Available online at www.sciencedirect.com

Journal of Molecular Catalysis B: Enzymatic 22 (2003) 271–277

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Application of lipase catalysis in organic solvents for selective protection–deprotection of bioactive compounds ☆

Daniela Lambusta, Giovanni Nicolosi*, Angela Patti, Claudia Sanfilippo

CNR Istituto Chimica Biomolecolare, Sezione di Catania, Via del Santuario 110, 95028 Valverde CT, Italy

Accepted 3 February 2003

Abstract

Lipases are useful catalysts to realise in non-conventional medium esterifications and alcoholysis of polyhydroxylated compounds with high level of regioselectivity. This paper describes some examples of regioprotection–deprotection of flavonoids and conduritols, realised in our laboratory, using *Pseudomonas cepacia*, *Mucor miehei* and *Candida cylindracea* (*C. rugosa*) lipases.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Lipase; Flavonoid; Conduritol; Biocatalysis

1. Introduction

Selectivity in the transformation of multifunctional compounds is an extremely important goal in organic synthesis, hence research of new selective methods is of current interest.

The discovery that lipases are able to act also in organic solvents paved the way to broad application of these biocatalysts in organic synthesis [1]. These enzymes possess high molecular recognition abilities and, therefore, they can be usefully employed in the selective modification of polyhydroxylated aromatic and aliphatic substrates.

In the last years, our group has developed a number of lipase applications aimed at having access to regioprotected phenols and cyclitols, flavonoids and conduritols, respectively, to be used directly or after appropriate transformation as modulators of enzymatic processes.

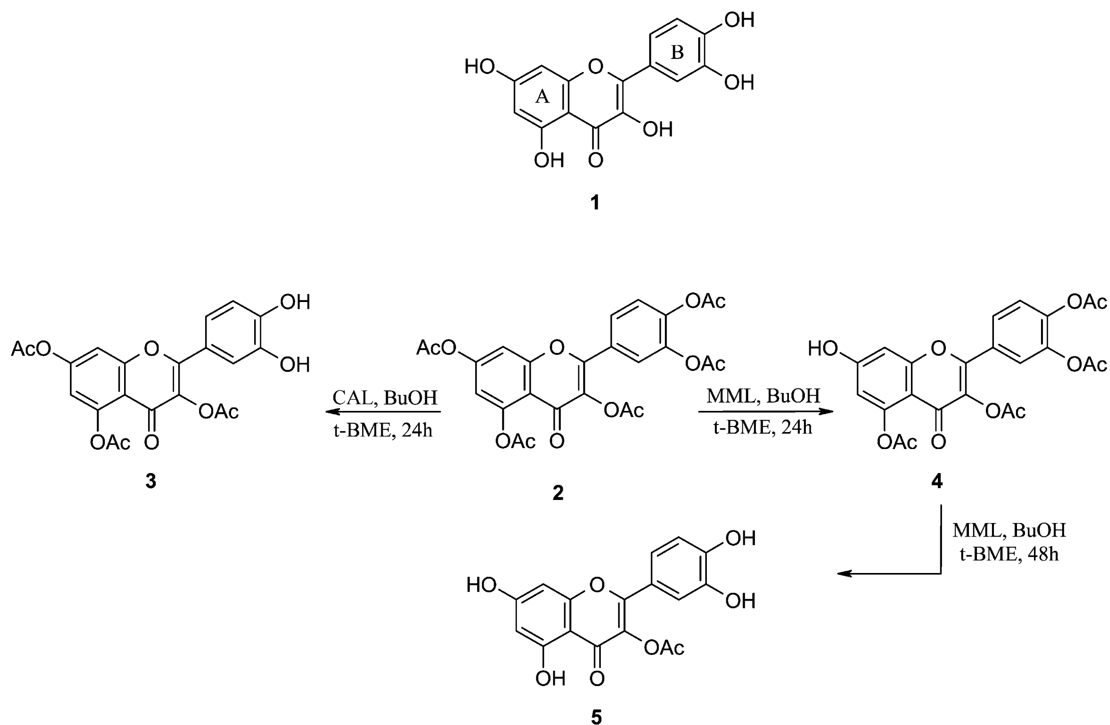
2. Regioprotection–deprotection of flavonoids

Flavonoids are important natural polyphenols widespread in the plant kingdom and having interesting biological properties. Their enzyme inhibitory activity and radical scavenger action have been evidenced [2]. These properties are strictly dependent on the location of the oxygenated functions at specific positions in the flavane framework [3], therefore, preparation of definite regioprotected flavonoids appears worthy of consideration. In our laboratory, we have considered the preparation of different esters of flavonoids, such as quercetin and catechin, in the light of their biological applications [4]. The poor

☆ Contribution at "Synthesis, Testing and Pharmacological Applications of Lipase Inhibitors" Meeting, Roma, 6 July 2001.

