Pergamon

Tetrahedron Letters 41 (2000) 9135–9138

TETRAHEDRON LETTERS

Comparative study of conformational effects on stereoselective lipase catalysed acetylation of *sec* hydroxy groups in diastereomeric 14-membered lactones and their acyclic analogs

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Received 5 June 2000; revised 15 September 2000; accepted 20 September 2000

Abstract

Stereoselective acetylation of *sec* hydroxy groups in a diastereomeric (1:1) mixture of macrocyclic lactones **14**, **15**, catalysed by seven microbial lipases in *n*-heptane afforded the C(7)-*O*-acetyl derivative **17** with up to 99% diastereomeric excess (d.e.), whereas acetylation of their acyclic analogs **10**, **11** afforded C(5)-*O*-acetyl derivative **13** with up to 93% d.e. Six lipases, from *Pseudomonas cepacia* (PCL), *Pseudomonas fluorescens* (PFL), *Geotrichum candidum* (GCL), *Candida cylindracea* (CCL) and *Pseudomonas cepacia* immobilised on ceramics (PS-C) predominantly acetylate cyclic diastereomer (3*S*,7*S*)-**15** to (3*S*,7*S*)-**17**; only *Candida antarctica*-B (CAL-B) lipase predominantly acetylates (3*S*,7*R*)-**14** to (3*S*,7*R*)-**16**. With acyclic analogs **10**, **11** the same set of lipases exhibited different diastereoselective bias; CAL-B, GCL and CCL predominantly acylate (1*S*,5*S*)-**11** to (1*S*,5*S*)-**13**, whereas PCL, PS-C, PFL and PSL acylate predominantly (1*S*,5*R*)-**10** to (1*S*,5*R*)-**12**. Only GCL exhibited higher stereoselectivity for an acyclic pair of stereoisomers with higher conformational flexibility, over cyclic diastereomeric substrates with a conformationally restricted macrocyclic ring. The preference of PCL for macrocyclic substrates is particularly interesting, in view of the recently suggested binding mode of a series of acyclic *sec* alcohols in the extended conformation.

Keywords: lipase catalysed acetylation; acyclic versus macrocyclic *sec* alcohols; conformational effect on stereoselectivity.

Recently we have reported a study of the lipase catalysed stereoselective acetylation of the diastereomeric mixture **14**, **15** and hydrolysis of the corresponding acetates **16**, **17**.[1,2] Prompted by the unexpectedly high stereoselectivity in both directions, in view of the distance of the perturbing groups from the stereogenic centre C(7), we have proposed that absolute conformation around a stereogenic centre determines stereoselectivity in these substrates, rather than 'large' and 'medium' groups, as defined by the known model of Kazlauskas.[3–5]

