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Enzymatic resolution of (±)-conduritol-B, a key intermediate for the synthesis of glycosidase inhibitors

Claudia Sanfilippo, Angela Patti and Giovanni Nicolosi *

Istituto CNR per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-Farmaceutico, Via del Santuario 110, I-95028 Valverde CT, Italy

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Abstract

Lipases from porcine pancreas, *Candida cylindracea* and *Mucor miehei* (adsorbed on support, Lipozyme® IM) catalysed in *t*-butylmethylether the alcoholysis of *rac*-conduritol-B peracetate, (±)-**1**, by *n*-butanol to give enantiopure (2*S*,3*S*)-diacetoxy-(1*R*,4*R*)-dihydroxycyclohex-5-ene, (−)-**3**, and (1*S*,2*R*,3*R*,4*S*)-tetraacetoxy-cyclohex-5-ene, (+)-**1**. The enantioforms (+)- and (−)-conduritol-B, obtained after chemical hydrolysis of (−)-**3** and (+)-**1**, respectively, may be employed to prepare both the enantiomers of conduritol-B epoxide and cyclophellitol, powerful inhibitors of glycosidases.

A remarkable interest in the preparation of chiral conduritols (5-cyclohexen-1,2,3,4-tetrols) exists today because these polyalcohols may be used as starting material in the preparation of densely-functionalised molecules, including inositol derivatives and pseudosugars, which exhibit important biological activity.[1]

The presence of four stereogenic carbon atoms in the framework of conduritols allows them to exist in 10 different stereoforms, two of which are *meso*-compounds, conduritol-A and -D, while the others constitute four enantiomeric couples, conduritol-B, -C, -E and -F, respectively. Due to their synthetic importance, the preparation of these tetrols in enantiopure form and the selective transformation of the OH functions present on the cyclohexene skeleton is particularly useful but not always readily performed. In this context biocatalysed procedures can afford easy and stereocontrolled access to enantiopure conduritols. Thus, an efficient desymmetrisation by esterification in the presence of *Pseudomonas* sp. lipase of *meso*-conduritol-A 1,4-diester, previously transformed into the corresponding 2,3-ketal, has been reported by Johnson et al.,[2] whereas in our laboratory a highly enantioselective asymmetrisation of conduritol-D tetraester has been obtained through transesterification mediated by *Mucor miehei* lipase.[3] The same lipase catalysed the enantiomeric alcoholysis of conduritol-E tetraacetate or 1,2-diacetate producing, at the same time, selective partially protected derivatives, profitable for synthetic purposes.[4]

* Corresponding author. E-mail: nicolosi@issn.ct.cnr.it

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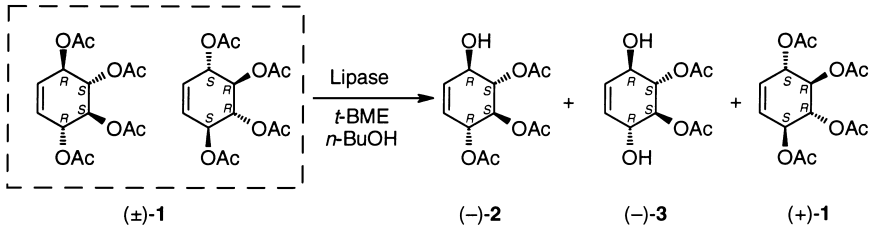
Table 1
Biocatalysed alcoholysis of conduritol-B tetraacetate, (±)-**1**[a]



(±)-**1** (−)-**2** (−)-**3** (+)-**1**

Lipase	Time, h	(−)-**2**% [b], (*ee*%)[c]	(−)-**3**,% [b]	(+)-**1**% [b], (*ee*%)
PPL	4	16 (>98)	5	79 (30)
PPL	24	14 (>98)	30	56 (75)
CCL	96	24 (>98)	18	58 (68)
Lipozyme	4	15 (>98)	15	70 (41)

[a]Experimental conditions: Enzyme (20 mg/ml), *n*-BuOH (0.012 ml, 0.13 mmol), substrate 10mg/ml), *tert*-butylmethyl ether (*t*-BME), 300 rpm, 40 °C. [b,c]Conversion and *ee* determined by GC analysis.

