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Microbiological transformations 50: selection of epoxide hydrolases for enzymatic resolution of 2-, 3- or 4-pyridyloxirane

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Abstract

A study aimed to select efficient epoxide hydrolases (EHs) allowing to achieve the enzymatic resolution of 2-, 3- and 4-pyridyloxirane (**1**–**3**) has been achieved, using 2-pyridyloxirane **1** as test substrate. Five thus selected EH-sources that showed interesting enantioselectivity were looked at in more detail for the conversion of **1**–**3**.

Keywords: Epoxide hydrolases; Enzymatic resolution; *Aspergillus niger*; Pyridyloxirane; Screening

1. Introduction

The preparation of enantiopure epoxides as well as of their corresponding vicinal diols is of great interest in synthetic organic chemistry, as these compounds represent highly versatile chiral synthons for the synthesis of many biologically active molecules. Various chemical ways using in particular heavy metal based catalysts have been developed in order to synthesize these important building blocks in enantiopure form, either via direct epoxidation of an olefin or, more recently, using a kinetic resolution process [1,2]. However, due to increased environmental and regulatory pressure and to the generally moderate turnover observed, these methods raise some issues at the preparative and a fortiori industrial level. We (and others) have recently developed studies towards a "green chemistry" strategy based on the use of microbial enzymes, i.e. epoxide hydrolases (EHs) (EC 3.3.2.3) [3–9]. Such enzymes were shown to be cofactor independent, a fact that makes them very easy to use, likewise lipases for example. Furthermore, some of them were shown to display complementary enantioselectivity, whereas some others exhibited opposite regioselectivity on the two enantiomers of one particular substrate, thus affording a so-called "enantioconvergent" process [5,10–12]. This could be exploited for the synthesis of enantiomerically pure bioactive compounds [5,6,13,14].

Owing to the interest of enantiopure 2-, 3-, and 4-pyridyloxiranes **1**–**3** as key-step building blocks for the synthesis of β-adrenergic receptor agonists or

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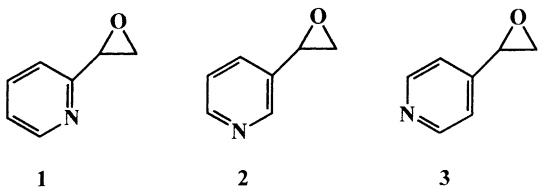


1 2 3

Scheme 1. Racemic epoxides studied: 2-, 3- and 4-pyridyloxirane.

anti-obesity drugs [15–17], we recently have focused our attention on the preparation of these chirons (Scheme 1). In this paper we describe our results focused on the selection of appropriate EHs, as well as the comparison of four of these enzymes as far as their enantio and regioselectivity are concerned for enzymatic hydrolysis of **1**–**3**.

2. Experimental

2.1. General

NMR spectra (^{1}H and ^{13}C) were recorded in $CDCl_3$ at 250 and 100 MHz, respectively. Chemical shifts are reported in δ from TMS as internal standard. For gas chromatography (GC) analysis, the chiral column Chiralsil Dex CB (25 mm × 0.25 mm, Chrompack) was used.

2.1.1. Synthesis of 2-pyridyloxirane *1*

This was synthesized following the procedure described by Hanzlik et al. [18]. From 10.3 ml 2-vinylpyridine (Fluka) 4.9 g of **1** were obtained (41% yield) [19]. Purification by flash chromatography (hexane/ethylacetate 4/6), followed by removal of solvent by bulb-to-bulb distillation (40 °C; 4×10^{-2} mbar). GC-condition: 110 °C; 1 kg/cm^2 helium; $R = 7.8$ min; $S = 8.6$ min.

2.1.2. Synthesis of 3-pyridyloxirane *2*

As described by Giannini et al. [20]. From 3.29 g 3-pyridylaldehyde (Fluka) 2.1 g of **2** were obtained (56% yield). DMSO was removed by washing the organic phase four times with distilled water. Purification by flash chromatography (hexane/ethylacetate 4/6), followed by removal of solvent by bulb-to-bulb distillation without heating (4×10^{-2} mbar). GC-condition: 110 °C; 1 kg/cm^2 helium; R=12.2 min; $S = 12.8$ min.