* Corresponding author. Tel.: +39-95-7212136;
fax: +39-95-7212141.
E-mail address: nicolosi@issn.ct.cnr.it (G. Nicolosi).

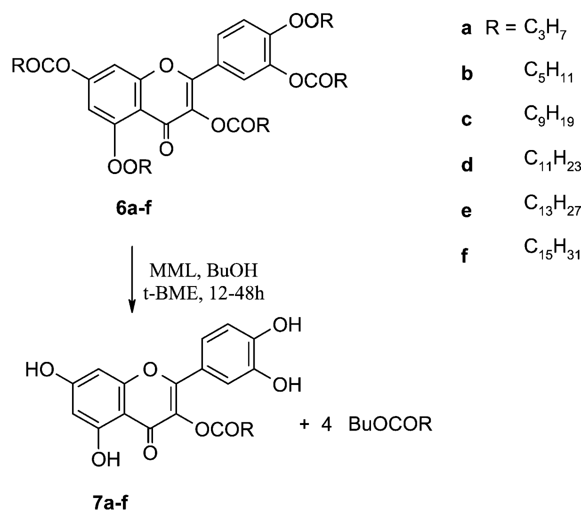
1381-1177/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1381-1177(03)00042-0



Scheme 1.

solubility of quercetin, **1**, in common organic solvents prompted us to consider as substrate the corresponding peracetate **2**, which was subject to alcoholysis in the presence of lipase from *Candida antarctica* (CAL) and *Mucor miehei* (MML). CAL recognizes preferentially the ester group at C-3′ and C-4′ positions and consequently catalyzes its alcoholysis to give triester **3** (Scheme 1). On the contrary, MML favours the alcoholysis of the acetyl group located on the A ring, thereby furnishing tetraester **4**.[1] Extension of the reaction time caused also the ester groups on B ring to be affected, apart from the one at C-3 vinyl position, giving monoester **5**.

MML recognition was observed also for quercetin derivatives with acyl groups longer then acetyl [5]. Thus, lipophylic 3-*O*-acylquercetins (**7a-f**) were prepared, all possessing free phenolic hydroxyls available for exploiting radical scavenger activity (Scheme 2). Recently, Saso and co-workers [6] have evidenced strong inhibitory action for these esters towards lipase from *Candida rugosa*. In particular, the inhibitory activity of C2–C16 3-*O*-acylquercetins increases linearly with the length of the chain, and appears more active than quercetin. Thus, these compounds may be useful

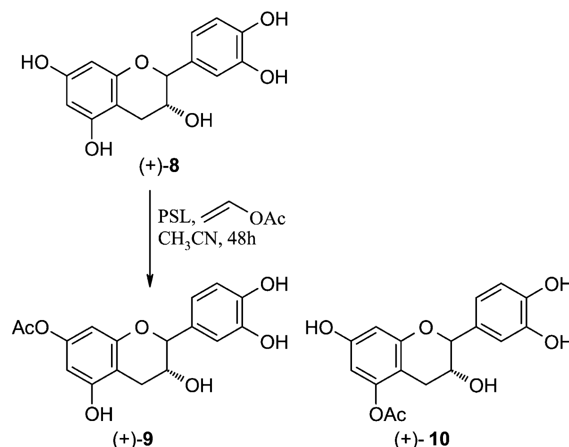


Scheme 2.

[1] Additional contributions regarding lipases mediated regioprotection–deprotections of flavonoids are also [4].

as adjuvant in the therapy of infective diseases due to lipase-producing microorganisms, and being more hydrophobic than the parent flavonoid, they could have more favourable pharmacokinetic properties, when applied to the skin or mucosae.

The unavailability of lipases with different regioselectivity in the preparation of partial esters of the natural catechin, (+)-**8**, has been overcome using a single enzyme, *Pseudomonas cepacia* lipase, in two complementary processes (Schemes 3 and 4) [7]. Direct esterification of catechin with vinyl acetate allowed to prepare in good yield derivatives protected at the A ring, namely (+)-**9** and (+)-**10**. Conversely, alcoholysis of peracetate **11** with butanol furnished triester **12**, which by extending the reaction time, is transformed into 3-*O*-acetylderivative **13**. The preservation of the ester group at C-3 was independent of the configuration of the catechin considered, thus four different 3-*O*-acetylcatechins were prepared.