Within our research project that foresees the development of enzymatic procedures to prepare enantiopure bioactive cyclitols, we have taken into consideration the preparation of both (+)- and (−)-conduritol-B by lipase mediated kinetic resolution of the corresponding racemic peracetate, (±)-**1**, readily available in high yield from benzoquinone.[5]

In a preliminary investigation tetraester (±)-**1**, dissolved in *t*-BME, has been treated with *n*-butanol in the presence of three different lipases. Lipase from porcine pancreas catalysed the alcoholysis of tetraacetate (±)-**1** with a moderate rate to give, after 4 h, a triacetate (−)-**2** as the main product, whose ^{1}H NMR spectrum was consistent with the presence of a free OH group in the allylic position of the molecule. GC analysis on a chiral column of this compound, as its peracetate (−)-**1** obtained by conventional acetylation, evidenced its enantiopure nature;[6] conversely its chemical hydrolysis gave (−)-conduritol-B, (−)-**4**, allowing assignment of the 1*R*,2*S*,3*S*,4*R* configuration to triester (−)-**2** (Table 1).

In a reaction prolonged for 24 h, in addition to (−)-**2**, diester (−)-**3** in 30% yield and enantiopure form was observed. The formation of this diester in the alcoholysis process is possible because triester (−)-**2** possesses a second function at C-4 of adapted configuration so becoming itself a new substrate for the lipase.

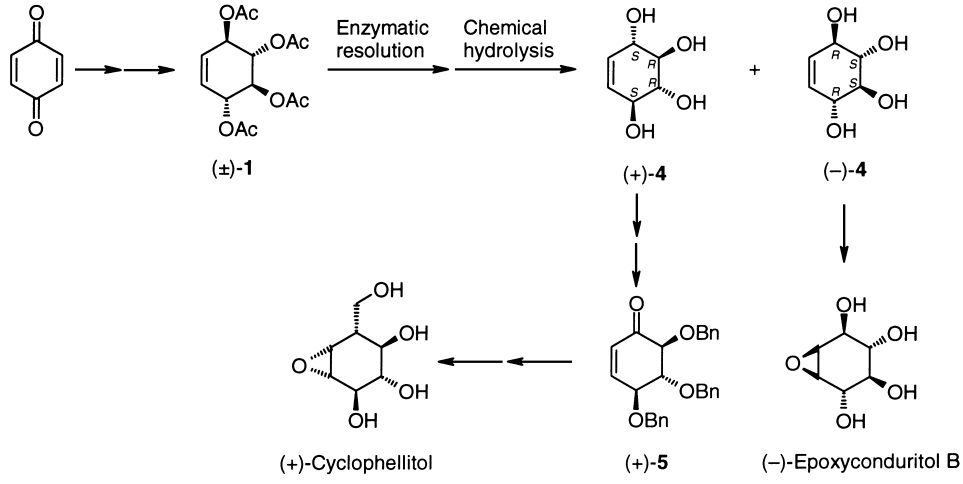
The reaction catalysed by lipase from *Candida cylindracea* gave comparable results in terms of products formed and enantioselectivity but showed a very low reaction rate. Conversely, when lipase from *Mucor miehei* adsorbed on support (Lipozyme® IM) was employed, after a 4 h reaction, (±)-**1** suffered 30% of conversion to give homochiral enantiopure (−)-**2** and (−)-**3** in equal amounts.[7]

All the lipases utilised possess *R* stereopreference. It is noteworthy that ester groups located on C-2 and C-3 in the enantiomer (+)-**1**, although having the suitable configuration, do not undergo any alcoholysis. This unreactivity can be interpreted in terms of steric hindrance exercised by the vicinal allylic ester group.