2.1.3. Synthesis of 4-pyridyloxirane *3*

As described by Carrol [22], Corey and Chaykovsky [21]. From 23.3 g 4-pyridylaldehyde (Fluka) 6.7 g **3** was obtained (25% yield). DMSO was removed by washing the organic phase four times with distilled water. Purification by flash chromatography (hexane/ethylacetate 4/6), followed by removal of solvent by bulb-to-bulb distillation without heating (4×10^{-2} mbar). GC-condition: 110 °C; 1 kg/cm^2 helium; $R = 12.9$ min; $S = 13.8$ min.

2.1.4. Racemic 2-pyridyldiol *1d*

As described by Hanzlik et al. [18]. 1.6 g **1** was hydrolyzed under acidic conditions (H_2SO_4) for 64 h to give 1.1 g **1d** (yield 60%). ^{1}H NMR (acetone D_6) δ: 3.64 (dd, 1H, H_2, $J_{gem} = 10.8$ Hz, $J_{1-2} = 5.6$ Hz); 3.82 (dd, 1H, H_2, $J_{gem} = 11.3$ Hz, $J_{1-2} = 6.1$ Hz); 3.97–3.94 (m, 1H, H_{OH}); 4.66 (d, 1H, H_{OH}, $J = 4.95$); 4.77–4.73 (m, 1H, H_1); 7.28–7.24 (m, 1H); 7.56 (d, 1H, $J = 7.8$ Hz); 7.82–7.75 (m, 1H); 8.51 (d, 1H, $J = 4.5$ Hz) [23]. ^{13}C NMR δ: 67.81; 75.06; 121.59; 123.07; 137.32; 149.12;162.57.

2.2. General procedure for the enzymatic hydrolysis of *1*–*3*. Analytical scale conversion

A substrate solution (5–80 mM) in a 0.1 M pH8 sodium phosphate-buffer was used and diethylendiethylglycolether (Aldrich) was added as an internal standard (12 mM). Before addition of the enzyme extract, a sample was taken for the 100% epoxide-value. The reaction was started with addition of the substrate solution to the enzyme extract. The kinetic resolution (28 °C, 750 rpm) was followed by taking aliquots from the reaction medium (1 vol. sample was added to 1 vol. of methanol and 2 vol. of $CHCl_3$). The organic phase was analyzed by GC to calculate the degree of conversion and the ee of the remaining epoxide (GC: 110 °C; internal standard = 4.6 min). After total extraction of the epoxide with $CHCl_3$ and saturation of the aqueous phase with NaCl, the diol was extracted using ethylacetate. In order to determine its ee, the diol was cyclized back to the epoxide following the procedure previously described by Golding et al. [24] (without change of its absolute configuration) as follows. To the dried diol, 100 μl HBr in glacial acetic acid (33%) were added. After 1.25 h at RT, the mixture was neutralized by addition of 500 μl water and

about 200 mg of solid Na_2CO_3 and extracted twice with 300 μl ethylacetate. The combined organic layers were evaporated and dried under vacuum. To this, 50 μl of KOH in MeOH (1 N) was added and after 30 min at RT-300 μl water were added. The epoxide was extracted with 300 μl $CHCl_3$ and analyzed by GC.

3. Results and discussion

3.1. Stability of epoxides 1–3

As epoxides **1**–**3** were previously described as being somewhat unstable [19,22,25] their stability under the experimental conditions we currently use for enzymatic hydrolysis (0.1 M sodium–phosphate buffer, pH8) was checked. Their half life time at 28 °C ($t_{1/2}$) were respectively 6.6 days for **1**, 2.6 days for **2** and 4.4 days for **3**. At lower temperature their stability increased significantly ($t_{1/2}$ at 0 °C: 103.8 days for **1**, 13.4 days for **2** and 46.3 days for **3**). Only **1** afforded the corresponding diol upon acid catalyzed hydrolysis, while **2** and **3** seemed to decompose to other products.

3.2. Enzymatic hydrolysis of 1 using different EH-sources

In order to determine the most suitable biocatalyst for performing the BHKR of **1**–**3**, we investigated the enzymatic hydrolysis of **1** using 14 enzyme extracts from our present EH collection including nine fungal enzymes and five from mammalian or plant origin. All these were effective for achieving enzymatic hydrolysis of **1**. The results are summarized in Table 1.

