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Efficient asymmetric hydrogenation of the C–C double bond of 2-methyl- and 2,3-dimethyl-*N*-phenylalkylmaleimides by *Aspergillus fumigatus*

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ABSTRACT

Eight *N*-phenylalkylmaleimides (four 2-methyl-*N*-phenylalkylmaleimides and four 2,3-dimethyl-*N*-phenylalkylmaleimides with an alkyl chain $(CH_2)_n$ (n = 1–4) between the imide N and the benzene ring) were subjected to biotransformation using the fungal strain *Aspergillus fumigatus* ATCC 26934. All compounds were reduced enantioselectively to their respective succinimides: (*R*)-2-methyl-*N*-phenylalkylsuccinimides and (2*R*,3*R*)-2,3-dimethyl-*N*-phenylalkylsuccinimides, with satisfactory conversion rates and high stereoisomeric excesses. NMR analysis using the chiral shift reagent Eu(hfc)$_3$ showed that enantiomeric excesses were >99%.

1. Introduction

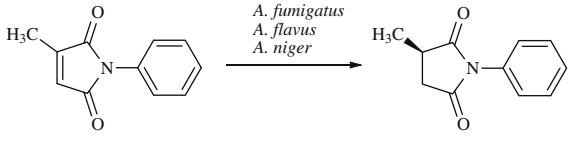
Biocatalysis has become an increasingly valuable tool for the easy preparation of chiral compounds, which are greatly needed in organic synthesis as well as in the pharmaceutical and agrochemical fields, due to the great differences observed in the biological activity of stereoisomers (enantiomers and diastereoisomers).[1] In these conversions, a particular compound is modified by transforming functional groups that are difficult or impossible to achieve through conventional chemical procedures.[1] In addition, biotransformations have great potential because of their sustainable methodology for the production of chemicals, that is, green chemistry.

To perform bioconversions, whole microorganisms or isolated enzymes can be utilized, but the use of microbial cells is very attractive since they simultaneously provide a number of stereoselective enzymes. Among them, whole fungal cells are highly advantageous biocatalysts because of their rapid growth in natural and synthetic media, their ease of handling, and simple scale-up. They play a leading role in 'chemo-enzymatic syntheses' because of their great diversity which produces a range of useful enzymes with catalytic abilities.[2]

Although the potential of fungi as biocatalysts has been demonstrated in some previous work,[1] new studies exploring the potentiality of fungal species as new routes to obtain enantiopure compounds are highly welcome.

2-Methyl-*N*-phenyl- and 2,3-dimethyl-*N*-phenylmaleimides **1** and **2** have proven to be good substrates for biocatalytic C–C double bond reduction leading to the introduction of one or two stereogenic centers into an achiral structure with high enantioselectivity. Culture plant cells of *N. tabacum*, *Cinechococcus* sp., and *Marchantia polymorpha* have been shown to possess the ability of hydrogenating the double bond of **1** to afford (*R*)-*N*-phenyl-3-methylsuccinimide **3** with a 99% ee.[3] In turn, *M. polymorpha* has been shown to enantioselectively hydrogenate **2** to yield *trans*-(2*R*,3*R*)-*N*-phenyl-2,3-dimethylsuccinimide **4** with 99% ee.[4] In a more recent paper, we have found that *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* are also efficient biocatalysts for the enantioselective hydrogenation of **1** and **2** into (*R*)-(+)-2-methyl-*N*-phenyl- and (2*R*,3*R*)-(+)-2,3-dimethyl-*N*-phenylsuccinimides **3** and **4** (Schemes 1 and 2) with 99% ee and conversions higher than 96%, thus demonstrating that *Aspergillus* spp. are efficient tools for the production of chiral succinimides, which could be used as asymmetric synthons for organic synthesis or useful biologically active compounds.[5] In the same paper, we reported that *Fusarium graminearum* and *Penicillium* sp. converted **2** to **4** with a lower reduction capacity and the same enantioselectivity.

On the basis of our earlier findings, we herein expand upon the preliminary report, by using analogues of **1** and **2** as substrates, all possessing an alkyl chain $(CH_2)_n$ between the *N* of maleimide and the benzene ring, whose length (n) varies from 1 to 4 (compounds



1 (*R*)-(+)-**3**

Scheme 1. Biotransformation of **1** by fungal species.

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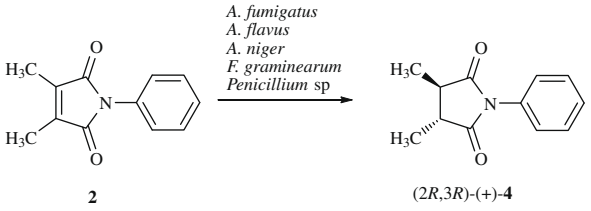


Scheme 2. Biotransformation of **2** by fungal species.