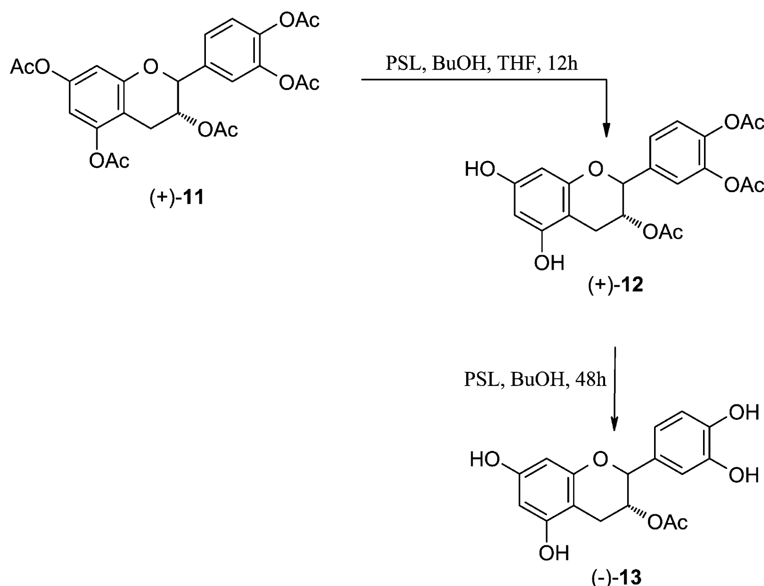


Scheme 3.

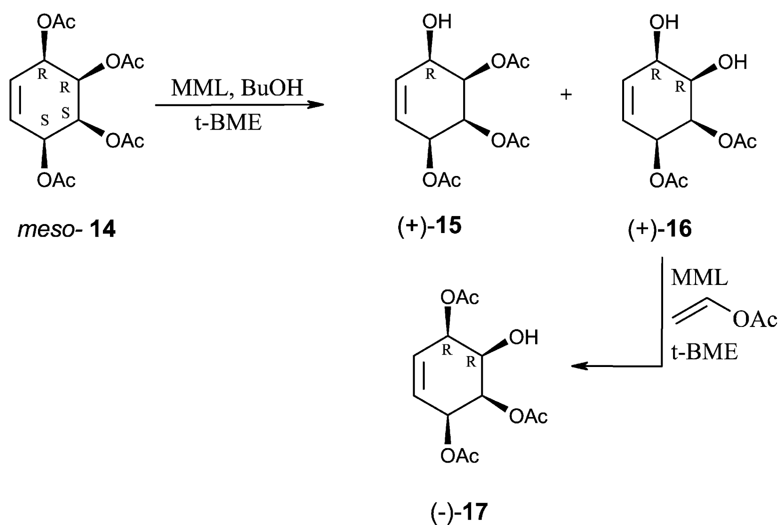
3. Regioprotection–deprotection of conduritols

Today there is an outstanding interest in the preparation of chiral conduritols (5-cyclohexen-1,2,3,4-tetrols), because these polyalcohols may be used as starting material in the synthesis of densely-functionalised molecules, including inositols and pseudosugars with significant biological activity [8].

The presence of four stereogenic carbon atoms in the framework of conduritols allows the existence of 10 stereoforms. In our studies, we have considered molecular recognition by MML in the alcoholysis of conduritol peracetates B, E and D, the latter being a *meso*-form while the other two exist as couples

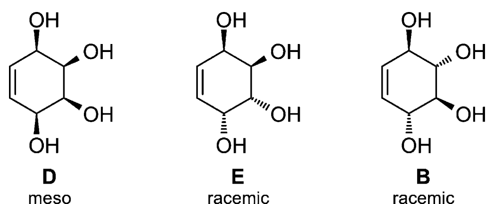


Scheme 4.



