


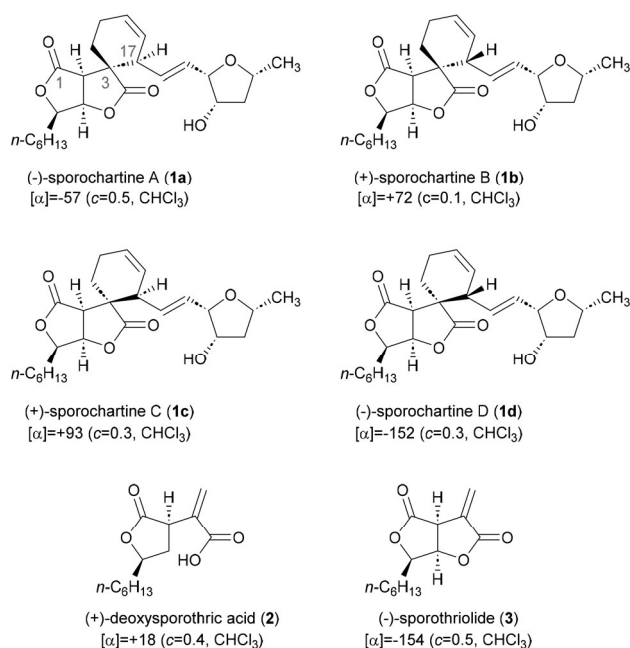
# Biomimetic-inspired synthesis of sporochartines through Diels–Alder reaction between enantiopure (–)-sporothriolide and (+)-trienylfuranol A†

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A biomimetic-inspired synthesis of sporochartines A–D involving a [4 + 2] Diels–Alder cycloaddition between (–)-sporothriolide and (+)-trienylfuranol A is described. For this study, we have realized the first enantiopure total synthesis of (+)-trienylfuranol A, containing an unusual and very sensitive terminal trienyl moiety. In this synthetic study, we have performed for the first time baker's yeast-mediated reduction of  $\alpha$ -oxo- $\gamma$ -butyrolactone derivatives with high stereoselectivities. To improve the enantioselectivity level, an enzymatic kinetic resolution with Amano lipase PS, from *Burkholderia cepacia*, allowed us to prepare (+)-trienylfuranol A with excellent enantiomeric excess (>99%). A Diels–Alder reaction between the two secondary metabolites (–)-sporothriolide and (+)-trienylfuranol A allowed us to observe the formation of sporochartines in 39% isolated yield. The relative distribution of the sporochartines formed showed a spectacular correlation with that obtained from the fermentation culture of *Hypoxyylon monticulosum* CLL-205 and allowed us to confirm that a Diels–Alder reaction is involved in the biosynthetic pathway for the formation of spiranic backbones of sporochartines.

## Introduction

Recently, we disclosed a new family of natural products named sporochartines due to the furofuran–dione framework, the basic core structure of sporothriolide. These new compounds were originally from a *Sphaerocladina* sponge, collected from the caves of Ti-Pari (Tahiti, France).<sup>1</sup> From this marine sponge, the fungus *Hypoxyylon monticulosum* CLL-205 was extracted and isolated. This *Hypoxyylon monticulosum* was cultivated in potato dextrose broth (5 days) and ethyl acetate extraction furnished an extract where sporochartines A–D **1a–d** were isolated (Fig. 1).<sup>2</sup> These new compounds were obtained in a mixture with (+)-deoxysporothric acid **2** and also with the well-known compound (–)-sporothriolide **3**. The (+)-deoxysporothric acid **2** was also recently observed during the fermentation culture of



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**Fig. 1** Structures of sporochartines A–D **1a–d**, deoxysporothric acid **2** and sporothriolide **3**.

the endophytic fungus *Nodulisporium* sp. A21 isolated from *Ginkgo biloba*.<sup>3</sup> The (–)-sporothriolide **3** has been known in the literature for many years, first isolated from *Sporothrix* sp. in 1994,<sup>4</sup> and more recently from *Hypoxyylon monticulosum* of tropical *Xylariaceae* originating from French Guiana and Thailand<sup>5</sup> and also from *Nodulisporium* sp. A21, an endophytic fungus in the leaves of *Ginkgo biloba*.<sup>6</sup> Racemic<sup>7</sup> and enantioselective<sup>8</sup> syntheses of sporothriolide **3** have been performed and its biosynthetic pathway was fully identified.<sup>9</sup>

Sporochartines exhibited activities against three human cancer cell lines,<sup>2b</sup> HCT-116 (human colon carcinoma cell line), PC-3 (prostate cancer cell line), MCF-7 (breast cancer cell line), and inhibited hyaluronidase in the same order of magnitude of glycyrrhizin.<sup>2a</sup>

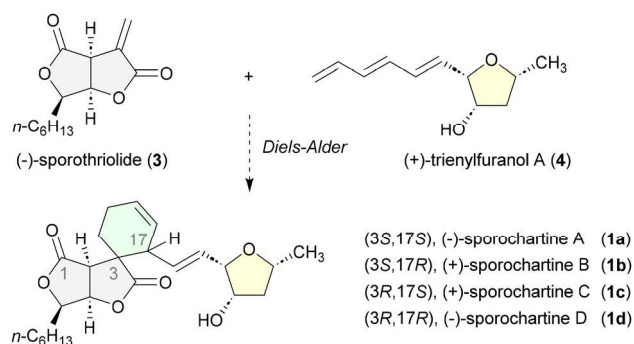
Recently, a strain of *Hypoxyylon submonticulosum* was isolated as an endophyte from a surface-sterilized leaf of a cultivated raspberry *Rubus idaeus*.<sup>10</sup> From a liquid extract of this strain, three new secondary metabolites with a furanic moiety were isolated. The major component was identified as a tetrahydro-5-methylfuran-3-ol compound with a trienyl side chain at the C-2 position and named trienylfuranol A **4** by the authors.