5–**12**). Of these, substrates 2-methyl and 2,3-dimethyl-*N*-phenylalkylmaleimides **6**–**8** and **10**–**12**, with $n = 2$, 3, and 4 have not been reported previously.

Considering that *A. fumigatus* ATCC 26934 showed the best efficiency (99% conversion rate and >99% ee) in the previous paper, we chose this fungus for the bioconversion of the new series of maleimides.

2. Results and discussion

Four 2-methyl-*N*-phenylalkylmaleimides **5**–**8** and four 2,3-dimethyl-*N*-phenylalkylmaleimides **9**–**12** with a $(CH_2)_n$ chain ($n = 1$–4) between the imide *N* and the benzene ring, obtained through reported procedures,[6] were submitted to biotransformation with the growing cells of *A. fumigatus* ATCC 26934 in Czapek medium under controlled conditions.

The results showed that *A. fumigatus* reduced the substrates **5**–**12** into their respective dextrorotatory succinimides **13**–**20** with the conversion rates ranging from 9.44% to 93.81% (Tables 1 and 3). Among them, dimethylated maleimide **12** ($n = 4$) was transformed to the highest extent (93.81%) followed by **11** ($n = 3$, 61.40%), **9** ($n = 1$, 52.12%), and **10** ($n = 2$, 29.20%). Similar results were obtained with monomethylated maleimides, although they were less readily accepted: maleimide **8** ($n = 4$) was transformed to the highest extent (42.07%) followed by **7** ($n = 3$, 18.91%), **5** ($n = 1$, 12.14%), and **6** ($n = 2$, 9.44%). Figure 1 shows the conversion rates of maleimides **5**–**12**, along with those obtained with maleimides **1** and **2** (reported previously) with the same strain of *A. fumigatus*.[5] For each series, a dependence of the conversion with the length of the alkyl chain was observed, with the highest rate occurring when $n = 0$ and the lowest one when $n = 2$, for each mono- and dimethylated maleimides.

2.1. Absolute configuration and enantiomeric excesses of 2-methyl-*N*-phenylalkylsuccinimides 13–16

To assist in the assignment of the absolute configuration of (+)-**13**–**16** obtained in the biotransformation process, (*R*)-2-methyl-*N*-phenylalkylsuccinimides **13**–**16** were prepared as standards from commercial (*R*)-methyl succinic acid **21** and the respective phenylalkylamines (Ph–$(CH_2)_n$–NH_2, $n = 1$–4) **22**–**25**, following the reported procedures.[7] All these synthetic enantiopure compounds were dextrorotatory indicating that the biotransformation products (+)-**13**–**16** had the (*R*)-configuration.

Enantiomeric excesses were determined by ^{1}H NMR spectroscopy using the chiral shift reagent, Eu(hfc)$_3$. For this purpose, *rac*-2-methyl-*N*-phenylalkylsuccinimides **13**–**16** were prepared by catalytic hydrogenation from the respective monomethylated maleimides **5**–**8**. The methyl protons of both enantiomers were clearly separated in the ^{1}H NMR spectra of **13**–**16** upon addition of Eu(hfc)$_3$, allowing the calculation of the ee of the biotransformation products with satisfactory precision. The spectrum of the ^{1}H NMR added to the shift reagent showed that the enantiomeric purities of (+)-**13**–**16** were higher than 99% for all bioconversions (Table 1).

2.2. Absolute configuration and enantiomeric excesses of 2,3-dimethyl-*N*-phenylalkylsuccinimides 17–20

The 2,3-dimethylsuccinimides **17**–**20** obtained by biotransformation of *N*-substituted 2,3-dimethylmaleimides **9**–**12** with *A. fumigatus* have optical activity (all were dextrorotatory, Table 3) clearly indicating that the (+)-*trans*-isomers were produced enantio- and diastereoselectively.

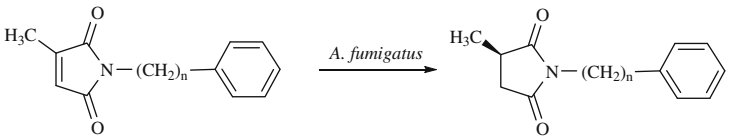
To investigate the diastereoisomeric excess (de) of the reaction, *rac*-*trans*- and *cis*-2,3-dimethyl-*N*-phenylalkylsuccinimides **17**–**20** were prepared from commercial dimethyl succinic acid **26** (mixture of ± and *meso*) and the respective phenylalkylamines (Ph–$(CH_2)_n$–NH_2, $n = 1$–4) **22**–**25**, according to the reported procedures.[7] The peaks of CH_3 and 2,3-H in the ^{1}H NMR spectra of each diastereoisomer were determined, showing that those of the *trans*-isomers appear at lower and higher fields, respectively, than those of the *cis*-isomer (Table 2).