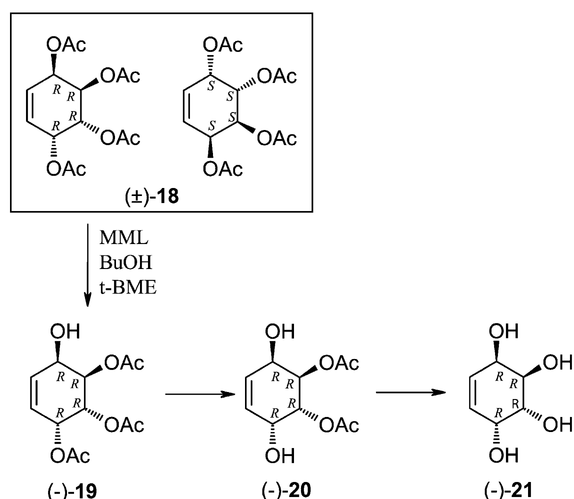
Scheme 5.

of enantiomers [9].[2]



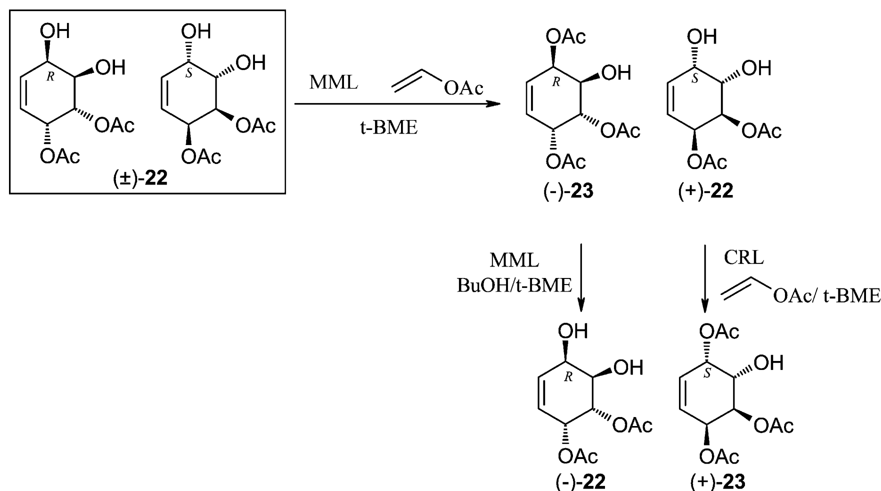
Alcoholysis of conduritol D peracetate, **14**, occurred with preferential removal of the allylic ester group located on the stereogenic centre possessing *R*-configuration, to give (+)-**15** (Scheme 5) [10]. Upon prolonging the reaction time, alcoholysis of a second acetyl group on an *R*-centre was observed, obtaining as final product of dialcohol (+)-**16**. It is to be emphasized that the double alcoholysis occurs by two subsequent steps, since the monoalcohol at C-2 was absent from the reaction products. This can be due to steric hindrance protecting the ester group at C-2 position, and in line with this hypothesis is the course of the esterification of dialcohol (+)-**16** in the presence of MML. As a result, triester (−)-**17** was quickly produced, without further formation of the peracetate, thus demonstrating the impossibility for the hydroxyl function at C-2 to be recognised by lipase from *M. miehei*.

In the case of conduritol E peracetate, (±)-**18**, MML showed enantiomeric discrimination between all-*R* and all-*S* stereoisomers, allowing kinetic resolution of the racemic mixture (Scheme 6) [11]. Due to the C_2 symmetry of conduritol E, recognition of all-*R* stereoforms results in the preferential alcoholysis of ester group at C-1 or C-4 to give initially alcohol



Scheme 6.

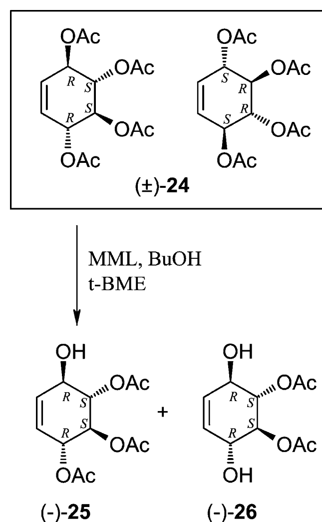
[2] Additional contributions regarding lipases mediated regioprotection–deprotections of conduritols are also [9].



Scheme 7.