3.2.1. Enantioselectivity

The *Aspergillus niger* GBCF 79 and GBCF 80 EHs exhibited the highest *E*-values (78 and 66 respectively). All other EH-sources showed only low to moderate enantioselectivity. As far as the

Table 1
Bioconversion of 1 by enzyme extracts from different sources

	Epoxide hydrolase source	*E*[a]	Epoxide **1**[b]	Diol **1d**[c]
1	*A. niger* LCP 48.521 [6]	**44**	*S*	*R*
2	*A. niger* GBCF 79 [28,29,30,31][d]	**78**	*S*	*R*
3	A. terreus *CBS 116-46* [6]	6.3	*S*	*R*
4	Strain GBCF 80 [6][e]	**66**	*S*	*R*
5	*Mortierella isabellina* ATCC 42619 [6]	1.2	*S*	–[f]
6	*Cunninghamella elegans* LCP 58–1543 [6]	1.1	*S*	–
7	*Beauveria bassiana* ATCC 7159 [6]	2	*S*	–
8	*Chaetomium globosum* LCP 47–679 [6]	1.4	*S*	*R*
9	*Syncephalastrum racemosum* MUCL 28766 [6]	1.9	*S*	*R*
10	Rat [32,33]	2	*S*	–
11	Human [33,34]	1.7	*R*	*R*
12	Soybean (*Glycine max*) [35]	1.3	*R*	–
13	*Solanum tuberosum* [33,36]	18	*R*	*R*
14	*Arabidopsis thaliana* [33,37]	2	*R*	*S*

[a] *E*-value calculated from the degree of conversion and the ee of the epoxide.
[b] Absolute configuration of the remaining epoxide [27].
[c] Absolute configuration of the preferred produced diol [27].
[d] The recombinant GBCF 79 strain is owned by the CNRS and is an in-house item of our laboratory. Any query about this strain can thus be addressed to the Marseille's group and may be available upon agreement. It has been constructed by Professor J. Visser (Wageningen Agricultural University, Section Molecular Genetics of Industrial Microorganisms, Wageningen, 6703H, The Netherlands) by cloning the epoxide hydrolase gene from the wild strain *Aspergillus niger* LCP 521 into an appropriate *Aspergillus* host (unpublished results). The enzyme has been previously purified in our laboratory [29] and further on sequenced and cloned in an *E. coli* host [30] The X-ray crystal structure of this enzyme has also been published recently [31].
[e] Strain GBCF 80 was newly isolated in our lab, but not yet characterized; the enzyme extract was prepared using the procedure described for *A. niger* LCP 48.521. Any query about this strain can thus be addressed to the Marseille's group and may be available upon agreement.
[f] Not determined.

enantiopreference is concerned, it clearly appeared that most EHs preferentially hydrolyzed the (*R*) enantiomer and led to recovery of the (*S*)-epoxide (entries 1–10). Surprisingly, even *Beauveria bassiana*, a fungus we had previously shown to be enantiocomplementary to *A. niger* for styrene oxide [12] behaved similarly. However, as can be seen at entries 11–14, the human, soybean (*Glycine max*), *Solanum tuberosum* and *Arabidopsis thaliana* enzymes hydrolyzed the (*S*)-epoxide preferentially, thus being enantiocomplementary to the other EHs.