The ^{1}H NMR spectroscopy data of the biotransformation products demonstrated that only *trans*-dimethylsuccinimides (+)-**17**–**20** were produced (de >99%) indicating the *anti*-addition of the hydrogen atoms to the C–C double bond of maleimides, in accordance with the fungal biotransformation of **2** recently reported.[5] This is an important result, since it shows a clear difference from the *cis*-product obtained by the catalytic hydrogenation of the double bond and could be a very useful tool for synthetic procedures.

Considering that the absolute configuration of *trans*-(+)-*N*-phenylsuccinimide **4** has been assigned as (2*R*,3*R*) in the previous reports,[4,5] (+)-**17**–**20** should be (2*R*,3*R*), since (+)-**17**–**20** differs from (+)-**4** only in the *N*-alkyl chain and not in the chiral imide ring.

The enantiomeric excesses were determined by ^{1}H NMR spectroscopy with Eu(hfc)$_3$ with the aid of synthetic *rac*-*trans*-2,3-dimethyl-*N*-phenylalkylsuccinimides **17**–**20**. The peak analyses of

Table 1
Biotransformation of monomethylated maleimides **5**–**8**



Substrate	Product	Conv (%)	$[\alpha]_D^{27}$	% ee
5: $n = 1$	(*R*)-(+)-**13**: $n = 1$	12.14	+19.7 ± 1.2 (*c* 0.73, $CHCl_3$)	>99
6: $n = 2$	(*R*)-(+)-**14**: $n = 2$	9.44	+7.0 ± 1.5 (*c* 0.73, $CHCl_3$)	>99
7: $n = 3$	(*R*)-(+)-**15**: $n = 3$	18.91	+1.5 ± 0.8 (*c* 1.50, $CHCl_3$)	>99
8: $n = 4$	(*R*)-(+)-**16**: $n = 4$	42.07	+1.3 ± 0.3 (*c* 0.45, $CHCl_3$)	>99

Conversion rates (Conv) were determined by GC by using the following equation: % of conversion = product TIC/(product TIC + substrate TIC) × 100; % ees were determined by ^{1}H NMR using Eu(hfc)$_3$.

Table 2
^{1}H NMR chemical shifts (δ, ppm) belonging to CH_3 and 2,3-H of *cis* and *trans*-2,3-dimethyl-*N*-phenylalkylsuccinimides **4**, **17**–**20**

	H-2,3		CH_3	
	cis	*trans*	*cis*	*trans*
4	3.11 (2H, m)	2.60 (2H, m)	1.33 (6H, d, J = 7.2 Hz)	1.44 (6H, d, J = 7.2 Hz)
17	2.93 (2H, m)	2.42 (2H, m)	1.20 (6H, d, J = 7.2 Hz)	1.32 (6H, d, J = 7.2 Hz)
18	2.84 (2H, m)	2.30 (2H, m)	1.12 (6H, d, J = 7.2 Hz)	1.27 (6H, d, J = 7.2 Hz)
19	2.83 (2H, m)	2.31 (2H, m)	1.18 (6H, d, J = 7.2 Hz)	1.28 (6H, d, J = 7.2 Hz)
20	2.90 (2H, m)	2.30 (2H, m)	1.20 (6H, d, J = 7.2 Hz)	1.32 (6H, d, J = 7.2 Hz)

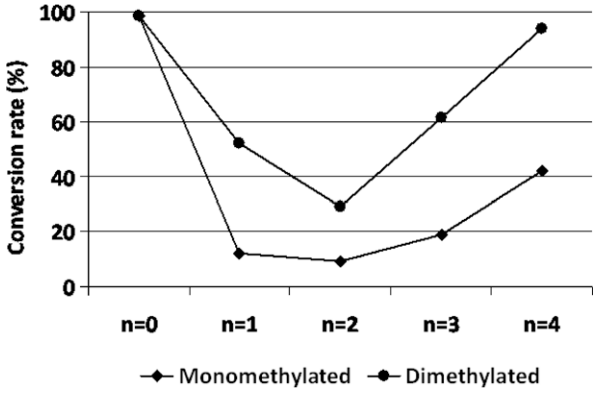


Figure 1. Dependence of the conversion rates with the length of the alkyl chain between *N* and the aromatic ring.

the spectra showed that the enantiomeric purities of (+)-*trans*-**17**–**20** obtained by biotransformation with *A. fumigatus* were higher than 99% (Table 3).

