Synthesis of Macrocyclic Dilactones through Lipases

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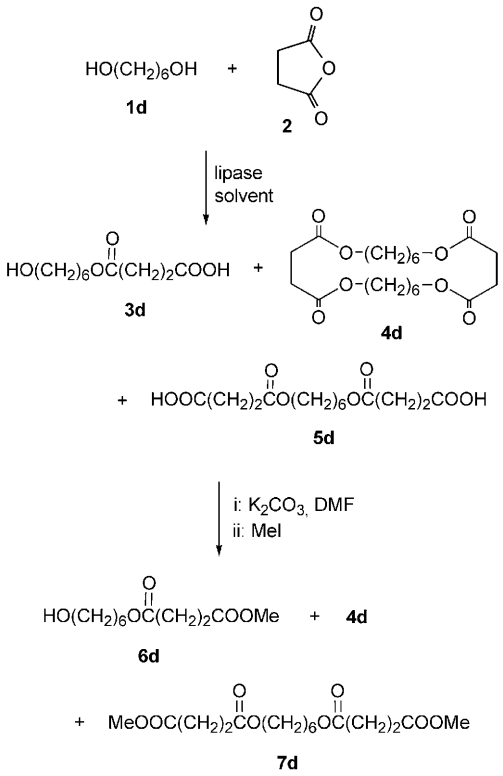
Abstract: We report herein a selective and efficient method to prepare macrocyclic dilactones in good yields from diols and succinic anhydride through a biocatalytic condensation reaction.

Key words: *Candida antarctica B*, macrocyclic dilactones, lipases, synthesis, biocatalysis

The synthetically challenging structures of macrolides have attracted the attention of organic chemists for a long time but only recently have efforts in this direction begun to gain momentum.[1] Particularly attractive are macrocyclic dilactones, not only because of their remarkable biological activities but also because of their ability to complex and transport alkali metal cations and their utilization in the perfume industry.[2] However, only a limited number of methods for preparation of macrocyclic dilactones are currently available. They comprise the reaction of the dicesium salts of pyridindicarboxylic acids with dibromides,[3] reaction of dioxodioic acid chlorides with oligoethylene glycols,[4] condensation of the dipotassium salt of maleic, citraconic and *o*-phthalic acids with alkyl or alkynyl dibromides,[5] condensation of benzyl bromides with homologous series of dicarboxylic acids,[6] reaction of substituted dicarboxylic acid chlorides with ω-bromoalcohols followed by reaction with lithium or potassium salts of dicarboxyxlic acids,[7] condensation of diacid chlorides with diols using metal carbonates and KF as bases under phase-transfer catalysis,[8] or via cyclization of (ω-carboxyalkyl)diphenylsulfonium salts in the presence of cesium carbonate under high dilution conditions.[9] However, many of these methods provide mixtures of dilactones and tetralactones, in some cases in low yields and under harsh reaction conditions. Only one case has been found in the literature in which macrocyclic lactones are prepared enzymatically by reaction of acyclic diacids with diols in the presence of *C. cylindracea*, *Pseudomonas sp*. and porcine pancreatic lipase.[10] However, even in this case mixtures of mono- and dilactones were obtained in moderate yields, the dilactones being produced in the range of 5–19% yield.

In this paper we present a simple and high yielding method to selectively prepare macrocyclic dilactones by reaction of 1,n-diols with succinic anhydride[11] in the presence of *Candida Antarctica B* lipase (*CALB*, *Novozyme* 435). Intramolecular lactonization of long chain hydroxyesters with lipase P (*Pseudomonas sp*.) to provide pheromone components of grain beetles[12] or a key intermediate for the synthesis of the anti-obesity agent tetrahydrolipstatin with PPL has been described.[13]

Initially, we tested the reaction of 1,6-hexanediol and succinic anhydride in the presence of several lipases (*PS*, *AP-6*, *Mucor miehei*, *Candida rugosa*, *CALB* and *Pseudomonas fluorescens*) in a number of solvents and under different experimental conditions.



