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TETRAHEDRON: *ASYMMETRY*

Microbial reduction of 2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-1,3-dione with *Schizosaccharomyces pombe* (NRRL Y-164)

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Abstract

A mixture of *cis*- and *trans*-2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-1,3-dione (±)-**10** was synthesized and incubated with *Schizosaccharomyces pombe* (NRRL Y-164) to give (+)-**11**, (+)-**12**, (−)-**13**, and (−)-**14** in 19, 13, 22, and 16% yields, respectively. Chromic acid oxidation of these microbiologically reduced products gave (−)-**10a**, (+)-**10b**, (+)-**10a**, and (−)-**10b**, respectively.

1. Introduction

In previous communications, we reported the resolution of racemic 2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4,5-trimethylcyclopenta-1,3-dione[1] **1** and 2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4,4-trimethylcyclopenta-1,3-dione[2] **4** by microbial reduction with *Schizosaccharomyces pombe* (NRRL Y-164), respectively (Scheme 1).

In view of these successful resolutions, 2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-1,3-dione **10** was prepared by condensation of **8**[3] and **9**.[4] Compound **10**, which comprised four components on chiral HPLC analysis,[5] was incubated with *S. pombe* (NRRL Y-164). Here we report the results obtained in this biotransformation reaction.

2. Results and discussion

Direct separation of the incubation mixture from **10** with *S. pombe* (NRRL Y-164) by silica gel column led to the isolation of (+)-**11** (19%), (+)-**12** (13%), and a diastereomeric mixture (43%) containing (−)-**13** and (−)-**14**, respectively (Scheme 2). The diastereomeric mixture of (−)-**13** and

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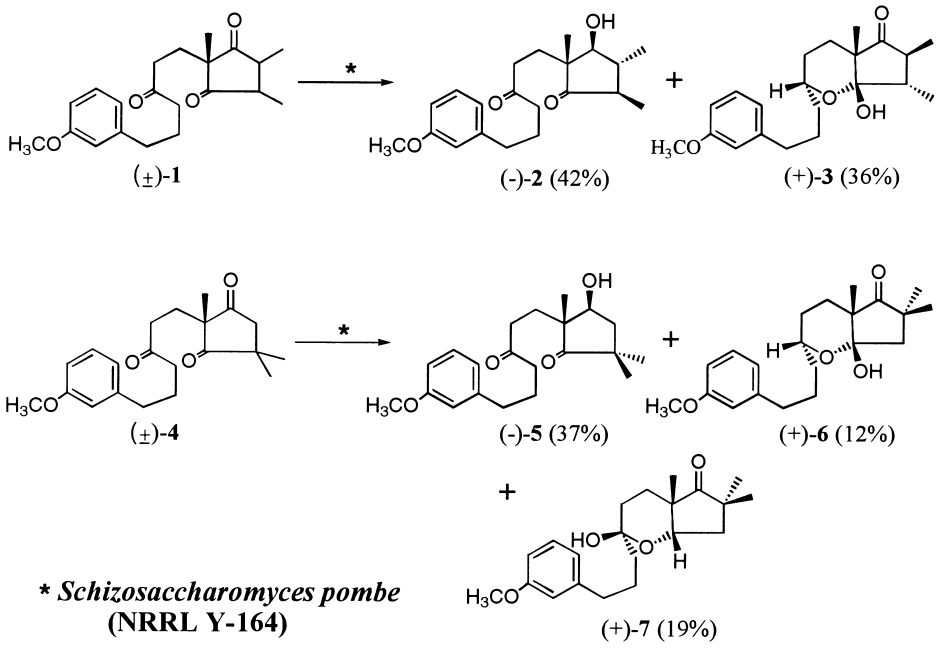
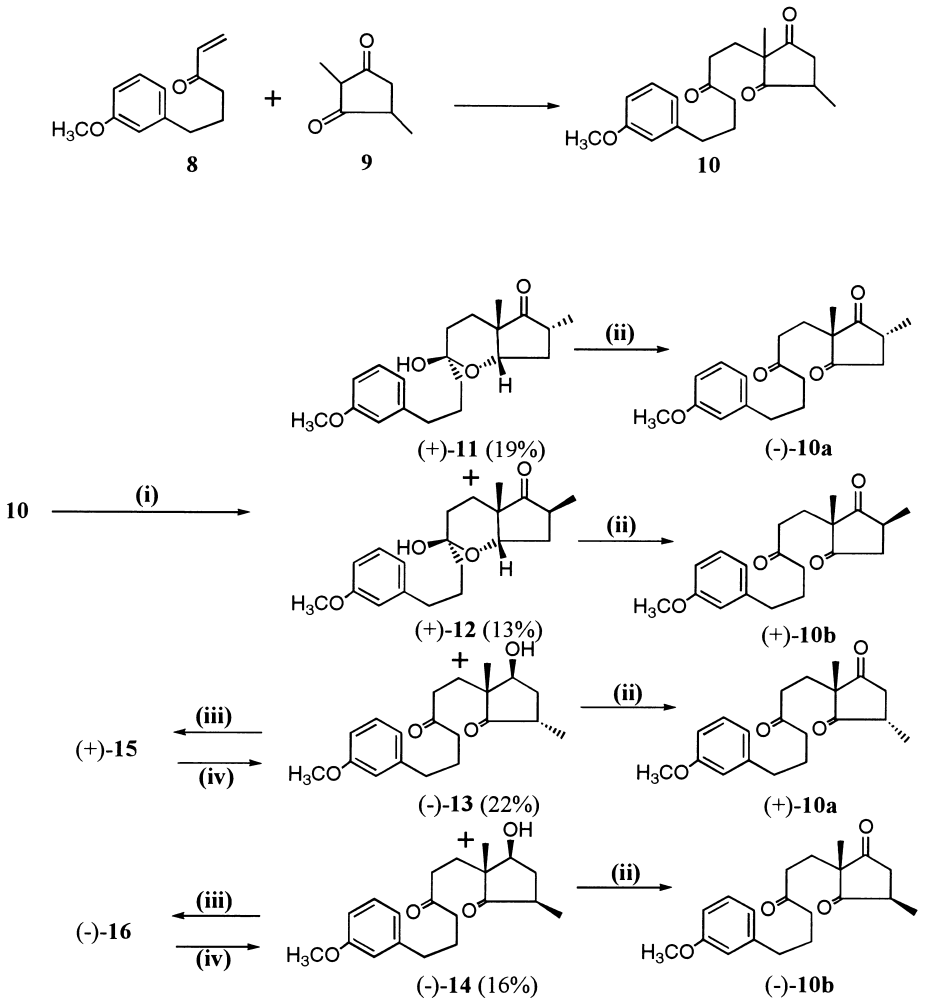


