Hz), 1.21 (d, 3H, J = 6.1 Hz), 1.13 (d, 3H, J = 6.2 Hz), 0.92 (s, 3H), 0.79 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.8, 170.8, 135.5, 128.6, 128.4, 128.3, 117.5, 104.1, 98.2, 95.4, 85.8, 82.5, 81.5, 77.2, 74.9, 74.0, 73.9, 73.7, 72.4, 68.4, 68.0, 66.4, 66.3, 64.7, 60.0, 59.8, 55.5, 50.9, 50.8, 45.6, 41.4, 37.1, 36.8, 36.3, 35.9, 35.0, 33.2, 32.6, 30.3, 30.2, 29.8, 27.4, 26.6, 26.5, 23.6, 22.7, 21.7, 19.4, 18.2, 9.0; HRMS calcd for $C_{50}H_{74}NO_{14}$ 912.5109, found 912.5101; HPLC (mobile phase B) retention time 8.1 min.

7b: In the reductive amination with β-alanine benzyl ester *p*-toluenesulfonate, two products were isolated: 32% of less polar **7b**, and 24% of **6b**. **7b** had the following spectral data: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.93 (s, 1H), 5.12 (d, 2H, J = 2.0 Hz), 4.94–4.84 (m, 4H), 4.78 (dd, 1H, J = 7.0 and 4.3 Hz), 4.12 (m, 1H), 4.01 (br, 1H), 3.83–3.63 (m, 3H), 3.39–3.29 (m, 2H), 3.23 (dd, 1H, J = 9.3 and 2.8 Hz), 3.09 (dd, 1H, J = 9.4 and 2.7 Hz), 3.05 (br, 1H), 2.82 (t, 1H, J = 6.4 Hz), 2.74–2.60 (m, 2H), 2.48 (t, 1H, J = 7.0 Hz), 2.33 (dd, 1H, J = 12.9 and 9.6 Hz), 2.76–1.25 (m, 28H), 1.21 (d, 3H, J = 6.2 Hz), 1.20 (d, 3H, J = 6.2 Hz), 1.11 (d, 3H, J = 7.4 Hz), 0.91 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.8, 172.3, 136.0, 128.6, 128.3, 128.2, 117.6, 104.0, 98.2, 95.4, 85.9, 82.6, 81.6, 77.3, 75.1, 74.1, 73.7, 72.5, 68.5, 68.1, 66.5, 66.3, 65.4, 55.5, 54.3, 50.1, 45.6, 41.4, 37.1, 36.7, 36.2, 36.0, 35.0, 33.2, 33.2, 32.6, 30.3, 30.2, 29.8, 27.4, 26.6, 26.5, 23.6, 22.2, 21.7, 19.4, 18.2, 9.0; FABMS (M + H)$^+$ at 926; HPLC (mobile phase B) retention time 9.0 min.

7c: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.92 (s, 1H), 5.11 (s, 2H), 4.90–4.83 (m, 5H), 4.23 (m, 1H), 4.16 (m, 1H), 4.01 (br, 1H), 3.83–3.73 (m, 3H), 3.39–3.29 (m, 2H), 3.23 (dd, 1H, J = 9.3 and 2.8 Hz), 3.12 (dd, 1H, J = 9.4 and 2.7 Hz), 3.10 (br, 1H), 2.74–2.54 (m, 3H), 2.45 (t, 1H, J = 7.0 Hz), 2.38 (t, 1H, J = 6.4 Hz), 2.33 (dd, 1H, J = 12.9 and 9.5 Hz), 2.20–1.25 (m, 28H), 1.21 (d, 3H, J = 6.2 Hz), 1.20 (d, 3H, J = 6.2 Hz), 1.11 (d, 3H, J = 7.4 Hz), 0.91 (s, 3H), 0.78 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.9, 173.4, 136.0, 128.6, 128.3, 128.2, 117.6, 104.0, 98.2, 95.4, 85.9, 82.5, 81.7, 77.3, 75.1, 73.8, 72.4, 68.5, 68.1, 68.0, 66.5, 66.4, 66.3, 65.5, 57.9, 55.6, 51.1, 45.6, 41.4, 37.1, 36.8, 36.7, 36.3, 36.0, 35.0, 33.2, 32.6, 30.3, 30.2, 29.8, 27.4, 26.6, 26.5, 23.6, 22.7, 21.7, 19.6, 18.2, 9.0; HRMS calcd for $C_{54}H_{78}NO_{14}$ 940.5422, found 940.5402; HPLC (mobile phase B) retention time 9.8 min.