Scheme 1 Enzymatic reaction of diol **1d** with succinic anhydride **2**

The reaction resulted in a mixture of variable amounts of starting diol **1d**, hydroxyacid **3d**, dilactone **4d** and diacid **5d**[14] (Scheme 1) by GC analysis after esterification with MeI/K_2CO_3.[15] Under these conditions, compounds **3d** and **5d** are converted into **6d** and **7d**, respectively (Table 1).

Among the lipases tested, *AP-6*, *C. rugosa* and *P. fluorescens* were completely unable to produce dilactones **4d**, but lipase *PS* (entry 2), *M. miehei* (entry 11) and especially *CALB* (entries 13–18) afforded variable amounts of the dilactones, the latter with every solvent used (toluene, methyl *tert*-butyl ether, diethyl ether and acetone). The

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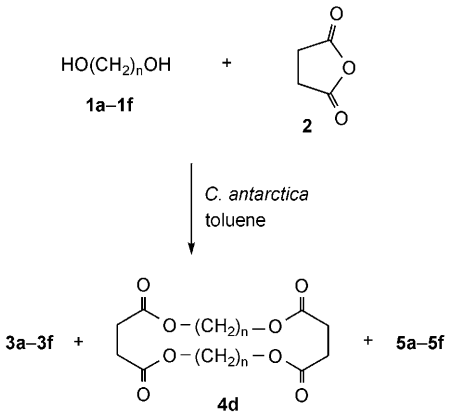
Table 1 Lipase-Catalyzed Reaction of 1,6-Hexanediol (**1d**) with Succinic Anhydride (**2**)

Entry	Lipase	Solvent	Time (h)	Temp (°C)	**1d**[a]	**6d**[a]	**7d**[a]	**4d**[a]
1	*PS*	$C_6H_5CH_3$	70	30	23	45	31	–
2	*PS*	MTBE	70	30	14	39	35	12
3	*AP-6*	$C_6H_5CH_3$	70	30	10	30	60	–
4	*AP-6*	$C_6H_5CH_3$	24	30	39	46	15	–
5	*AP-6*	MTBE	120	30	100	–	–	–
6	*AP-6*	MTBE	24	30	100	–	–	–
7	*AP-6*	Et_2O	24	30	34	58	8	–
8	*AP-6*	Et_2O	24	40	9	59	32	–
9	*AP-6*	Et_2O	48	30	7	39	54	–
10	*AP-6*	Me_2CO	24	30	38	45	17	–
11	*Mucor miehei*	$C_6H_5CH_3$	7	30	17	35	40	8
12	*C. rugosa*	$C_6H_5CH_3$	79	30	5	44	55	–
13	*CALB*	$C_6H_5CH_3$	1	20	38	44	8	10
14	*CALB*	$C_6H_5CH_3$	2	20	21	5	21	53
15	*CALB*	$C_6H_5CH_3$	24	30	4	3	1	92
16	*CALB*	MTBE	2	30	40	30	22	8
17	*CALB*	Et_2O	2	30	60	12	18	10
18	*CALB*	Me_2CO	2	30	35	25	25	15
19	*P. fluorescens*	$C_6H_5CH_3$	72	30	3	37	60	–

[a] By GC analysis.

effect of the solvent was variable; whereas toluene with *CALB* provided the best yield of **4d** (92%, entry 15), with lipase *PS* the reaction completely failed. In contrast, MTBE with lipase *PS* gave 12% of the dilactone (entry 2), no dilactone with *AP-6* and only 8% (entry 11) with *M. miehei*, the poorest result of all obtained with this enzyme. The reaction did not proceed in the absence of enzyme.



Scheme 2 *C. antarctica*-catalyzed reaction of diols **1a–f** with succinic anhydride (**2**)

The best conditions found were then applied to other 1,n-diols (n = 2, 3, 4, 8, 10) to explore the scope of the reaction (Scheme 2). The reactions were run at 30 °C for 24 hours. In all cases they led to formation of the corresponding dilactones **4a**, **4b**, **4c**, **4e** and **4f** in good isolated yields (Table 2).[16] The open chain acids **3a–f** and **5a–f** were obtained in very low, almost negligible, amounts. It should be noted that the efficiency of the lactonization is independent of the size of the macrocycle to be formed, and thus analogous good yields were obtained when the ring contained 16 (n = 2) or 32 atoms (n = 10).