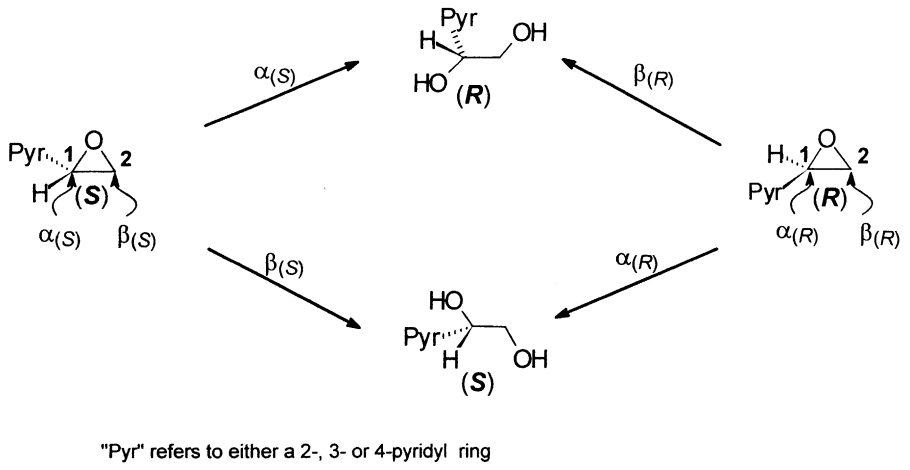
3.2.2. Regioselectivity

As an epoxide can be attacked at both carbon atoms of the oxirane ring, and owing to the fact that the regioselectivity of this attack can be different from one enantiomer to the other, determination of the regioselectivity involved for each enantiomer of a given substrate must be achieved in order to afford a correct analysis of the reaction. Interestingly, a variation of regioselectivity between two enantiomers will lead to a process qualified as being enantioconvergent, i.e affording (theoretically) a 100% yield of enantiopure diol (Scheme 2) [26]. Thus, we observed that the enzymatic attack occurred at the less substituted carbon atom (β carbon) of **1** in most cases, the (*R*)-diol being very preferentially formed from the (*R*) enantiomer. Similarly, the *A. thaliana* EH preferably afforded the (*S*)-diol by hydrolysis of the (*S*)-epoxide, thus showing again a preferential attack at the less substituted carbon atom. To the opposite, both human and *S. tuberosum* EHs led to the diol of (*R*) absolute configuration upon hydrolysis of the (*S*) enantiomer, thus revealing a preferential attack at the more substituted carbon atom (α carbon) (entries 11 and 13). To determine the regioselectivity coefficient [26] further studies were conducted on the three substrates **1**, **2** and **3** using the best four EHs, i.e. *A. niger* GBCF 79, *A. niger* GBCF 80, *S. tuberosum* and *A. thaliana* (Table 2).

The α(*S*)/β(*S*) and α(*R*)/β(*R*) ratios for **1** were determined by studying separately the stereochemical outcome of the enzymatic hydrolysis of enantiopure (*S*)-**1** as well as of racemic **1** [26]. It thus can be observed that, in the case of *S. tuberosum*, the (*R*) enantiomer is predominantly attacked at the β carbon atom, whereas the regioselectivity of the (*S*) enantiomer is rather at the α carbon atom.

3.3. Enzymatic hydrolysis of epoxides 2 and 3

Here again, the best enantioselectivity for **2** and **3** was obtained using the fungal EHs, **2** showing comparatively the lowest enantioselectivity. As for **1**, the α(*S*)/β(*S*) and α(*R*)/β(*R*) ratios corresponding to each enantiomer of **2** and **3** were determined by studying separately the stereochemical outcome of the hydrolysis of their enantiopure (*S*) enantiomer as well as of the racemic substrate (see Table 2). The *A. niger* GCBF 79 EH as well as the extract from strain GBCF 80



Scheme 2. Possible enantioconvergent processes due to complementary enantio and regioselectivity implied during enzymatic hydrolysis of an epoxide.

Table 2
Parameters of biohydrolysis (enzymatic hydrolysis) of **1**–**3** using four different EHs.

Substrate	Enzyme extract	Remaining epoxide[a]	Diol[b]	*E*-value[c]	Activity[d]	α(*S*)/β(*S*)[e]	α(*R*)/β(*R*)[e]
1	*A. niger* GBCF 79	*S*	*R*	78	190	3/97	3/97
	Strain GBCF 80	*S*	*R*	66	9	14/86	2/98
	Solanum tuberosum	*R*	*R*	18	3	85/15	20/80
	Arabidopsis thaliana	*R*	*S*	2	104	2/98	20/80
2	*A. niger* GBCF 79	*S*	*R*	27	944	7/93	7/93
	Strain GBCF 80	*S*	*R*	9	19	8/92	7/93
	Solanum tuberosum	*R*	*R*	9	3	70/30	0/10
	Arabidopsis thaliana	*S*	*S*	≈1	110	11/89	34/66
3	Strain GBCF 79	*S*	*R*	47	556	11/89	10/90
	Strain GBCF 80	*S*	*R*	14	13	8/92	7/93
	Solanum tuberosum	*R*	*R*	15	4	77/23	7/93
	Arabidopsis thaliana	*R*	*S*	11	66	8/92	11/89