3. Conclusions

We have shown that *A. fumigatus* ATCC 26934 has the ability of enantioselectively hydrogenating the endocyclic C–C double bond of 2- and 2,3-dimethyl-*N*-phenylalkylmaleimides to afford (*R*)-(+)-methyl- and *trans*-(2*R*,3*R*)-(+)-dimethyl-*N*-phenylalkylsuccinimides with satisfactory conversion rates and excellent stereoselectivities. The data obtained in the present work, added to those of our previous paper,[5] show that this fungus has the versatility to accept not only 2-methyl or 2,3-dimethyl-*N*-phenylmaleimide substrates but also analogues containing an alkyl chain of variable length between the imidic N and the benzene ring.

The biotransformation of 2,3-dimethyl-*N*-phenylalkylmaleimides was easy to perform and shows a clear difference with the C–C reduction by catalytic hydrogenation, where only the *cis*-isomer (*meso*) is obtained, thus being a very useful tool for synthetic procedures.

This work provides new evidence that biocatalysis provides great opportunities to prepare useful chiral compounds by an environmentally viable alternative. In addition, the findings reported herein are a valuable contribution to the challenge of discovering new biocatalysts for the stereoselective reduction of prochiral building blocks. In terms of diastereo- and enantioselectivities, *A. fumigatus* appears as a promising fungus for deeper biochemical and biocatalytic studies.

4. Experimental

4.1. Synthesis

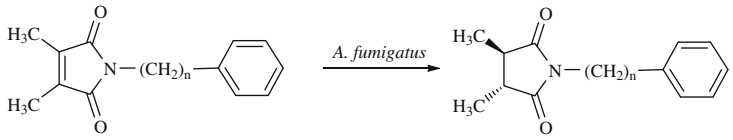
4.1.1. General

Solvents and reagents were purchased from Sigma (St. Louis, MO, USA) and were purified in the usual manner. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz (Karlsruhe, Germany). Compounds were dissolved in deuterated solvents from commercial sources (Sigma) with tetramethylsilane (TMS) as the internal standard. Chemical shifts are reported in ppm (δ) relative to the solvent peak ($CHCl_3$ in $CDCl_3$ at 7.26 ppm for protons and at 77.0 ppm for carbons). Signals are designated as follows: s, singlet; d, doublet; dd, doublets of doublets; t, triplet; m, multiplet; q, quartet; quint, quintuplet. Gas chromatograms were obtained in a CG–MS Turbo Mass Perkin Elmer (Waltman, USA), column PE1 30 m × 0.25 mm of inner diameter, film 0.1 μm, ionization energy 70 eV. Elemental analyses were performed on a Carlo Erba EA 1108 analyzer (Milano, Italy). Percentages of C, H, and N were in agreement with the product formula. Melting points were obtained in an Electrothermal apparatus (Southern on Sea-UK) and were uncorrected. Optical rotations were measured in a Jasco DIP-1000 polarimeter (Easton, USA). The reported data refer to the Na-line value (589 nm). IR spectra were recorded using a spectrophotometer Shimadzu Prestige 21 (Kyoto, Japan).

4.1.2. Substrates

4.1.2.1. 2-Methyl-*N*-phenylalkylmaleimides 5–8. Monomethylated maleimides were prepared from the reaction mixtures containing commercial methylmaleic anhydride (5.0 mmol) in 5 mL

Table 3
Biotransformation of dimethylated maleimides **9**–**12**



Substrate	Product	Conv (%)	$[\alpha]_D^{27}$	% de	% ee
9: n = 1	(2*R*,3*R*)-(+)-**17**: n = 1	52.12	+38.0 ± 2.2 (c 0.31, $CHCl_3$)	>99	>99
10: n = 2	(2*R*,3*R*)-(+)-**18**: n = 2	29.20	+40.5 ± 0.6 (c 0.36, $CHCl_3$)	>99	>99
11: n = 3	(2*R*,3*R*)-(+)-**19**: n = 3	61.40	+39.4 ± 0.9 (c 0.36, $CHCl_3$)	>99	>99
12: n = 4	(2*R*,3*R*)-(+)-**20**: n = 4	93.81	+38.3 ± 0.6 (c 0.36, $CHCl_3$)	>99	>99

Conversion rates (Conv) were determined by GC by using the following equation: % of conversion = product TIC/(product TIC + substrate TIC) × 100; % ees were determined by ^{1}H NMR using $Eu(hfc)_3$.