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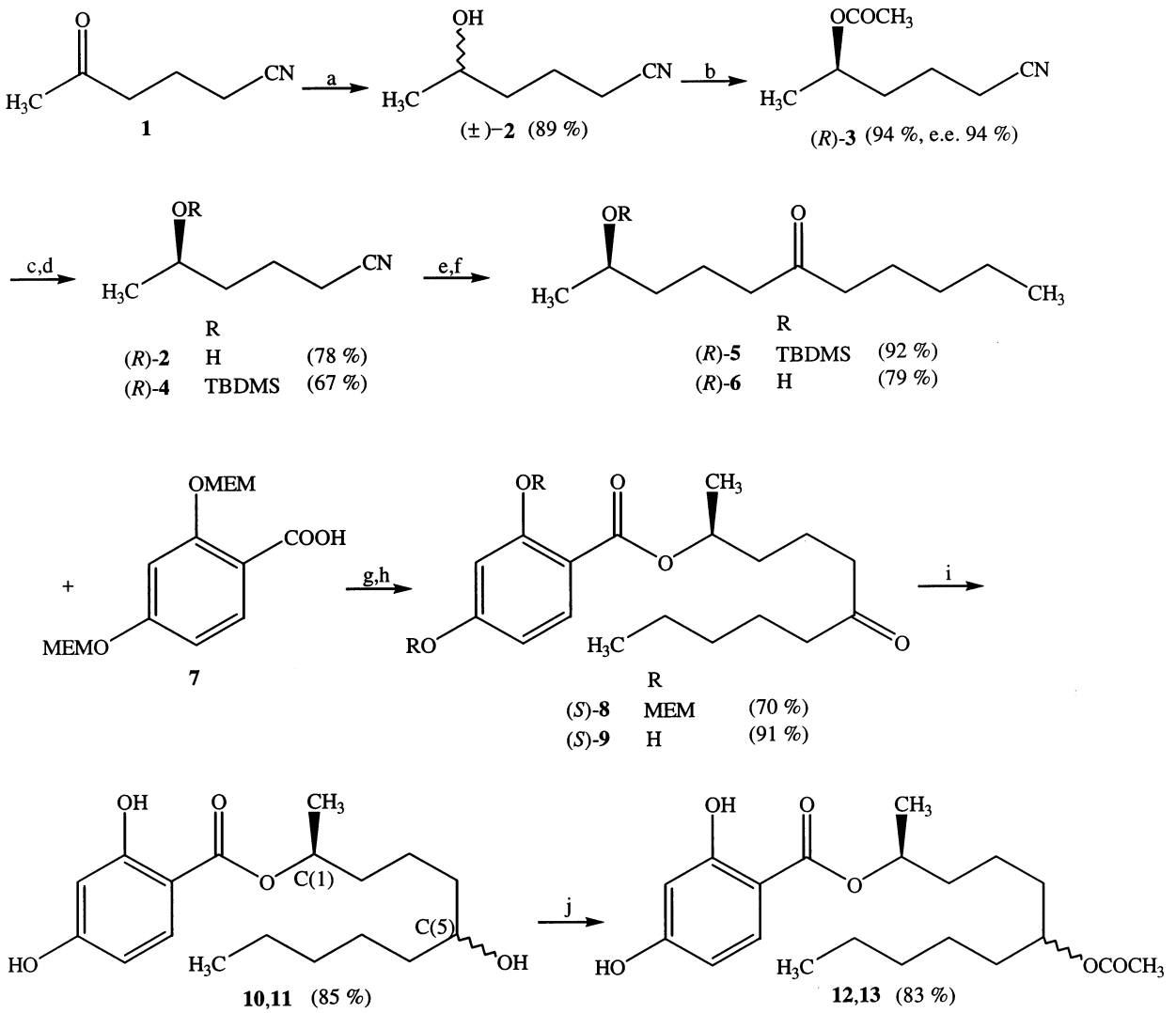
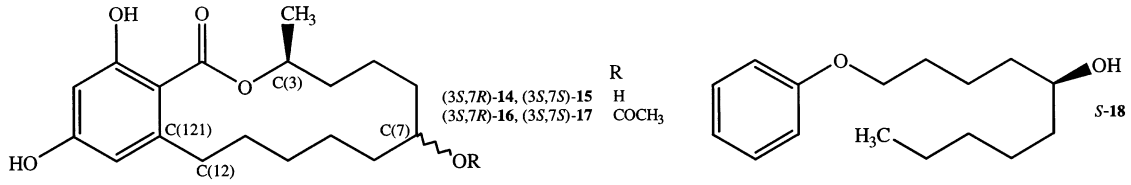
0040-4039/00/$ - see front matter
PII: S0040-4039(00)01634-8

To test this finding further, and also prompted by the potential biological activity of the resorcillic esters **10** and **11**, open-chain analogs of the macrocyclic lactones **14** and **15**, we have undertaken their synthesis and biocatalytic separation. They are formally derived from the macrocyclic counterparts **14** and **15** by cleaving the C(12)–C(121) bond. Compound **14** (generic name α-zearanol) has found application as an anabolic agent in meat-production;[6] its 7β-isomer **15** is eliminated from the commercial product because of its estrogenic side effects.[7] The possibility that their open-chain analogs **10**, **11** retain anabolic activity whilst avoiding the undesired side effects was an additional motive for undertaking this study. Open-chain diastereomers **10**, **11** (1:1) were prepared with the (1*S*) configuration, which corresponds to biosynthetically generated (3*S*) configuration in **14** and **15**, Scheme 1.[8]



Scheme 1. (a) $Al(OCH(CH_3)_2)_3$/2-PrOH/Δ; (b) PS-C/vinyl acetate/*n*-hexane/30°C; (c) KOH/MeOH/rt; (d) *t*-$Bu(CH_3)_2SiCl$/imidazole/CH_2Cl_2/0°C; (e) $CH_3(CH_2)_4Br/Mg/I_2/Et_2O$–toluene/Δ; (f) 1% HCl/EtOH/rt; (g) PPh_3/DEAD/Et_2O/rt; (h) 5% HCl/THF (1:2)/rt; (i) $NaBH_4$/MeOH; (j) PS-C/vinyl acetate/*n*-heptane/30°C

Key steps in this synthesis comprise the efficient kinetic resolution of **2** by PS-C lipase that affords *R*-**3** with >94% e.a., the subsequent Grignard reaction of the TBDMS-protected *R*-**4**, and acylation of *R*-**6** with inversion of configuration (Mitsunobu reaction) by MEM-protected resorcillic acid **7**; the overall yield from **1** to a 1:1 mixture **10**, **11** was 17%. A standard sample of **12**, **13** (1:1) was prepared by exhaustive enzymatic acetylation.