We are assuming that the biosynthetic pathway of sporochartines **1a–d** may result from a [4 + 2] cycloaddition between sporothriolide **3** and trienylfuranol A **4** with the concomitant formation of the spiranic backbone (Fig. 2).

In order to study the [4 + 2] cycloaddition operational feasibility, the two components sporothriolide and trienylfuranol A are needed.

## Results and discussion

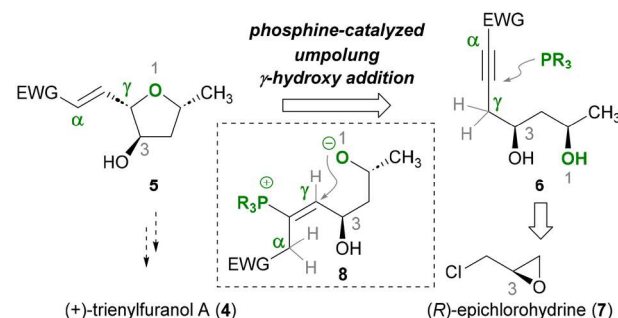
The sporothriolide is readily available by fermentation culture of *Hypoxyylon monticulosum* CLL-205 (see ESI Part III.1†). In contrast, trienylfuranol A **4** is produced in a small amount and the availability of this compound is complicated by its intrinsic poor stability due to highly susceptible polymerization or degradation of the very sensitive terminal trienyl moiety.<sup>10</sup>



**Fig. 2** Plausible biosynthetic pathway of sporochartines **1a–d** through Diels–Alder cycloaddition between (–)-sporothriolide **3** and (+)-trienylfuranol A **4**.

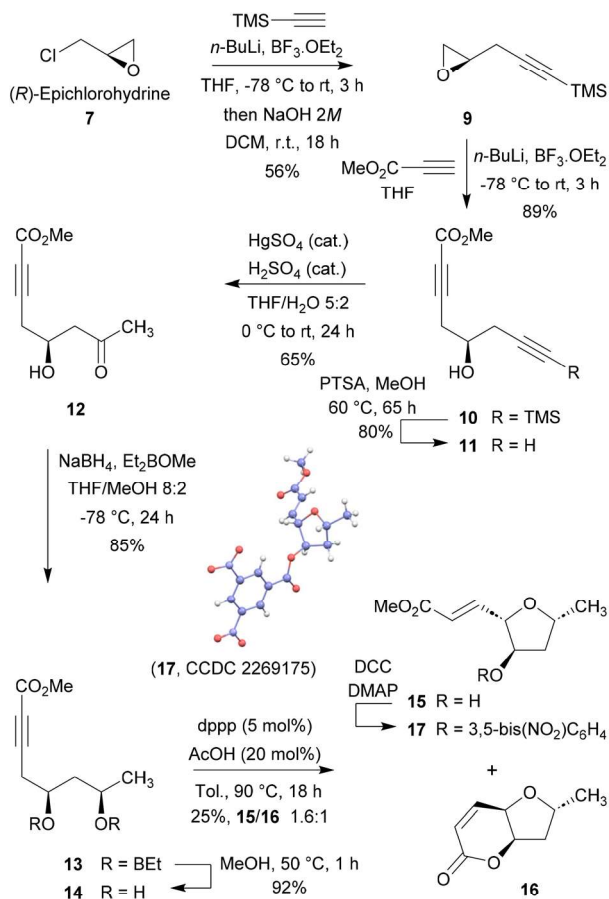
To obtain enantiopure (+)-trienylfuranol A **4**, a synthetic approach was developed starting from enantiopure (*R*)-epichlorohydrin **7** as a chiral source for the C-3 hydroxy function (furan numbering) (Fig. 3). Epichlorohydrin is a valuable chiral precursor for the synthesis of various polyhydroxylated natural products with the 1,3-diol pattern.<sup>11</sup> The furanic compound **5**, a precursor of trienylfuranol A **4**, should be obtained from an acyclic 5,7-dihydroxy-alkynoate compound **6** under a phosphine-catalyzed umpolung  $\gamma$ -hydroxy addition onto an alkynoate moiety. Indeed, phosphines, as Lewis base catalysts, are able to activate electron-poor alkynes by Michael-type additions. After the phosphine 1,4-addition, followed by a [1,3]-proton shift, the electrophilic vinyl phosphonium intermediate **8** was generated. The use of a suitable tethered alcohol on the substrate allows the interception of this key intermediate **8** by an oxygen nucleophilic addition with the formation of the desired furanic framework. After phosphine  $\beta$ -elimination, the C-2 exocyclic double bond of derivative **5** was generated with the release of the phosphine catalyst.<sup>12</sup>

The synthesis of methyl 5,7-dihydroxyoct-2-ynoate **14**, a precursor of the furanic compound **15**, began with the commercially available (*R*)-epichlorohydrin **7** (Scheme 1).<sup>13</sup> The more reactive epoxide function of epichlorohydrin reacted with the lithiated TMS-acetylene in the presence of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ <sup>14</sup> and the resulting ring-opening chlorohydrin was treated in basic media to afford the cyclised epoxide **9** in 56% yield.<sup>15</sup> A similar second alkylation reaction was carried out with the lithiated methyl propiolate to afford the bisalkynyl ester **10** in 89% yield. Then selective hydration reaction on the more electron-rich alkyne was initiated on compounds **10** and **11**, its TMS-deprotected counterpart, where this compound **11** with a terminal alkyne was anticipated as more reactive. The use of different noble (Au, Ag, and Pt) and non-noble (Fe and Hg) organometallic complexes or metallic salts was investigated (see ESI Tables S1 and S2†). The most selective and efficient hydration reaction was achieved on compound **10**, without deprotection of the silyl group, with mercury sulfate as a catalyst (3 mol%) in acidic media.<sup>16</sup> We observed a positive effect of the TMS group on the alkyne, which could be involved in the stabilization of the cationic intermediate.<sup>17</sup> Under these con-



**Fig. 3** Retrosynthetic pathway of enantiopure (+)-trienylfuranol A **4** by phosphine-catalyzed umpolung  $\gamma$ -hydroalkoxylation of the alkynoate derivative.