of $CHCl_3$ and equimolar amounts of the appropriate phenylalkylamines [Ph–$(CH_2)_n$–NH_2; n = 1–4] **22**–**25** dissolved in 1 mL of $CHCl_3$ and stirred during 1 h. The solid, maleamic acid, which precipitated out of the reaction mixture was filtered off. The whole amount of maleamic acid was dissolved in 5 mL of acetic anhydride, and 100 mg of sodium acetate was added. The mixture was heated for 2 h at reflux. The reaction mixture was cooled and the reaction was quenched with water, then, the aqueous solution was extracted with Et_2O, dried with Na_2SO_4, filtered, and the solvent was evaporated. The product was purified by silica gel column chromatography using a mixture of hexane and ethyl acetate (9:1) as eluent. Spectroscopic data of compound **5** were identical to those previously reported.[6,8]

2-Methyl-*N*-phenethylmaleimide **6**: White crystals. Mp: 54–56 °C. Yield: 86%. IR ν_{max}/cm^{-1} (KBr): 1712. ^1H NMR ($CDCl_3$; 300 MHz): δ 2.06 (3H, d, J = 1.8 Hz, CH_3); 2.90 (2H, t, J = 7.5 Hz, ArCH_2); 3.75 (2H, t, J = 7.5 Hz, NCH_2); 6.29 (1H, q, J = 1.8 Hz, H-3); 7.21–7.57 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 10.9. (*C*H_3); 34.6 (Ar*C*H_2); 39.2 (N*C*H_2); 126.6 (C-4′); 127.2 (C-3); 128.5 (C-3′,5′); 128.8 (C-2′,6′); 138.0 (C-1′); 145.5 (C-2); 170.7 (C-4); 171.6 (C-1) ppm. MS: m/z = 215 (M^+). Anal. Calcd for $C_{13}H_{13}NO_2$: C, 72.5; H, 6.0; N, 6.5. Found: C, 70.2; H, 6.1; N, 6.7.

2-Methyl-*N*-propylphenylmaleimide **7**: White crystals. Mp: 45–46 °C. Yield: 65%. IR ν_{max}/cm^{-1} (KBr): 1713. ^1H NMR ($CDCl_3$; 300 MHz): δ 1.95 (2H, quint, J = 7.5 Hz, $CH_2CH_2CH_2$); 2.06 (3H, d, J = 1.8 Hz, CH_3); 2.64 (2H, t, J = 7.5 Hz, ArCH_2); 3.56 (2H, t, J = 7.5 Hz, NCH_2); 6.28 (H, q, J = 1.8 Hz, H-3); 7.10–7.40 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 10.9 (*C*H_3); 29.8 ($CH_2CH_2CH_2$); 33.1 (Ar*C*H_2); 37.7 (N*C*H_2); 125.9 (C-4′); 127.2 (C-3); 128.3 (C-3′,5′); 128.4 (C-2′,6′); 141.1 (C-1′); 145.4 (C-2); 170.9 (C-4); 171.9 (C-1) ppm. MS: m/z = 229 (M^+). Anal. Calcd for $C_{14}H_{15}NO_2$: C, 73.3; H, 6.5; N, 6.1. Found: C, 73.3; H, 6.5; N, 6.3.

2-Methyl-*N*-butylphenylmaleimide **8**: White crystals. Mp: 46–47 °C. Yield: 71%. IR ν_{max}/cm^{-1} (KBr): 1710. ^1H NMR ($CDCl_3$; 300 MHz): δ 1.50–1.75 (4H, m, $CH_2(CH_2)_2CH_2$); 2.08 (3H, d, J = 1.8 Hz, CH_3); 2.64 (2H, t, J = 7.2 Hz, ArCH_2); 3.54 (2H, t, J = 7.2 Hz, NCH_2); 6.30 (H, q, J = 1.8 Hz, H-3); 7.14–7.30 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 10.9 (*C*H_3); 28.2 (ArCH_2CH_2); 28.5 (NCH_2CH_2); 35.3 (Ar*C*H_2); 37.7 (N*C*H_2); 125.8 (C-4′); 127.3 (C-3); 128.3 (C-3′,5′); 128.4 (C-2′,6′); 142.0 (C-1′); 145.5 (C-2); 170.9 (C-4); 171.9 (C-1) ppm. MS: m/z = 243 (M^+). Anal. Calcd for $C_{15}H_{17}NO_2$: C, 74.0; H, 6.9; N, 5.8. Found: C, 73.6; H, 7.2; N, 5.8.