7d: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.93 (s, 1H), 5.11 (s, 2H), 4.90–4.83 (m, 5H), 4.23 (m, 1H), 4.16 (m, 1H), 4.01 (br, 1H), 3.83–3.73 (m, 3H), 3.39–3.29 (m, 2H), 3.23 (dd, 1H, J = 9.3 and 2.8 Hz), 3.12 (dd, 1H, J = 9.4 and 2.7 Hz), 3.11 (br, 1H), 2.74–2.54 (m, 3H), 2.45 (t, 1H, J = 7.3 Hz), 2.38 (t, 1H, J = 7.3 Hz), 2.31 (dd, 1H, J = 12.9 and 9.5 Hz), 2.20–1.25 (m, 30H), 1.22 (d, 3H, J = 6.2 Hz), 1.21 (d, 3H, J = 6.2 Hz), 1.13 (d, 3H, J = 7.4 Hz), 0.91 (s, 3H), 0.79 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.9, 173.4, 136.0, 128.6, 128.2, 117.6, 104.0, 98.2, 95.4, 85.9, 82.5, 81.7, 77.2, 75.1, 73.8, 72.4, 68.5, 68.1, 68.0, 66.5, 66.4, 66.2, 65.5, 58.4, 55.6, 50.9, 45.6, 41.4, 37.1, 36.7, 36.3, 35.9, 35.0, 34.0, 33.2, 32.6, 30.3, 30.2, 29.8, 27.4, 26.6, 26.5, 23.6, 22.7, 21.7, 19.6, 18.2, 9.0; HRMS calcd for $C_{53}H_{80}NO_{14}$ 954.5579, found 954.5559; HPLC (mobile phase B) retention time 10.3 min.

Hydrolysis Study of Digoxin Esters. Esters **6a–e** and **7a–e** (1 mg) were dissolved in CH_3CN (1 mL), and 150 μL of this solution was combined with 350 μL of 50 mM phosphate buffer (pH 6.0, 7.0, or 8.0) and incubated for 24 h at ambient temperature. Enzymatic hydrolyses included lipase (2 mg/mL) premixed into the phosphate buffer. The course of hydrolysis was followed by HPLC by direct injection of an aliquot of the reaction mixture onto the column. The results are presented in Table 1.

A competitive hydrolysis between esters **6e** and **7e** was carried out by combining 150 μg of each ester in 300 μL of acetonitrile with 700 μL of pH 8.0 buffer containing 1.4 mg of lipase. The mixture was allowed to incubate for 20 h. Aliquots were removed at 0, 6, and 20 h and analyzed by HPLC (condition B[9]). The results are presented in Figure 1.

Hydrolysis of 6b to Acid 4b. Ester **6b** (38 mg) was dissolved in CH_3CN (2 mL) and combined with phosphate buffer (pH 8.0, 3 mL). After stirring for 144 h at ambient temperature, the CH_3CN was evaporated in a stream of N_2.[8] The remaining aqueous solution was lyophylized, and the resulting residue was suspended in 30% MeOH/$CHCl_3$ and filtered. The concentrate was chromatographed on silica gel (25% MeOH/$CHCl_3$), providing 31 mg (77%) of acid **4b**: ^{1}H NMR (DMSO-d_6) δ 5.81 (s, 1H), 4.88–4.75 (m, 5H), 4.20–3.81 (m, 6H), 3.80–3.65 (m, 4H), 3.24–3.08 (m, 4H), 2.75–2.63 (m, 3H), 2.30–2.16 (m, 2H), 2.08–1.26 (m, 28H), 1.10 (m, 6H), 1.09 (d, 3H, J = 5.8), 0.84 (s, 3H), 0.64 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 176.9, 174.5, 173.9, 115.8, 104.3, 98.9, 95.3, 84.3, 81.8, 80.9, 73.3, 73.2, 73.1, 72.9, 72.0, 67.6, 67.5, 66.2, 64.6, 55.7, 54.4, 50.3, 45.1, 40.4, 40.2, 38.3, 37.9, 36.3, 35.3, 34.6, 33.7, 31.6, 30.1, 29.7, 29.6, 26.8, 26.7, 26.4, 26.0, 23.6, 21.3, 19.5, 18.0, 9.4; ESMS (M + H)$^+$ at 836; HPLC (mobile phase B) retention time 2.8 min.