**Scheme 1** Synthesis of trisubstituted furane **15** (dppp = 1,3-bis(diphenylphosphino)propane).

ditions, the desired methyl ketone **12** was obtained selectively in a satisfactory 65% yield and without the formation of the undesired double hydration product.

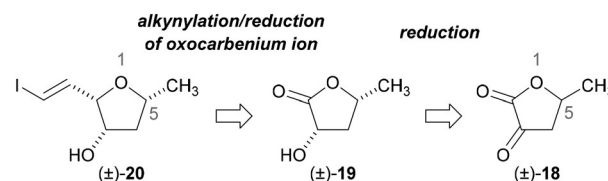
The next step, the diastereoselective *syn*-reduction of  $\beta$ -hydroxyketone **12**, was achieved using a boron-chelation method developed by Narasaka<sup>18</sup> and Prasad.<sup>19</sup> Treatment of **12** with diethylmethoxyborane and the successive reduction with sodium borohydride at  $-78\text{ }^\circ\text{C}$  in a solvent mixture THF/MeOH did not provide the expected *syn*-1,3-diol compound **14**, but produced the derived *cis*-*B*-ethyl-dioxaborinane **13** in 85% yield. It can be noted that several stable cyclic ethyl boronic esters, obtained by the Narasaka/Prasad procedure<sup>20</sup> or using a superhydride  $\text{LiBHET}_3$  reagent,<sup>21</sup> have also been previously reported in the literature. Simple methanolysis of this ethylboronate **13** under evaporation with  $\text{CH}_3\text{OH}$  at  $50\text{ }^\circ\text{C}$  afforded **14** with the free *syn*-1,3-diol moiety.

The first attempts to produce **15** from **14** were made using optimized conditions developed by Trost<sup>12a</sup> and applied with success on similar cyclization reactions for the synthesis of spirotryprostatins<sup>22</sup> and robustanoids A,<sup>23</sup> which involved the use of 5 mol% of phosphine catalyst and 20 mol% acetic acid in toluene at  $90\text{ }^\circ\text{C}$ . With the use of triarylphosphine, an incomplete reaction was observed with the formation of the

desired furanic compound **15** and the fused bicyclic furanopyranone **16** with low yields and selectivity (see ESI Table S3†). The structure of the targeted furanic compound **15** was confirmed by the formation of its 3,5-dinitro derivative **17** and recrystallization for obtaining crystals suitable for X-ray crystallography.<sup>24</sup> After screening several phosphine Lewis base catalysts, we found that the bisphosphine catalyst allowed a complete conversion of the alkyne substrate **14** (see ESI Table S3†). The use of bisphosphine as the catalyst is well-known to favor this intramolecular hydroalkoxylation process by stabilization of the key electrophilic cationic intermediate **8**, due to an extra phosphine moiety.<sup>12a,25</sup> In our case, the optimal distance between the two phosphine moieties was observed with dppp and dppb, while dppe led to incomplete reaction. However, despite a complete reaction, the desired product **15** is isolated in only 15% yield. Moreover, this low yield was associated with difficulties in separation from the undesired bicyclic compound **16**. The use of other experimental conditions, inspired by the work of Fu,<sup>12b</sup> failed to improve the results for the formation of compound **15**.

These disappointing results of this organocatalytic approach led us to reconsider our previously published racemic synthesis of trienylfuranol A.<sup>26</sup> This racemic approach implies the use of  $\alpha$ -oxo- $\gamma$ -butyrolactone ( $\pm$ )-**18** as a starting material (Fig. 4). This unsaturated compound **18** was firstly reduced in lactone ( $\pm$ )-**19** under a 1,3-induced asymmetric hydrogenation to set up the hydroxyl function in a *cis* relative configuration. Then, the C-2 vinyl moiety of ( $\pm$ )-**20** was stereoselectively introduced, taking advantage of the two stereocenters already set up on **19**, and based on an alkylation/reduction of an oxocarbenium ion sequence and using an organic hydride donor.<sup>26</sup> So, to obtain the  $\alpha$ -hydroxy-lactone **19** in an enantiomeric manner from ( $\pm$ )-**18**, the enantio-enriched (+)-trienylfuranol A **4** should be prepared.

To perform this stereoselective transformation for obtaining enantiomerically enriched **19**, we considered the use of baker's yeast (*Saccharomyces cerevisiae*) for the stereoselective reduction of ( $\pm$ )-**18**. Baker's yeast-mediated reactions are well-established whole-cell biotransformations, easily performed on lab scale with the added option to scale up for industrial applications to produce fine chemicals under mild conditions, with high yields and excellent selectivities.<sup>27</sup> This readily available biocatalyst, with low cost, is well known to reduce carbonyl groups, but other chemical transformations have also been carried out using baker's yeast, including organocatalyzed reactions<sup>28</sup> and multicomponent reactions.<sup>29</sup>

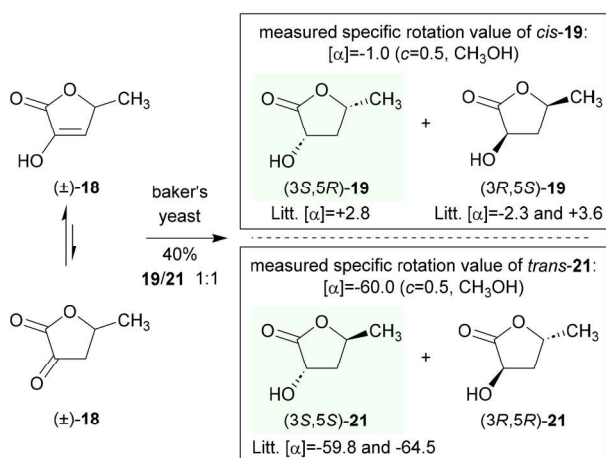


**Fig. 4** Previous synthetic approach of ( $\pm$ )-trienylfuranol A **4** for the stereocontrol of all-*cis*-relationship orientation of substituents on the furan ring.