4.1.2.2. 2,3-Dimethyl-*N*-phenylalkylmaleimides 9–12. Dimethylated maleimides were prepared from commercial 2,3-dimethylmaleic anhydride and phenylalkylamines [Ph–$(CH_2)_n$–NH_2; n = 1–4] **22**–**25** as described for monomethylated maleimides. Spectroscopic data of compound **9** were identical to those previously reported.[8]

2,3-Dimethyl-*N*-phenethylmaleimide **10**: White crystals. Mp: 54–56 °C. Yield 78%. IR ν_{max}/cm^{-1} (KBr): 1698. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.96 (6H, s, CH_3); 2.90 (2H, t, J = 7.8 Hz, ArCH_2); 3.74 (2H, t, J = 7.8 Hz, NCH_2); 7.20–7.38 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.6 (*C*H_3); 34.8 (ArCH_2); 39.1 (N*C*H_2); 126.5 (C-4′); 128.5 (C-2′,6′); 128.8 (C-3′,5′); 137.1 (C-1′); 138.2 (C-2,3); 172.0 (C-1,4) ppm. MS: m/z = 229 (M^+). Anal. Calcd for $C_{14}H_{15}NO_2$: C, 73.3; H, 6.6; N, 6.1. Found: C, 76.2; H, 6.8; N, 6.3.

2,3-Dimethyl-*N*-propylphenylmaleimide **11**: Colorless oil. Yield 68%. IR ν_{max}/cm^{-1} (KBr): 1711. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.87–2.10 (8H, m, $CH_2CH_2CH_2$ and CH_3); 2.45 (2H, t, J = 7.8 Hz, ArCH_2); 3.36 (2H, t, J = 8.0 Hz, NCH_2); 7.10–7.35 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ 8.7 (*C*H_3). 29.8 ($CH_2CH_2CH_2$); 33.1 (Ar*C*H_2); 37.7 (N*C*H_2); 125.9 (C-4′); 128.3 (C-2′,6′); 128.3 (C-3′,5′); 137.0 (C-1′); 141.1 (C-2,3); 172.3 (C-1,4) ppm. MS: m/z = 243 (M^+). Anal. Calcd for $C_{15}H_{17}NO_2$: C, 74.0; H, 6.9; N, 5.8. Found: C, 73.9; H, 7.0; N, 5.9.

2,3-Dimethyl-*N*-butylphenylmaleimide **12**: White crystals. Mp: 62–64 °C. Yield 62%. IR ν_{max}/cm^{-1} (KBr): 1709. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.60–1.65 (4H, m, $CH_2(CH_2)_2CH_2$); 2.38 (s, 6H, CH_3); 2.66 (2H, t, J = 7.2 Hz, ArCH_2); 3.66 (2H, t, J = 7.2 Hz, NCH_2); 7.10–7.36 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ 26.4 (*C*H_3); 28.6 (ArCH_2CH_2); 28.7 (NCH_2CH_2); 35.4 (Ar*C*H_2); 44.8 (N*C*H_2); 125.9 (C-4′); 128.3 (C-2′,6′); 128.4 (C-3′,5′); 137.0 (C-1′); 142.1 (C-2,3); 173.2 (C-1,4) ppm. MS: m/z = 257 (M^+). Anal. Calcd for $C_{16}H_{19}NO_2$: C, 74.7; H, 7.4; N, 5.4. Found: C, 75.1; H, 7.6; N, 5.6.

4.1.3. Standards

4.1.3.1. (R)-(+)-2-Methyl-*N*-phenylalkylsuccinimides 13–16. A mixture of (*R*)-(+)-methylsuccinic acid (5.0 mmol in water) **21** and phenylalkylamines (Ph–$(CH_2)_n$–NH_2; n = 1–4) **22**–**25** was kept at 170 °C for 2 h and then cooled to 20 °C. The aqueous solution was extracted with Et_2O, dried with Na_2SO_4, filtered, and the solvent was evaporated.[7] The mixtures were subjected to silica gel column chromatography using a mixture of hexane and ethyl acetate (9:1) as eluent. Spectroscopic data of compound **13** were identical to those previously reported.[3]

2-Methyl-*N*-phenethylsuccinimide **14**: White crystals. Mp: 52–54 °C. Yield: 59%. IR ν_{max}/cm^{-1} (KBr): 1712. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.27 (3H, d, J = 7.2 Hz, CH_3); 2.24 (1H, dd, J = 17.2 Hz; 4.5, H-3a); 2.70–2.97 (2H, m, H-2 and H-3b); 2.91 (2H, t, J = 7.8 Hz, ArCH_2); 3.77 (2H, quint, J = 7.8 Hz, NCH_2); 7.18–7.34 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 16.8 (*C*H_3); 33.4 (ArCH_2); 34.5 (C-2); 36.3 (C-3); 39.8 (N*C*H_2); 126.7 (C-4′); 128.4 (C-2′,6′); 128.9 (C-3′,5′); 137.7 (C-1′); 176.2 (C-1); 180.4 (C-4) ppm. MS: m/z = 217 (M^+). Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.9; H, 6.9; N, 6.5. Found: C, 71.5; H, 7.1; N, 6.2. $[\alpha]_D^{27} = +5.3 \pm 1.2$ (c 0.54, $CHCl_3$).