[a] Absolute configuration determined as described earlier [27].
[b] Absolute configuration determined as described earlier [27].
[c] Determined using the ee of the epoxide and the conversion ratio, at 28 °C and 4–8 mM substrate concentration.
[d] Expressed in nmol/min/mg enzyme extract. This was calculated from the derived second order polynomial regression of conversion ratio against time, up to 40% conversion.
[e] Regioselectivity coefficient for either (*S*) or (*R*) enantiomer. These values were calculated from the equation $\alpha(R) = \alpha(S)$-eef [26].

showed clearly, for all three epoxides, high β(*S*) and β(*R*) values (90–97%), resulting in retention of configuration for both diols. As a consequence, the ee of the diol decreased throughout the reaction to afford a nearly racemic product at total conversion. To the opposite, an interesting enantioconvergent process was observed for both *A. thaliana* and *S. tuberosum* enzymes. Indeed, although the *A. thaliana* EH showed a very poor enantioselectivity for both substrates, the final ee-value of the diol obtained from **2** was approximately 20%. This feature was significantly more pronounced for the *S. tuberosum* extract. Indeed, for both substrates **2** and **3**, the final ee-value of the diol was about 70%; due to preferential attack at the α-carbon atom of the (*S*) enantiomer and to a nearly exclusive attack at the β-carbon of the (*R*) enantiomer. Interestingly, all these variations of enantio and regioselectivity are only linked to the position of the nitrogen atom in the pyridine ring, indicating that some electronic factors must be implied in the positioning of the substrate into the active site and/or in the kinetic behavior of these substrates. Also, it is interesting to notice that the activities observed with the three substrates **1** to **3** and the *A. niger* GBCF 79 enzyme also depend on this structural feature. Thus, a three-fold activity increase was observed from **1** to **3**, whereas the activity against **2** showed an increase of a factor 5 as compared to **1**.

4. Conclusion

Different fungal and other known EHs from various sources were tested for performing the enzymatic resolution of 2-, 3- and 4-pyridyloxirane **1**–**3**. All the enzymes hydrolyzed these substrates, although with different enantio and regioselectivities. The enzyme extract from the *A. niger* wild type (LCP 48.521), from the strain GBCF 79 overexpressing the same enzyme as well as the one from the newly discovered GBCF 80 strain showed the best *E*-values, i.e. respectively 44, 78 and 66 for substrate **1**, thus allowing to precisely tune the reaction for obtaining the unreacted (*S*) enantiomer of these epoxides in enantiomerically pure form. Interestingly, we have observed that obtention of these enantiopure epoxides was not possible using the best presently known conventional chemistry approaches [27]. Furthermore, we observed that the EH from *S. tuberosum* led to an enantioconvergent process, thus allowing in principle to prepare the (*R*)-diol with a 70% ee and a 100% yield starting from racemic **2**.