Since Lipozyme catalysed the alcoholysis with the best rate, it was selected to perform the alcoholysis of conduritol (±)-**1** on a two gram scale. After 24 h the reaction mixture contained (−)-**3** and the

unreacted (+)-**1** in a 1:1 ratio. Chromatographic purification on Silica-gel column (eluting with ethyl acetate:hexane, 6:4) furnished (−)-**3** (44% yield, >98% ee)[8] and (+)-**1** (47% yield, >98% ee).[9,10] Treatment of diester (−)-**3** and tetraester (+)-**1** with MeOH:NH_4OH, 9:1, afforded, quantitatively, (1*R*,2*S*,3*S*,4*R*)-1,2,3,4-tetrahydroxy-5-cyclohexene, (−)-**4**, and (1*S*,2*R*,3*R*,4*S*)-1,2,3,4-tetrahydroxy-5-cyclohexene, (+)-**4**, respectively.[11]

From a synthetic point of view, the C_2 symmetry of conduritol-B allows the exploitation of (+)- and (−)-**4** to prepare, in a highly diastereospecific manner, two different cyclitols (see Scheme 1), already proven as having inhibitory activity versus glycosidase. Their direct oxidation with 3-chloroperbenzoic acid can give both the enantiomers of conduritol-B epoxide.[12] The selective oxidation with PtO_2 of one of the allylic OH functions present in conduritol-B enantiomers, followed by exhaustive benzylation, leads to the corresponding enones (+)- and (−)-**5**,[13] of which the former has been employed as the chiral key-intermediate in an elegant synthesis for (+)-cyclophellitol, recently reported by Letellier et al.[14]



Scheme 1.

Further investigations are being carried out in our laboratory to prepare regioselective partial enantiopure esters of conduritol-B, to be used as starting material in the preparation of new chiral cyclitols.

Acknowledgements

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5. Guo, Z.-X.; Haines, A. H.; Pyke, S. M.; Pyke, S. G.; Taylor, R. J. K. *Carbohydrate Res.* **1994**, *264*, 147–153.
6. Enantiomeric excesses (ee) were determined by GC analysis on chiral capillary column (Megadex DMP β, dimethylpenthyl β-CDX, OV1701 0.25 μm×0.25 mm×25 m). The ee of tetracetylconduritol-B was determined by direct injection. After conventional acetylation, the same method was utilised for excess determinations of (−)-**2** and (−)-**3**.

7. For comparison of the alcoholysis of the conduritol-E tetraacetate see: Ref. 4a.
8. $[\alpha]_D$ −90.8 (*c* 0.3, CH_3Cl); ^1H NMR ($CDCl_3$): δ 2.15 (6H, s), 2.53 (2H, d, *J*=5.5 Hz), 4.44 (2H, ddd, *J*=2.3, 5.0 and 5.5 Hz), 5.04 (2H, dd, *J*=2.3 and 5.0 Hz), 5.76 (2H, s); ^{13}C NMR ($CDCl_3$): δ 22.2, 72.2, 76.8, 130.4, 172.7.
9. $[\alpha]_D$ +170.6 (*c* 1.2, $CHCl_3$; lit.[10] $[\alpha]_D$ −172.4, *c* 1.1, $CHCl_3$ for the *R* enantiomer).
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11. The monoalcohol (−)-**2**, which, having a single free allylic hydroxyl group is of considerable synthetic interest, is recovered in low yield by all the above alcoholysis processes. In order to prepare triester (−)-**2** in higher yields an alternative synthetic way can be the direct esterification of diester (−)-**3** with vinyl acetate in the presence of Lipozyme. Although hydroxyl functions in C-1 and C-4 are both located on an *R*-configuration centre and (−)-**3** has C_2 symmetry, the concentration of desired (−)-**2** reaches 70% yield before significant concentration of the total esterified product appears in the reaction medium. (−)-(1*R*,2*S*,3*S*,4*R*)-1-Hydroxy-2,3,4-triacetyloxycyclohex-5-ene, (−)-**2**: $[\alpha]_D$ −111.8, (*c* 0.1 $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.07 (3H, s), 2.08 (3H, s), 2.14 (3H, s), 2.59 (1H, d, *J*=6.2 Hz), 4.46 (1H, ddd, *J*=8.0, 6.2 and 2.3 Hz), 5.07 (1H, dd, *J*=10.8 and 8.0 Hz), 5.33 (1H, dd, *J*=10.8 and 8.0 Hz), 5.63 (2H, m), 5.85 (1H, dd, *J*=10.2 and 2.0); ^{13}C NMR ($CDCl_3$): δ 21.9, 22.1, 22.2, 72.0, 72.5, 73.1, 76.9, 126.8, 132.4, 171.4, 171.6, 172.7.
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