2-Methyl-*N*-propylphenylsuccinimide **15**: White crystals. Mp: 53–55 °C. Yield: 49%. IR ν_{max}/cm^{-1} (KBr): 1712. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.30 (3H, d, J = 7.2 Hz, CH_3); 1.94 (2H, quint, J = 7.2 Hz, $CH_2CH_2CH_2$); 2.22 (1H, dd, J = 17.3 Hz; 3.6, H-3a); 2.65 (2H, t, J = 7.2 Hz, ArCH_2); 2.70–2.86 (2H, m, H-2 and H-3a); 3.58 (2H, t, J = 7.2 Hz, NCH_2); 7.12–7.32 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 16.7 (*C*H_3); 28.7 ($CH_2CH_2CH_2$); 33.2 (ArCH_2); 34.6 (C-2); 36.4 (C-3); 38.7 (N*C*H_2); 126.0 (C-4′); 128.3 (C-2′,6′); 128.4 (C-3′,5′); 140.9 (C-1′); 176.5 (C-1); 180.6 (C-4) ppm. MS: m/z = 231 (M^+). Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.7; H, 7.4; N, 6.1. Found: C, 71.5; H, 7.4; N, 6.2. $[\alpha]_D^{27} = +1.3 \pm 0.9$ (c 0.27, $CHCl_3$).

2-Methyl-*N*-butylphenylsuccinimide **16**: White crystals. Mp: 70–72 °C. Yield: 52%. IR ν_{max} (KBr) 1697 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.34 (3H, d, J = 7.2 Hz, CH_3); 1.57–1.69 (4H, m, $CH_2(CH_2)_2CH_2$); 2.30 (1H, dd, J = 17.1 Hz, 3.6, H-3a); 2.65 (2H, t, J = 7.2 Hz, ArCH_2); 2.74–7.78 (2H, m, H-2 and H-3b); 3.53 (2H, t, J = 6.9 Hz, NCH_2); 7.14–7.33 (5H, m, H_{Ar}). ^{13}C NMR ($CDCl_3$, 75 MHz): 16.7 (*C*H_3); 27.3 (ArCH_2CH_2); 28.5 (NCH_2CH_2); 34.6 (C-2); 35.2 (*C*H_2); 36.4 (C-3); 38.5 (N*C*H_2); 125.8 (C-4′); 128.3 (C-2′,6′); 128.4 (C-3′,5′); 141.9 (C-1′); 176.5 (C-1); 180.6 (C-4). MS: m/z = 245 (M^+). Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.4; H, 7.7; N, 5.7. Found: C, 71.6; H, 7.9; N, 5.5. $[\alpha]_D^{27} = +2.7 \pm 1.25$ (c 0.23, $CHCl_3$).

4.1.3.2. rac-2-Methyl-*N*-phenylalkylsuccinimides 13–16. Each monomethylated maleimide **5**–**8** (2 mmol) was dissolved in CH_2Cl_2 (2 mL) and 5% Pd/C (catalyst) was added. Then, the mixture was submitted to an H_2 atmosphere at room temperature for 2 h. The crude reaction mixture was filtered, the solvent was evaporated, and the product was purified by silica gel column chromatography using a mixture of hexane and ethyl acetate (9:1) as eluent.

4.1.3.3. rac-trans-2,3-Dimethyl-*N*-phenylalkylsuccinimides 17–20. Dimethylated succinimides *trans*-(±)-**17**–**20** were prepared from 2,3-dimethylsuccinic acid **26** (mixture of ± and meso) and phenylalkylamines (Ph–$(CH_2)_n$–NH_2; n = 1–4) **22**–**25** as described

for (*R*)-(+)-2-methyl-*N*-phenylalkylsuccinimides. The mixtures were subjected to silica gel column chromatography using a mixture of hexane and ethyl acetate (9:1) as eluent to give *rac-trans*-2,3-dimethyl-*N*-phenylalkylsuccinimides **17**–**20**.

trans-2,3-Dimethyl-*N*-bencylsuccinimide **17**: White crystals. Mp: 123–126 °C. Yield: 47%. IR ν_{max}/cm^{-1} (KBr): 1695. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.32 (6H, d, J = 7.2 Hz, CH_3); 2.36–2.47 (2H, m, H-2,3); 4.65 (2H, s, CH_2); 7.22–7.41 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 14.9 (*C*H_3); 42.1 (*C*H_2); 43.1 (C-2,3); 127.9 (C-4′); 128.6 (C-2′,6′); 128.7 (C-3′,5′); 136.0 (C-1′); 179.2 (C-1,4) ppm. MS: m/z = 217 (M^+). Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.8; H, 6.9; N, 6.5. Found: C, 72.3; H, 6.8; N, 6.7.