When the reaction was extended to substituted diols, such as 2,2-dimethyl-1,3-propanediol, or unsaturated diols, such as (*Z*)-1,4-butenediol, no traces of dilactone were obtained. As shown by X-ray crystallography, *CALB*, compared to other lipases, has very limited space available at the active site pocket, which is composed of two channels, one hosting the acyl part and the other hosting the alcohol moiety of the substrate.[17] The acyl channel is more spacious than the alcohol channel, and therefore the enzyme is expected to exert a broad specificity towards acyl

Table 2 Reaction of Diols **1a–f** with Succinic Anhydride in the Presence of *CALB* in Toluene[a]

Diol	n	Recovered diol[b]	Hydroxy acid[b]	Diacid[b]	Dilactone[b]	Dilactone[c]
1a	2	**1a** (–)	**3a** (7)	**5a** (5)	**4a** (88)	**4a** (79)
1b	3	**1b** (5)	**3b** (5)	**5b** (5)	**4b** (85)	**4b** (77)
1c	4	**1c** (–)	**3c** (4)	**5c** (2)	**4c** (94)	**4c** (86)
1d	6	**1d** (4)	**3d** (3)	**5d** (1)	**4d** (92)	**4d** (84)
1e	8	**1e** (3)	**3e** (4)	**5e** (3)	**4e** (90)	**4e** (81)
1f	10	**1f** (4)	**3f** (5)	**5f** (2)	**4f** (89)	**4f** (80)

[a] All reactions were run at 30 °C for 24 h.
[b] GC yield of the corresponding methyl ester.
[c] Isolated yield.

donors and a much higher degree of selectivity towards alcohol substrates.[18] These considerations are in line with our observations.

In summary, we have developed a selective and high yielding biocatalytic method for the synthesis of macrocyclic dilactones (16–32 atoms) under very mild conditions and using readily available starting materials.