While baker's yeast-mediated reductions of  $\beta$ -ketoesters, including dynamic kinetic resolution,<sup>30</sup> are widely used to produce 3-hydroxyesters as valuable chiral building blocks in the field of polyketide synthesis, the reductions of  $\alpha$ -keto esters have received less attention. For the asymmetric biocatalytic reductions of  $\alpha$ -oxo- $\gamma$ -butyrolactone derivatives, the literature data were focused on converting ketopantoyl lactone into D(-)-pantoyl lactone, a common synthetic starting material of vitamin B<sub>5</sub>. Despite the intensive screening of biocatalysts, modest enantiomeric excesses, lower than 87% ee, were obtained for this transformation.<sup>31</sup> On the other hand, asymmetric biocatalytic reductions of unsubstituted  $\alpha$ -oxo- $\gamma$ -butyrolactone also showed modest 82% ee.<sup>32</sup> In our studied baker's yeast-mediated reductions of chiral racemic  $\alpha$ -oxo- $\gamma$ -butyrolactone ( $\pm$ )-**18**, with a stereogenic center at the C-5 position due to the methyl substituent, we expected a kinetic resolution and a reaction with total conversion in a diastereoselective manner.

Under optimized conditions and on a 9 mmol scale (see ESI Table S4†), 20% yields of each of the compounds *cis*-**19** and *trans*-**21** were isolated after purification (Scheme 2). These two diastereoisomers were separated by column chromatography and clearly identified by comparison with literature data. The compound *cis*-**19** adopts an enveloped conformation and places the C-3 hydroxyl and C-5 methyl substituents in a pseudo-equatorial orientation. In this case, the chemical shifts of the two protons of C4 methylene were highly discriminated in the <sup>1</sup>H NMR spectrum ( $\delta$  = 2.71 and 1.85 ppm) as observed by us<sup>26</sup> and others.<sup>33</sup> In contrast, the compound *trans*-**21** adopts a more planar conformation associated with a lower deformation angle which induces a lower discrimination for the C4 methylene ( $\delta$  = 2.38 and 2.22 ppm).<sup>33a,b</sup>

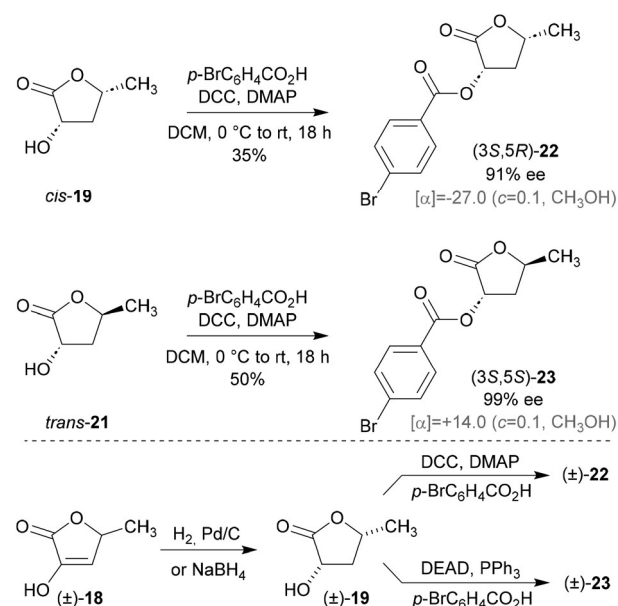
For the *trans*-**21** compound, the absolute stereochemistry was easily established as (3*S*,5*S*)-**21** by the measured specific optical rotation value  $[\alpha] = -60.0$  and comparison with literature data ( $[\alpha] = -59.8$ <sup>33a</sup> and  $-64.5$ <sup>33b</sup>). On the other hand, for the desired *cis*-**19** compound, the measured specific optical



**Scheme 2** Enantioselective reduction of  $\alpha$ -oxo- $\gamma$ -butyrolactone ( $\pm$ )-**18** using baker's yeast.

rotation value was close to zero ( $[\alpha] = -1.0$ ) and still difficult to compare with contradictory literature data ( $[\alpha] = +2.8$ <sup>33c,d</sup> for (3*S*,5*R*)-**19**, while  $[\alpha] = +3.6$ <sup>33a</sup> and  $-2.3$ <sup>33c,d</sup> were reported for (3*R*,5*S*)-**19**). For this reason and in order to determine the enantiomeric excesses, compounds *cis*-**19** and *trans*-**21** were derivatized into their *p*-bromobenzoate esters *cis*-**22** and *trans*-**23** under DCC/DMAP conditions (Scheme 3). A similar behaviour of C-4 methylene in the <sup>1</sup>H NMR spectra of these bromobenzoate esters was observed, which indicates a high discrimination of *cis*-**22** ( $\delta$  = 2.92 and 2.00 ppm) and a much lower discrimination of *trans*-**23** ( $\delta$  = 2.51 and 2.43 ppm). The racemic samples were also prepared starting from  $\alpha$ -oxo- $\gamma$ -butyrolactone ( $\pm$ )-**18**, which is reduced by hydrogenation to afford the  $\alpha$ -hydroxy- $\gamma$ -butyrolactone ( $\pm$ )-**19** with a *cis* relative configuration as already mentioned in our previous racemic synthesis.<sup>26</sup> It can also be mentioned that the same transformation can be realized by the treatment of with NaBH<sub>4</sub> to afford the reduced compound ( $\pm$ )-**19** with high diastereoselectivity. From this same compound ( $\pm$ )-**19**, *cis* ( $\pm$ )-**22** and *trans* ( $\pm$ )-**23** racemic samples were prepared using DCC/DMAP or Mitsunobu conditions, respectively. The enantiomeric excesses of *cis*-**22** and *trans*-**23** were determined by chiral HPLC analysis (Chiralpak ID column, see ESI Part VII.1 and VII.2†). To our delight, the ee values measured were satisfactory at 91% for (3*S*,5*R*)-**22** and excellent at 99% for (3*S*,5*S*)-**23**. The absolute stereochemistry of the desired (3*S*,5*R*)-**22** was determined from its acetate derivative and comparison of its specific rotatory power with literature data (see ESI Part III.3†).<sup>33c</sup>