(−)-**19**. This triester suffers a second alcoholysis to give (−)-**20**. Removal of the allylic ester groups decreased the shielding of the acetoxyls at C-2 and C-3, so that tetrol (−)-**21** was obtained as final product. No alcoholysis was observed for the enatioform all-*S*. This restriction in the preparation of the regioprotected *S*-enantioform derivatives can be overcome by using dialcohol (±)-**22** as starting material [12]. Two lipases, from *M. miehei* and *Candida cylindracea* (later renamed *C. rugosa*, CRL), are able to catalyse the esterification of this alcohol, the former possessing a high *R*-selectivity and the latter being weakly *S*-enantioselective (Scheme 7). These lipases can be advantageously employed together to obtain regioprotected derivatives of both conduritol E enantiomers. In the first step, enantiomeric separation of (±)-**22** was realised through esterification catalysed by MML, to give enantiopure (−)-**23** along with unreacted (+)-**22**. Subsequent alcoholysis of (−)-**23** in the presence of MML furnished (−)-**22**, while CRL catalysed esterification of the single all-*S* (+)-**22** gave (+)-**23**. This represents a typical case in which a lipase, although possessing low enantio-discrimination, can be employed advantageously for synthetic purposes.

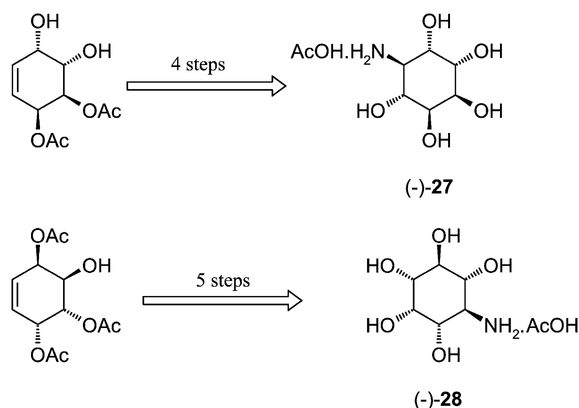
Finally, we wish to report the case of conduritol B, also possessing C_2 symmetry, but with a different reciprocal disposition of hydroxyl groups (Scheme 8) [13]. The use of MML in the alcoholysis of (±)-**24** results initially in the removal of the acetyl group at C-1 position in the *RSSR* enantioform, followed by successive alcoholysis at the symmetrical C-4 position. Further alcoholysis did not occur due to the unsuitable configuration of the acetoxyl groups at C-2 and C-3, so that (−)-**25** and (−)-**26** were the regioprotected final products. In the same conditions, the *SRRS* enantioform, (+)-**24**, in which the allylic positions have the wrong stereochemistry while the inner acetoxyl groups are strongly shielded, was recovered untransformed.



Scheme 8.

The obtained chiral regioprotected conduritols can be utilised as key intermediates in the synthesis of inositol analogues, since they contain four stereogenic centres of defined configuration and a double bond suitable to introduce two additional ones. Moreover, selective protection of all but one of the hydroxyl groups can be used for the conversion of the free OH into a nitrogen substituent or to creating discriminatory effects at the vinylic positions. This is the case of triester (−)-**23** and diester (+)-**22**, stereo- and regio-selectively transformed into 4-amino-4-deoxy-*myo*-inositol, (−)-**27**, and 4-amino-4-deoxy-*chiro*-inositol, (−)-**28**, respectively [14].

In conclusion, we have reported here examples of synthetic applications of lipase-catalysed reactions in



the preparation of regioprotected polyhydroxylated compounds. The selectivity of lipases can be a powerful tool in organic synthesis, allowing the ready preparation of molecules possessing specific features or useful for further modification to give more complex molecules.

1. *Experimental procedure for the synthesis of* 3 *and* 4

To a solution of **2** (200 mg) in *t*-butyl methyl ether (5 ml) 50 μl of *n*-butanol and CAL (30 mg) were added and the suspension incubated at 45 °C under shaking (300 rpm). After 24 h the reaction was stopped, the enzyme filtered off and the solvent evaporated under reduced pressure. Chromatographic purification of the residue afforded 48 mg of **3** (yield 29%). NMR (DMSO-d_6) 7.57 (d, $J = 2.0$ Hz, H-8), 7.30 (d, $J = 2.2$ Hz, H-2′), 7.29 (dd, $J = 2.2$, 8.5 Hz, H-6′), 7.10 (d, $J = 2,0$ Hz, H-6), 6.90 (d, $J = 8.5$ Hz, H-5′), 2.31–2.30 (bs, Ac × 3). UV (absorption EtOH) λ_{max} 248 nm (ε 18800), 345 nm (ε 13150). Analytically calculated for $C_{21}H_{16}O_{10}$: C 58.88, H 3.76; found: C 59.00, H 3.88.