The results of a parallel study of the stereoselective acetylation of **10**, **11** and **14**, **15** by a series of microbial lipases in *n*-heptane are presented in Table 1.

Table 1
Stereoselectivity of acetylation of a 1:1 mixture of acyclic alcohols **10**, **11** and macrocyclic alcohols **14**, **15** by various microbial lipases

Comp.	Lipase	*t* (h)	*T* (°C)	Conv. (%)	D.e. (%) alcohol	D.e. (%) acetate	***D***[a]
10, **11**	*Pseudomonas cepacia*	48	+50	42.9	24.0 **11**	31.8 **12**	2.4
	Pseudomonas cepacia	120	+30	33.8	16.8 **11**	32.9 **12**	2.3
	Pseudomonas fluorescens	72	+30	41.5	21.8 **11**	30.8 **12**	2.3
	Pseudomonas species	48	+30	46.9	29.3 **11**	33.2 **12**	2.6
	Pseudomonas cepacia-C	3	+30	43.6	5.0 **11**	9.5 **12**	1.3
	Candida antarctica B	120	+30	36.9	6.0 **10**	10.2 **13**	1.3
	Geotrichum candidum	6	+30	35.7	51.5 **10**	92.5 **13**	42.7
	Candida cylindracea	24	+30	49.2	44.6 **10**	46.2 **13**	4.1
14, **15**	*Pseudomonas cepacia*	5.5	+50	27.1	35.5 **14**	95.4 **17**	60
	Pseudomonas cepacia	48	+30	33.6	48.1 **14**	95.1 **17**	64
	Pseudomonas fluorescens	24	+30	45.6	79.5 **14**	94.7 **17**	89
	Pseudomonas species	24	+30	45.1	73.2 **14**	89.0 **17**	38
	Pseudomonas cepacia-C	6	+30	40.4	39.1 **14**	57.8 **17**	5.4
	Candida antarctica B	48	+30	50.0	42.1 **15**	42.1 **16**	3.6
	Geotrichum candidum	48	+30	3.6	3.7 **14**	99.9 **17**	>200
	Candida cylindracea	48	+30	36.4	56.6 **14**	98.7 **17**	>200

[a] *D*-value defines diastereoselectivity according to the Sih's equation, developed for enantioselectivity (*E*-value).[9]

Higher stereoselectivity was observed for most lipases with the cyclic substrates **14**, **15**. The exceptional selectivity of GCL is particularly interesting; it shows a higher *D*-value and much higher affinity for the acyclic pair **10**, **11**; in spite of the high *D*-value for the macrocyclic pair **14**, **15**, calculated at <4% conversion after 48 h, they can be regarded as nonsubstrates.

Most lipases accept the macrocyclic pair of diastereomers **14**, **15** better, and on average exhibit higher diastereoselectivity (d.e. 58–99%) in favour of the (3*S*,7*S*) diastereomer, whereas the acyclic analogs are less well accepted and the stereoselectivity is generally lower (d.e. 46–93%), Table 1.[10] The stereoselectivity of PCL catalysed acetylation of both pairs of stereoisomers is similar after 48 h at +50°C and after 120 h at +30°C, revealing that a larger temperature effect on the conformational mobility of the more flexible open-chain analogs is not reflected in enhanced stereoselectivity at lower temperatures.

The results reveal two interesting aspects of PCL and GCL stereoselectivity, however. First, GCL although known to possess one large cavity that extends from the active site up to the surface of the protein, substantially longer than in other lipases, and completely non-polar,[12,13] accommodates acyclic substrates **10**, **11** better than the macrocyclic ones, and acetylates them with a *D*-value of ca. 43. This indicates that the acyclic pair **10**, **11**, presumably bound on the GCL active site in an extended conformation, is accommodated better than their cyclic analogs. Secondly, it has been argued recently that PCL exhibits higher stereoselectivity in acylation of acyclic substrates that have substituents at the stereogenic centre, which differ significantly in shape but not in volume,[14] and it is stated that acyclic substrates do not fit the hydrophobic binding regions of the active site of PCL in a folded conformation. Our results, however, reveal the macrocyclic *sec* alcohols **14**, **15** as better substrates, though they are equal to the folded conformers of their acyclic counterparts **10**, **11**.

To explain the above results and to get a better insight into the origin of stereoselectivity we are presently performing computer docking of both pairs of stereoisomers, **10**, **11** and **14**, **15**, in the PCL and GCL active site.

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