Hydrolysis of 6c to Acid 4c. Ester **6c** (37 mg) was dissolved in CH_3CN (2 mL) and combined with phosphate buffer (pH 8.0, 3 mL). After stirring for 384 h at ambient temperature, the CH_3CN was evaporated in a stream of N_2.[8] The remaining aqueous solution was lyophylized, and the resulting residue was suspended in 30% MeOH/$CHCl_3$ and filtered. The concentrate was chromatographed on silica gel (25% MeOH/$CHCl_3$), providing 25 mg (70%) of acid **4c**: ^{1}H NMR (DMSO-d_6) δ 5.81 (s, 1H), 4.88–4.75 (m, 5H), 4.20–3.65 (m, 10H), 3.24–3.08 (m, 4H), 2.75–2.63 (m, 2H), 2.38 (t, 2H, J = 7.0 Hz), 2.26–1.26 (m, 31H), 1.10 (m, 6H), 1.04 (d, 3H, J = 6.2), 0.84 (s, 3H), 0.64 (s, 3H); ^{13}C NMR (d_6DMSO) δ 176.9, 175.4, 173.9, 115.8, 104.3, 98.9, 95.3, 84.3, 81.8, 80.9, 73.3, 73.0, 72.1, 67.7, 67.5, 66.3, 66.1, 65.0, 57.8, 57.7, 55.7, 50.8, 45.2, 40.5, 38.4, 37.9, 36.3, 35.4, 34.7, 33.3, 32.4, 31.6, 30.2, 29.7, 29.6, 26.8, 26.4, 26.0, 23.7, 23.0, 21.4, 19.5, 18.0, 9.4; ESMS (M + H)$^+$ at 850; HPLC (mobile phase B) retention time 2.9 min.

Lipase-Mediated Hydrolysis of 7e to 4e. Ester **7e** (42 mg) in CH_3CN (9 mL) was combined with phosphate buffer (pH 8.0, 21 mL) containing lipase (42 mg). The mixture was stirred at ambient temperature until the starting ester was consumed (as

(7) Zervas, L.; Winitz, M.; Greenstein, J. P. *J. Org. Chem.* **1957**, *22*, 1515.

determined by HPLC: 48 h). The organic solvent was evaporated under a stream of N_2.[8] The remaining aqueous solution was lyophylized, and the resulting residue was suspended in 30% MeOH/$CHCl_3$ and filtered. The concentrate was chromatographed on silica gel (25% MeOH/$CHCl_3$), providing 26 mg (70%) of acid **4e**: ^{1}H NMR (CD_3OD) δ 5.90 (s, 1H), 5.02–4.84 (m, 5H), 4.27–4.22 (m, 2H), 4.12–4.09 (m, 1H), 4.00 (br, 1H), 3.88–3.76 (m, 2H), 3.80–3.65 (m, 4H), 3.44–3.20 (m, 5H) 3.08–2.93 (m, 3H), 2.42–2.30 (m, 1H), 2.22 (t, 2H, J = 7.2 Hz), 2.18–1.26 (m, 32H), 1.10 (m, 9H), 0.94 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (CD_3OD) δ 178.5, 178.4, 177.3, 117.7, 103.8, 96.9, 86.8, 83.8, 83.0, 75.6, 75.4, 74.5, 74.4, 69.8, 69.4, 68.4, 68.3, 62.9, 58.7, 57.3, 50.2, 48.6, 47.0, 42.2, 39.0, 38.9, 38.0, 37.2, 36.2, 33.6, 33.4, 33.3, 31.4, 31.0, 28.4, 27.8, 27.5, 27.4, 26.3, 25.5, 24.3, 24.2, 22.9, 22.8, 20.3, 18.5, 9.9; ESMS $(M + H)^+$ at 878; HPLC (mobile phase B) retention time 3.1 min.

(8) A gentle stream of nitrogen was more effective in evaporating the acetonitrile. Rotary evaporation caused foaming, due to presence of the lipase.

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