Although the  $\alpha$ -hydroxy-lactone *cis*-**19** was obtained in its desired (3*S*,5*R*) enantiomeric form, the 91% ee value could cause complications through the formation of a mixture of dia-



**Scheme 3** Derivatization of *cis*-**19** and *trans*-**21** into their bromobenzoate derivatives *cis*-**22** and *trans*-**23** and preparation of bromobenzoate racemic samples ( $\pm$ )-**22** and ( $\pm$ )-**23** from ( $\pm$ )-**18**. ee values were determined by HPLC on a Chiralpak ID column.

stereoisomers during the [4 + 2] cycloaddition reactions with the optically pure (–)-sporothriolide **3**. Considering our previous synthetic racemic approach,<sup>26</sup> we then turned our attention to a more advanced stage of synthesis and envisaged to perform an enzymatic kinetic resolution on vinyl iodide (±)-**20** using enzymatic catalysis.

Biocatalysis holds numerous advantages and leads to many applications in the food, nutraceutical and pharmaceutical industries.<sup>34</sup> The desired product is commonly formed in high yields due to the specificity and selectivity of enzymes allowing the use of polyfunctional substrates. Moreover, the purification is greatly facilitated by little or no generation of by-products. The racemic secondary alcohol (±)-**20**, obtained earlier during our previous racemic synthesis of (±)-trienylfuranol A,<sup>26</sup> was converted to the corresponding acetate (±)-**24** (see ESI Part III.4†), as acetate ester consistently provided better resolution results when esterases or lipases were used as catalysts.<sup>35</sup> Then, this racemic ester (±)-**24** was screened for a wide range of commercially available enzymes, such as acylases, esterases and lipases, to perform an enzymatic kinetic resolution at pH 7.4 with KH<sub>2</sub>PO<sub>4</sub> as buffer.

The first attempts to achieve the kinetic resolution of (±)-**24** were made with acylase I from porcine kidneys, a broadly applicable enzymatic catalyst for the hydrolytic cleavage of unnatural α-amino acids,<sup>36</sup> and also with lipase from wheat germ, commonly used for enzymatic transesterification or hydrolysis and accepting a wide range of secondary alcohols (Table 1, entries 1 and 2).<sup>37</sup> Both of these enzymes failed to

discriminate between the two enantiomers of (±)-**24** as a racemic hydrolyzed product (±)-**20** was obtained in quantitative yield. The use of esterase from the porcine liver<sup>38</sup> or horse liver<sup>39</sup> gave disappointing results as conversion rates are greater than 50%, displaying a low discrimination between the two enantiomers (entries 3 and 4).

In contrast, a low conversion rate was observed with the use of lipase from *Candida cylindracea* (entry 5).<sup>40</sup> Better conversion was achieved with the use of acylase I from *Aspergillus melleus*<sup>36b,37</sup> with a product/substrate ratio of 41:59 determined by crude <sup>1</sup>H NMR analysis (entry 6). As the conversion rate was satisfactory for this trial, the hydrolysed free alcohol **20** and the unreacted acetate ester **24** were separated by column chromatography and the enantiomeric excesses were independently determined by chiral HPLC analysis (Chiralpak ID column, see ESI Part VII.3 and VII.4†) of these two compounds. The enantiomeric excesses were found to be 42% and 26% for **20** and **24**, respectively, leading to a weakly selective reaction with an *E*-value calculated as 3.1 for this enzyme.<sup>41</sup> The use of esterase from the hog liver<sup>42</sup> led to an optimal conversion as a 1:1 mixture of **20** and **24** was obtained (entry 7). The enantiomeric excesses were found to be 96% and >99% for **20** and **24**, respectively, leading to an satisfactory *E*-value of 260.

Finally, the use of Amano lipase PS, from *Burkholderia cepacia*,<sup>43</sup> formerly known as *Pseudomonas cepacia* lipase, led to an optimal conversion giving a 1:1 mixture of **20** and **24** with a highly enantiomer selective kinetic resolution (*E*-value >

**Table 1** Enzymatic kinetic resolution of acetate ester (±)-**24** and synthesis of both enantiomers of trienylfuranol A (–)-**4** and (+)-**4**