trans-2,3-Dimethyl-*N*-phenethylsuccinimide **18**: White crystals. Mp: 136–138 °C. Yield: 28%. IR ν_{max} (KBr) 1703 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.27 (6H, d, J = 7.2 Hz, CH_3); 2.25–2.37 (2H, m, H-2,3); 2.90 (2H, t, J = 7.5 Hz, ArCH_2); 3.75 (2H, t, J = 7.5 Hz, NCH_2); 7.16–7.34 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 15.1 (*C*H_3); 32.3 (Ar*C*H_2); 39.6 (N*C*H_2); 42.9 (C-2,3); 126.6 (C-2′,6′); 128.4 (C-4′); 128.9 (C-3′,5′); 137.7 (C-1′); 179.3 (C-1,4) ppm. MS: m/z = 231 (M^+). Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.7; H, 7.4; N, 6.1. Found: C, 73.1; H, 7.8; N, 6.0.

trans-2,3-Dimethyl-*N*-propylphenylsuccinimide **19**: White crystals. Mp: 130–133 °C. Yield: 40%. IR ν_{max} (KBr) 1706 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.28 (6H, d, J = 7.2 Hz, CH_3); 1.95 (2H, quint, J = 7.2 Hz, $CH_2CH_2CH_2$); 2.25–2.36 (2H, m, H-2,3); 2.65 (2H, t, J = 7.2 Hz, ArCH_2); 3.56 (2H, t, J = 7.2 Hz, NCH_2); 7.15–7.36 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 14.9 (*C*H_3); 28.8 ($CH_2CH_2CH_2$); 33.2 (Ar*C*H_2); 38.6 (N*C*H_2); 42.9 (C-2,3); 125.9 (C-2′,6′); 128.3 (C-4′); 128.4 (C-3′,5′); 137.0 (C-1′); 179.5 (C-1,4) ppm. MS: m/z = 245 (M^+). Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.4; H, 7.7; N, 5.7. Found: C, 71.3; H, 7.8; N, 5.6.

trans-2,3-Dimethyl-*N*-butylphenylsuccinimide **20**: White crystals. Mp: 122–125 °C. Yield: 67%. IR ν_{max} (KBr) 1705 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.32 (6H, d, J = 7.2 Hz, CH_3); 1.55–1.74 (4H, m, $CH_2(CH_2)_2CH_2$); 2.26–2.34 (2H, m, H-2,3); 2.45 (2H, t, J = 7.2 Hz, ArCH_2); 3.52 (2H, t, J = 7.2 Hz, NCH_2); 7.16–7.24 (5H, m, H_{Ar}) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 14.8 (*C*H_3); 28.6 ($ArCH_2CH_2$); 28.8 (NCH_2CH_2); 33.2 (Ar*C*H_2); 38.6 (N*C*H_2); 42.9 (C-2,3); 125.8 (C-2′,6′); 128.2 (C-4′); 128.3 (C-3′,5′); 137.1 (C-1′); 179.4 (C-1,4) ppm. MS: m/z = 259 (M^+). Anal. Calcd for $C_{16}H_{21}NO_2$: C, 74.1; H, 8.1; N, 5.4. Found: C, 72.4; H, 7.9; N, 5.5.

4.2. Biotransformations

A. fumigatus ATCC 26934 was grown on a plate with an agarized Czapek culture medium for 3 days at 30 °C until well sporulated. Suspensions of conidia (2–5 × 10^6 CFU/mL) were used to inoculate 2L erlenmeyer flasks containing Czapek broth medium (1 L). The cultures were incubated at 30 °C for 72 h on an orbital shaker (150 rpm; Innova 4000, New Jersey, USA).

The substrates (125 mg) in DMSO (5 mL) were poured into flasks containing the fungal biomass and the reaction mixtures were incubated at 30 °C for 72 h on an orbital shaker (150 rpm). A flask with fungal biomass and 5 mL DMSO instead of substrate was taken as fungal growth control. After incubation, the mixtures were filtered, and the aqueous phases were bulked and extracted with ethyl acetate (3 × 250 mL). The organic phases were dried over Na_2SO_4 and the products were analyzed by TLC and GC. Conversion rates of the products were determined by GC analysis of the crude extracts, and determined by using the following equation: percentage of conversion: product TIC (total ion current)/(product TIC + substrate) × 100.

The compounds were purified by silica gel column chromatography using a mixture of hexane and ethyl acetate (9:1) as eluent. The structure of each product was elucidated by NMR and MS analysis and the spectra were identical to the previously synthesized ones.

The enantiomeric purity of the products was determined on the basis of the peak analysis of the methyl proton signals of the ^1H NMR with Eu(hfc)$_3$. This was first performed for racemic mixtures and, then, in the same conditions, for biotransformation products.

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