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(14) As a typical example, in a 25 mL Erlenmeyer flask was placed a mixture of 0.118 g (1 mmol) of hexanediol, 0.100 g (1 mmol) of succinic anhydride, 15 mL of toluene and 0.100 g of *Candida antarctica B* lipase. The Erlenmeyer flask was capped, placed in a tempered bath at 30 °C, and shaken at 80 U. The reaction was monitored by GC and when the transformation was complete (24 h reaction), the mixture was filtered and the enzyme washed with Et_2O and MeOH. The solvent was stripped off and the resulting crude purified by column chromatography on silica gel eluting with hexane–Et_2O mixtures to furnish 0.337 g (84%) of lactone **4d**. Mp 96–98 °C. IR (KBr): 2984, 2945, 2864, 1728, 1268, 1172 cm^{-1}. ^1H NMR (300 MHz): δ = 4.06 (t, *J* = 6.6 Hz, 8 H, 4 CH_2O), 2.58 (s, 8 H, 4 CH_2CO), 1.54 (m, 8 H, 4 CH_2), 1.34 (m, 8 H, 4 CH_2). ^{13}C NMR (75 MHz): δ = 172.01 (CO), 64.51 (OCH_2), 29.42, 29.28, 28.44, 28.37, 25.47. Anal. Calcd for $C_{20}H_{32}O_8$: C, 59.98; H, 8.05. Found: C, 60.12; H, 8.08. MS (CI): *m/z* (%) = 401 (100) [M^+ + 1], 429 (8) [M^+ + 29], 441 (5) [M^+ + 41].
(15) Esterification was achieved by addition of anhyd DMF (0.90 mL) and K_2CO_3 (93 mg, 0.65 mmol) to the crude lipase-catalyzed reaction (52 mg). The suspension was magnetically stirred for 20 min, then MeI (0.300 mL, 4.81 mmol) was added and the mixture stirred for 16 h. After conventional work up, a mixture of the methyl esters **6d** and **7d**, starting material **1d**, and dilactone **4d** were obtained in almost quantitatively yield.
(16) Spectroscopic and analytical data of compounds **4a–f**.
Compound **4a**: mp 84 –87 °C. IR (KBr): 2963, 1727, 1265, 1182 cm^{-1}. ^1H NMR (300 MHz): δ = 4.28 (s, 8 H, 4 CH_2O), 2.66 (s, 8 H, 4 CH_2CO). ^{13}C NMR (75 MHz): δ = 171.53 (CO), 62.46 (CH_2O), 29.31. Anal. Calcd for $C_{12}H_{16}O_8$: C, 50.00; H, 5.59. Found: C, 49.89; H, 5.47. MS (CI): *m/z* (%) = 289 (100) [M^+ + 1].
Compound **4b**: mp 86–88 °C. IR (KBr): 2967, 1722, 1275, 1193 cm^{-1}. ^1H NMR (300 MHz): δ = 4.14 (t, *J* = 5.7 Hz, 8 H, 4 CH_2O), 2.62 (s, 8 H, 4 CH_2CO), 1.94 (q, *J* = 6.0 Hz, 4 H, 2 CH_2). ^{13}C NMR (75 MHz): δ = 171.77 (CO), 60.83 (CH_2O), 29.59, 27.67. Anal. Calcd for $C_{14}H_{20}O_8$: C, 53.16; H, 6.37. Found: C, 53.05; H, 6.22. MS (CI): *m/z* (%) = 317 (100) [M^+ + 1].
Compound **4c**: mp 88–92 °C. IR (KBr): 2964, 1716, 1265, 1176 cm^{-1}. ^1H NMR (300 MHz): δ = 4.09 (m, 8 H, 4 CH_2O), 2.62 (s, 8 H, 4 CH_2CO), 1.68 (m, 8 H, 4CH_2). ^{13}C NMR (75

MHz): δ = 171.94 (CO), 64.36 (CH_2O), 29.53, 25.24. Anal. Calcd for $C_{16}H_{24}O_8$: C, 55.81; H, 7.02. Found: C, 55.82; H, 7.11. MS (CI): *m*/*z* (%) = 345 (100) [M^+ + 1].
Compound **4e**: mp 87–91 °C. IR (KBr): 2948, 1726, 1265, 1173 cm^{-1}. ^1H NMR (300 MHz): δ = 4.06 (t, *J* = 6.0 Hz, 8 H, 4 CH_2O), 2.60 (s, 8 H, 4 CH_2CO), 1.52 (m, 8 H, 4 CH_2), 1.30 (s, 16 H, 8 CH_2). ^{13}C NMR (75 MHz): δ = 177.11 (CO), 64.71 (CH_2O), 29.54, 29.10, 28.54, 25.75. Anal. Calcd for $C_{24}H_{40}O_8$: C, 63.14; H, 8.83. Found: C, 63.24; H, 8.94. MS (CI): *m*/*z* (%) = 457 (100) [M^+ + 1].
Compound **4f**: mp 69–72 °C. IR (KBr): 2923, 1729, 1266, 1172 cm^{-1}. ^1H NMR (300 MHz): δ = 4.08 (t, *J* = 6.6 Hz, 8 H, 4 CH_2O), 2.62 (s, 8 H, 4 CH_2CO), 1.60 (m, 8 H, 4 CH_2), 1.29 (s, 24 H, 12 CH_2). ^{13}C NMR (75 MHz): δ = 172.15 (CO), 64.79 (CH_2O), 29.51, 29.38, 29.30, 29.18, 28.56, 25.83. Anal. Calcd for $C_{28}H_{48}O_8$: C, 65.60; H, 9.44. Found: C, 65.66; H, 9.41. MS (CI): *m*/*z* (%) = 513 (100) [M^+ + 1].

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