Entry	Enzyme used for kinetic resolution of (±)- <b>24</b>	Ratio <sup>c</sup> <b>20/24</b>	% ee <sup>d</sup> (–)- <b>20</b>	Yield <sup>e</sup> (–)- <b>20</b>	% ee <sup>d</sup> (–)- <b>24</b>	Yield <sup>e</sup> (–)- <b>24</b>	<i>E</i> <sup>f</sup>
1 <sup>a</sup>	Acylase I from porcine kidneys	100 : 0	NA	ND	NA	NA	NA
2 <sup>a</sup>	Lipase from wheat germ	100 : 0	NA	ND	NA	NA	NA
3 <sup>a</sup>	Esterase from porcine liver	71 : 29	ND	ND	ND	ND	ND
4 <sup>a</sup>	Esterase from horse liver	65 : 35	ND	ND	ND	ND	ND
5 <sup>a</sup>	Lipase from <i>Candida cylindracea</i>	22 : 78	ND	ND	ND	ND	ND
6 <sup>a</sup>	Acylase I from <i>Aspergillus melleus</i>	41 : 59	42	ND	26	ND	3.1
7 <sup>a</sup>	Esterase from hog liver	50 : 50	96	ND	>99	ND	260
8 <sup>a</sup>	Amano lipase PS from <i>Burkholderia cepacia</i>	50 : 50	>99	ND	>99	ND	>1000
9 <sup>b</sup>	Amano lipase PS from <i>Burkholderia cepacia</i>	50 : 50	>99	31%	>99	35%	>1000

<sup>a</sup> Reactions performed on a 0.02 mmol scale with 5 mg of enzyme in 20 mM aqueous KH<sub>2</sub>PO<sub>4</sub> (5 mL) at pH 7.4, 28 °C, and 150 rpm, for 5 h.

<sup>b</sup> Reactions performed on a 0.44 mmol scale with 130 mg of enzyme in 20 mM aqueous KH<sub>2</sub>PO<sub>4</sub> (130 mL) at pH 7.4, 28 °C, and 150 rpm, for 6 h.

<sup>c</sup> Determined by <sup>1</sup>H NMR of the crude reaction mixture. <sup>d</sup> Determined by HPLC analysis of a chiral stationary phase. <sup>e</sup> Isolated yield.

<sup>f</sup> Enantiomeric ratio:  $E = \ln[(1 - ee_s)(1 + ee_s/ee_p)] / \ln[(1 + ee_s)(1 + ee_s/ee_p)]$ .

1000), where the hydrolysed free alcohol **20** and the unreacted acetate ester **24** were obtained in excellent enantiomeric excesses (>99%) and in satisfactory isolated yields, 31% and 35%, respectively (entries 8 and 9).

The lipase from *Burkholderia cepacia* is an extracellular enzyme containing 320 amino acid residues (PDB: 3LIP)<sup>44</sup> and is widely used as a biocatalyst in biotechnological processes.<sup>43b,45</sup> The active site of *Burkholderia cepacia* lipase-catalyzed hydrolysis is characterized by the catalytic triad Ser87/Asp264/His286, which ensures rate and stereoselectivity.<sup>44</sup> The combination of Asp264 and His286 allows the nucleophilic addition of the hydroxyl side chain of Ser87 to the carbonyl ester moiety of the acetate substrate with the formation of short-lived oxyanion intermediates, followed by a rapid hydrolysed product formation. Most of the lipases display the same enantio-preference towards esters derived from secondary alcohols, and Kazlauskas has proposed an empirical model for chiral recognition by lipases based on the relative sizes of the substituents.<sup>46</sup> The same author, as part of the study for the determination of the absolute configuration of secondary alcohols using lipase-catalyzed kinetic resolutions, has shown that its predictive model can also be applied for the use of *Burkholderia cepacia* lipase.<sup>47</sup> On this basis, we are able to anticipate the fast-reacting enantiomer featuring the displacement of the acetate moiety in the stereogenic center as depicted in Fig. 5.

Thus, the hydrolytic kinetic resolution of racemic acetate ( $\pm$ )-**24**, catalyzed by lipase from *Burkholderia cepacia*, could provide the enantiomerically enriched trienylfuranol **4**. The unnatural antipode (–)-**4** was directly obtained by a Stille cross-coupling reaction<sup>48</sup> between vinyl iodide (–)-**20** and the previously prepared dienylstannane<sup>26</sup> under mild conditions, which means at room temperature and in a short time. These mild reaction conditions are essential for a clean formation of the unusual and very sensitive terminal trienyl unit,<sup>10</sup> and afforded (–)-**4** in 56% yield. The unreacted acetate ester (–)-**24** was saponified with  $K_2CO_3$  in methanol, and this Ac-deprotected product (+)-**20** was directly engaged in a Stille cross-

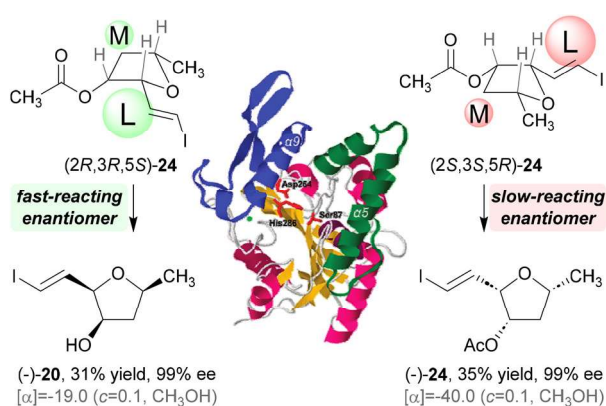
coupling reaction with dienylstannane to afford the natural enantiomer trienylfuranol A (+)-**4** in a satisfactory 51% yield (two steps).

The optical purities of both synthesized enantiomers of trienylfuranol **4** were checked by chiral SFC analysis (Chiralpak IA column, see ESI Part VII.5†). The enantiomeric excesses of both the unnatural antipode (–)-**4** [ $[\alpha] = -8.0$  ( $c = 0.1$ ,  $CH_3CN$ )] and the natural product (+)-**4** [ $[\alpha] = +9.0$  ( $c = 0.1$ ,  $CH_3CN$ )] were measured at 99% ee.