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References

[1] Y.N. Ito, T. Katsuki, Bull. Chem. Soc. Jpn. 71 (1999) 603.
[2] J.M. Ready, E.N. Jacobsen, J. Am. Chem. Soc. 123 (2001) 2687.
[3] A. Archelas, R. Furstoss, Curr. Opin. Chem. Biol. 5 (2001) 112.
[4] A. Archelas, R. Furstoss, in: W.D. Fessner (Eds.), Biocatalysis, from Discovery to Application, Springer, Berlin (Heidelberg), 1999, pp. 159–192.
[5] K.M. Manoj, A. Archelas, J. Baratti, R. Furstoss, Tetrahedron 57 (2001) 695.
[6] M. Cleij, A. Archelas, R. Furstoss, J. Org. Chem. 64 (1999) 5029.
[7] F. Zocher, M.M. Enzelberger, U.T. Bornscheuer, B. Hauer, W. Wohlleben, R.D. Schmid, J. Biotechnol. 77 (2000) 287.
[8] W.J. Choi, C.Y. Choi, J.A.M. de Bont, C.A.G.M. Weijers, Appl. Microbiol. Biotechnol. 54 (2000) 641.
[9] J.H.L. Spelberg, R. Rink, R.M. Kellogg, D.B. Janssen, Tetrahedron Asymmetry 9 (1998) 459.
[10] S. Pedragosa-Moreau, C. Morisseau, J. Baratti, J. Zylber, A. Archelas, R. Furstoss, Tetrahedron 53 (1997) 9707.
[11] R.V.A. Orru, W. Kroutil, K. Faber, Tetrahedron Lett. 38 (1997) 1753.
[12] S. Pedragosa-Moreau, A. Archelas, R. Furstoss, J. Org. Chem. 58 (1993) 5533.
[13] A. Archelas, J.P. Delbecque, R. Furstoss, Tetrahedron Asymmetry 4 (1993) 1445.
[14] R.V.A. Orru, I. Osprian, W. Kroutil, K. Faber, Synthesis (1998) 1259.
[15] R.J. Mathvink, A.M. Barritta, M.R. Candelore, M.A. Cascieri, L. Deng, L. Tota, C.D. Strader, M.J. Wyvratt, M.H. Fisher, A.E. Weber, Bioorg. Med. Chem. Lett. 9 (1999) 1869.
[16] K.M. Devries, R. L. Dow, S. W. Wright, Patent: WO9821184A1 (CAN 129: 27896), 1998.
[17] M.H. Fisher, E.M. Naylor, D. Ok, A.E. Weber, T. Shih, H. Ok, Patent US5561142 (CAN 125: 275666), 1996.
[18] R.P. Hanzlik, M. Edelman, W.J. Michaely, G. Scott, J. Am. Chem. Soc. 98 (1976) 1952.
[19] K. Kloc, E. Kubicz, J. Mlochowski, Heterocycles 22 (1984) 2517.
[20] M. Giannini, G. Bonacchi, M. Fedi, F. Sesto, German Patent: DE 3425477 A1 (CAN 103: 71336), 1985.
[21] E.J. Corey, M. Chaykovsky, J. Am. Chem. Soc. 87 (1965) 1353.
[22] F.I. Carrol, Org. Prep. Proc. Int. 3 (1971) 121.
[23] G. Chelucci, M.A. Cabras, A. Saba, Tetrahedron Asymmetry 5 (1994) 1973.
[24] B.T. Golding, D.R. Hall, S. Sarkrikar, J. Chem. Soc. Perkin Trans. I (1973) 1214.
[25] R.P. Hanzlik, W.J. Michaely, J. Chem. Soc. Chem. Commun. (1975) 113.
[26] P. Moussou, A. Archelas, J. Baratti, R. Furstoss, Tetrahedron Asymmetry 9 (1998) 1539.
[27] Y. Genzel, A. Archelas, Q.B. Broxterman, B. Schulze, R. Furstoss, J. Org. Chem. 66 (2001) 538.
[28] A. Archelas, M. Arand, J. Baratti, R. Furstoss, French Patent Application No. 9905711, (1999) and International Patent Application No. WO 00/68394 (CAN 133: 360460), 2000.
[29] C. Morisseau, A. Archelas, C. Guitton, D. Faucher, R. Furstoss, J.C. Baratti, Eur. J. Biochem. 263 (1999) 386.
[30] M. Arand, H. Hemmer, H. Durk, J. Baratti, A. Archelas, R. Furstoss, F. Oesch, Biochem. J. 344 (1999) 273.
[31] J.-Y. Zou, M. Hallberg, T. Bergfors, F. Oesch, M. Arand, S.L. Mowbray, T.A. Jones, Struct. London 8 (2000) 111.
[32] G.M. Lacourciere, V.N. Vakharia, C.P. Tan, D.I. Morris, G.H. Edwards, M. Moos, R.N. Armstrong, Biochemistry 32 (1993) 2610.
[33] D.R. O'Reilly, L.K. Miller, V.A. Luckow, in: Baculovirus Expression Vectors, A Laboratory Manual, WH Freeman Co., New York, 1992.
[34] J.K. Beetham, T. Tian, B.D. Hammock, Arch. Biochem. Biophys. 305 (1993) 197.
[35] E. Blée, F. Schuber, Biochem. J. 282 (1992) 711.
[36] A. Stapleton, J.K. Beetham, F. Pinot, J.E. Garbarino, D.R. Rockhold, M. Friedman, B.D. Hammock, W.R. Belknap, Plant J. 6 (1994) 251.
[37] T. Kiyosue, J.K. Beetham, F. Pinot, B.D. Hammock, K. Yamaguchi-Shinozaki, K. Shinozaki, Plant J. 6 (1994) 259.