To a solution of **2** (200 mg) in *t*-butyl methyl ether (5 ml) 50 μl of *n*-butanol and MML (100 mg) was added, and the suspension shaken (300 rpm) at 45 °C for 24 h. Work-up of the reaction mixture as furnished above 50 mg of **4** (yield 27%). ^{1}H NMR data were in agreement with those reported in the literature [15]. An analogous reaction carried out for 48 h gave 122 mg of **5** (yield 90%). ^{1}H NMR data were in agreement with those reported in the literature [15].

References

[1] A.M. Klibanov, Nature 409 (2001) 241–246;
G. Carrea, S. Riva, Angew. Chem. Int. Ed. 39 (2000) 2227–2254.

[2] J.A. Manthey, Microcirculation 7 (2000) S29–S30;
J.B. Harborne, C.A. Williams, Phytochemistry 55 (2000) 481–504;
C.F. Skibola, M.T. Smith, Free Radical Biol. Med. 29 (2000) 375–383.

[3] M. Foti, M. Piattelli, M.T. Baratta, G. Ruberto, J. Agric. Food Chem. 44 (1996) 497–501;
C. Rice-Evans, Curr. Med. Chem. 8 (2001) 797–807.

[4] V.S. Parmar, A.K. Prasad, N.K. Sharma, K.S. Bisht, R. Sinha, P. Taneja, Pure Appl. Chem. 64 (1992) 1135–1139;
V.S. Parmar, A.K. Prasad, N.K. Sharma, S.K. Singh, H.N. Pati, S. Gupta, Tetrahedron 31 (1992) 6495–6498;
K.S. Bisht, O.D. Tyagi, A.K. Prasad, N.K. Sharma, S. Gupta, V.S. Parmar, Bioorg. Med. Chem. 2 (1994) 1015–1020.

[5] D. Lambusta, G. Nicolosi, A. Patti, M. Piattelli, EP1088094, 2001 (http://ec.espacenet.com/espacenet/).

[6] M.T. Gatto, S. Falcocchio, E. Grippa, G. Mazzanti, L. Battinelli, G. Nicolosi, D. Lambusta, L. Saso, Bioorg. Med. Chem. 10 (2002) 269–272.

[7] D. Lambusta, G. Nicolosi, A. Patti, M. Piattelli, Synthesis 11 (1993) 1155–1158.

[8] B. Vanhaesebroeck, S.J. Leevers, K. Ahmadi, J. Timms, R. Katso, P.C. Driscoll, R. Woscholski, P.J. Parker, M.D. Waterfield, Ann. Rev. Biochem. 70 (2001) 535–602;
L. Cocco, A.M. Martelli, O. Barnabei, F.A. Manzoli, Adv. Enzyme Regul. 41 (2001) 361–384.

[9] R.C. Jonhson, P.A. Pie, J.P. Adams, J. Chem. Soc. Chem. Commun. 15 (1991) 1006–1007;
S. Takano, M. Moriya, Y. Higashi, K. Ogasawara, J. Chem. Soc. Chem. Commun. 2 (1993) 177–178;
H. Yoshizaki, J.E. Backvall, J. Org. Chem. 63 (1998) 9339–9341;
O. Block, G. Klein, H.J. Altenbach, D.J. Brauer, J. Org. Chem. 65 (2000) 716–721;
Y.U. Kwon, S.K. Chung, Org. Lett. 3 (2001) 3013–3016.

[10] A. Patti, C. Sanfilippo, M. Piattelli, G. Nicolosi, Tetrahedron: Asymmetry 7 (1996) 2665–2670.
[11] C. Sanfilippo, A. Patti, M. Piattelli, G. Nicolosi, Tetrahedron: Asymmetry 8 (1997) 1569–1573.
[12] C. Sanfilippo, A. Patti, M. Piattelli, G. Nicolosi, Tetrahedron: Asymmetry 8 (1997) 2083–2084.
[13] C. Sanfilippo, A. Patti, M. Piattelli, G. Nicolosi, Tetrahedron: Asymmetry 10 (1999) 3273–3276.
[14] C. Sanfilippo, A. Patti, M. Piattelli, G. Nicolosi, Tetrahedron: Asymmetry 9 (1998) 2809–2817.
[15] M. Natoli, M. Piattelli, G. Nicolosi, J. Org. Chem. 57 (1992) 5776–5778.