Having in hand the two desired compounds, the (–)-spirothriolide **3** produced through biofermentation of *Hypoxyylon monticulosum* CLL-205 and the synthesized (+)-trienylfuranol **4**, we continued our efforts to confirm the biosynthetic pathway of spirochartines **1**. The spiranic backbone could arise from a [4 + 2] cycloaddition reaction involving the *exo*-methylene of (–)-spirothriolide **3** and the terminal trienic side chain of (+)-trienylfuranol **4**. The Diels–Alder [4 + 2] cycloaddition reaction between a conjugated 1,3-diene and a dienophile (activated alkene) is an efficient chemical process to provide substituted six-membered rings.<sup>49</sup> Moreover, the use of a substrate with an *exo*-methylene function as a dienophile led to a spiro-fused quaternary stereogenic center and it has been recently used in [4 + 2] cycloaddition reactions with dienes to access structurally diverse spirocyclic compounds.<sup>50</sup>

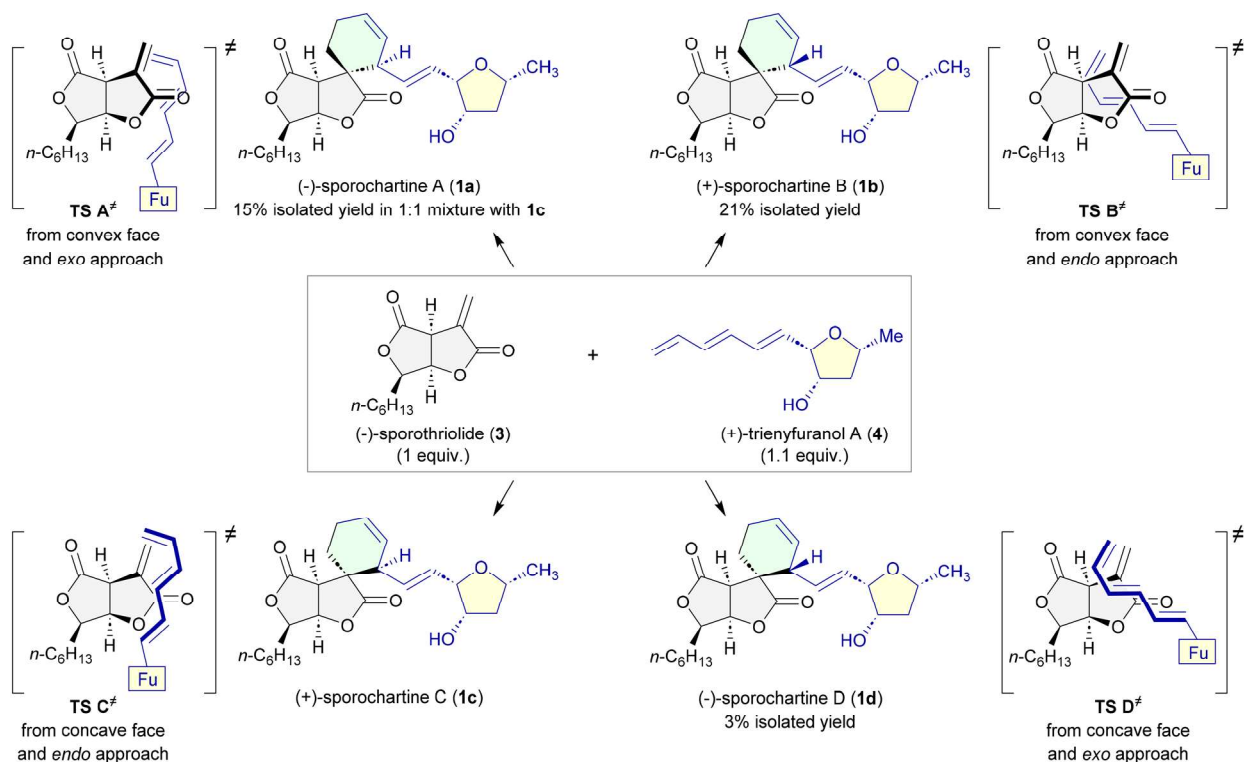
Despite the numerous natural products containing cyclohexenes or substituted cyclohexanes, the connection with enzymes able to catalyse the Diels–Alder reaction and involved in their biosynthesis was initially poorly documented.<sup>51</sup> The behavior and efficiency of enzymes are clearly defined in reactions that involve charge development and stabilization of the transition-state structure. Thus, for enzyme-catalyzed reactions, both the starting substrate and product must have less affinity than the transition state towards the enzyme. In contrast, for the pericyclic Diels–Alder reactions, the ordered transition state is close to the structure of the cycloadduct product. Moreover, such enzymes can be involved in other chemical processes such as oxidation<sup>51,52</sup> or reductive hydride transfer<sup>53</sup> on an acyclic precursor, before the subsequent Diels–Alder reactions. For these reasons, enzyme-catalyzed biosynthetic Diels–Alder reactions for structurally complex natural products remained elusive for a long period. However, since the late 1990s, a few enzymes have been identified to catalyse [4 + 2] reactions, which have been named Diels–Alderases, and are involved in the biosynthetic pathway of three natural products, two for the formation of the decalin unit of the phytotoxins solanapyrones<sup>54</sup> and the fungal metabolite lovastatin,<sup>55</sup> and in the aromatic fungal metabolite macrophomate including the formation of a Diels–Alder adduct followed by decarboxylation and dehydration.<sup>56</sup> Since these pioneering works, Diels–Alderases are increasingly involved in the biosynthesis of complex natural products, and to date we can find more than fifteen reviews<sup>51,57</sup> and perspectives<sup>52,53,58</sup> on this topic.

The reaction between the cultivated (–)-spirothriolide **3** and the synthesized (+)-trienylfuranol **4** was first attempted in dichloromethane, toluene, tetrahydrofuran and also water at 30 °C, the temperature used for the cultivation of the fungal



**Fig. 5** Kazlauskas' model for kinetic resolution of acetate ( $\pm$ )-**24** using *Burkholderia cepacia* lipase and the structure of this lipase in its open-active conformation.





**Scheme 4** Diels–Alder cycloaddition reactions between (–)-sporothriolide **3** and (+)-trienylfuranol **4**.

strain of *Hypoxydon monticulosum* CLL205.<sup>2</sup> Under these experimental conditions, we were not able to identify the expected [4 + 2] cyclized product and only starting materials were recovered. In contrast to R. J. Cox observations,<sup>9</sup> at higher temperature (until 100 °C) in organic solvent, we observed only the consumption of trienylfuranol **4** without the formation of the cyclized product, despite the use of 2 equiv. of **4**. In contrast, the same reaction performed in a mixture of water/THF, 4 : 1, at 100 °C and in an open flask to allow the evaporation of THF led cleanly to the expected sporochartines **1** (Scheme 4). These additive- and metal-free mild thermal conditions are especially designed for substrates bearing acid- or base-sensitive functionalities, specifically trienylfuranol **4** and its terminal trienyl moiety. Moreover, these mild thermal conditions allow a slow evaporation of THF in order to perform the reaction in pure water and can provide the required heat activation to trigger the [4 + 2] cyclization. These mild thermal conditions have been successfully applied to biomimetic synthetic studies on lankacidins<sup>59</sup> and chejuenolides,<sup>60</sup> involving a Mannich-based macrocyclization.

The beneficial effect of water on this cycloaddition will be issued on phenomenon by the hydrophobic effect, which results in the aggregation of the two nonpolar substrates, thus promoting intermolecular reaction.<sup>61</sup> Water as solvent for the Diels–Alder reaction is also known to form a hydrogen-bond network with the dienophile carbonyl group to reduce the LUMO energy and enhance the reactivity of this activated dienophile.<sup>51b</sup> Under these conditions and after purification with

a preparative reversed-phase HPLC system (Sunfire C18 column, see ESI Part VII.6<sup>†</sup>), we were able to isolate 21% of sporochartine B, 15% of 1 : 1 mixture of sporochartines A and C, and 3% of sporochartine D (Scheme 4). The resulting chromatogram of this crude mixture displays a spectacular correlation with that obtained from the fermentation culture of *Hypoxydon monticulosum* CLL-205.

Due to the 5/5-*cis*-fused ring system of sporothriolide, the diene approach from the less hindered convex face of the bicyclic system is favored (TS **A<sup>‡</sup>** and **B<sup>‡</sup>** versus **C<sup>‡</sup>** and **D<sup>‡</sup>**) to deliver sporochartines A and B. Sporochartine B was isolated as the major Diels–Alder adduct resulting from a favorable *endo* approach of the diene moiety in the transition state **B<sup>‡</sup>**. Sporochartines A and C, resulting from the convex face/*exo* approach TS **A<sup>‡</sup>** and concave face/*endo* approach TS **C<sup>‡</sup>**, respectively, were isolated in a 1 : 1 mixture in lower quantities. Sporochartine D, resulting from the concave face/*exo* approach TS **D<sup>‡</sup>**, was isolated as the minor Diels–Alder adduct.

## Conclusions

In summary, we have realized the first enantiopure total synthesis of (+)-trienylfuranol **4**, a secondary metabolite containing an unusual and very sensitive terminal trienyl moiety. Firstly, a phosphine-catalyzed umpolung  $\gamma$ -hydroalkoxylation of an alkynoate derivative was studied to set-up the all-*cis* orientation of the substituents on the furan ring. Then, a

second synthetic approach was engaged based on our previous racemic synthesis of trienylfuranol A. To prepare (+)-trienylfuranol A in an enantiopure form, we took advantage of baker's yeast-mediated reduction to produce an enantioenriched synthesis intermediate. While baker's yeast-mediated reduction is well known for the reduction of  $\beta$ -ketoesters, we have used for the first time this biocatalyst to perform stereoselective reduction of  $\alpha$ -oxo- $\gamma$ -butyrolactone derivatives with high stereoselectivities. To improve the enantioselectivity level, we then turned our attention to lipase-catalyzed hydrolysis cleavage for kinetic resolution on a more advanced stage intermediate of synthesis. For this enzyme-catalyzed reaction, the use of Amano lipase PS, from *Burkholderia cepacia*, formerly known as *Pseudomonas cepacia* lipase, allowed us to prepare (+)-trienylfuranol A with excellent enantiomeric excess (>99%).

A Diels–Alder reaction between the two secondary metabolites (–)-sporothriolide **3**, produced in our lab through biofermentation of *Hypoxyton monticulosum* CLL-205 and the synthesized (+)-trienylfuranol A **4**, allowed us to observe the formation of sporochartines. Although aqueous mild thermic conditions used for the [4 + 2] cycloaddition are far from the cellular biosynthetic environment, they conveniently provided [4 + 2] cycloadducts in an acceptable combined isolated 39% yield. Sporochartine B was isolated in 21% yield, sporochartines A and C in 15% yield as a 1 : 1 mixture and sporochartine D in 3% yield. This relative distribution of sporochartines formed shows a spectacular correlation with that obtained from the fermentation culture of *Hypoxyton monticulosum* CLL-205. These observations allowed us to confirm that the spiranic backbones of sporochartines are formed by a [4 + 2] cycloaddition reaction, with probable involvement of a Diels–Alderase enzyme in their biosynthesis.

## Author contributions

G. A. and T. M. performed chemical synthesis, obtained characterization data of compounds and performed HPLC or SFC analysis. E. V. E. performed single crystal X-ray analysis and interpreted the structural data. J. A. and J.-F. B. supervised the project and wrote the manuscript. All authors commented on the manuscript and gave approval to the final version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.

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