Scheme 1.

(−)-**14** was converted to the corresponding trimethylsilyl ether mixture by reacting with chlorotrimethylsilane, separated on a silica gel column, to give, after acidic hydrolysis, (−)-**13** (22%) and (−)-**14** (16%).

Compound (+)-**11** has a molecular formula $C_{20}H_{28}O_4$ as deduced from its HREIMS. Its IR absorption suggested the presence of a hydroxyl and a carbonyl function. The ^{13}C NMR spectrum revealed a sole carbonyl carbon (δ 223.1, s), a dioxygenated carbon (δ 96.6, s), and an oxygenated methine (δ 76.4, d) which couples directly to a carbinoyl proton (δ 4.18), identified by an HMQC spectrum. The molecular formula of (+)-**11** provided seven ring- and double-bond equivalents, six of which are easily identified from the presence of an aryl ring, a carbonyl group, and the five-membered ring in the molecule. The HMBC spectrum (Fig. 1) showed that the oxygenated methine coupled to the 6-Me singlet and 9-H_2, and the carbonyl carbon coupled to 8α-Me and 6β-Me. This afforded the exact substitution pattern for the five-membered ring, and the dioxygenated carbon to be a hemiketal carbon ether-linked likely to C-3 to form a pyran ring accounting for the seventh equivalent. The ^1H NMR spectrum showed a D_2O exchangeable doublet at δ 1.79, which couples to H-4α (an axial proton) (δ 1.26, ddt), identified by the D_2O exchange and double resonance at δ 1.79, both of which collapsed H-4α to a double triplet ($J = 13.5$, 5.0 Hz). These results locate 3β-OH which H-bonds to pyran oxygen (O-2) to make a W-coupling between O*H* and H-4α. These data suggest that (+)-**11** is structurally similar to (+)-**7** which was obtained during the microbial reduction of (±)-**4** with this microorganism. An NOESY study also confirmed the *cis*-relationship of H-1 to both 3β-OH and 6β-Me, and the *trans*-relationship of H-1 to 8α-Me. Based on these results, the structure of (+)-**11** was established as (1*S*,3*R*,6*S*,8*R*)-3-hydroxy-3-(3-*m*-methoxyphenylpropyl)-6,8-dimethyl-2-oxabicyclo[4.3.0]nonan-7-one.

Compound (+)-**12** has a molecular formula $C_{20}H_{28}O_4$ as deduced from its HREIMS. Its IR absorption suggested the presence of a hydroxyl and a carbonyl function. The ^{13}C NMR spectrum



Scheme 2. Reagents and conditions: (i) *Schizosaccharomyces pombe* (NRRL Y-164); (ii) CrO_3–H^+, 0°C; (iii) Me_3SiCl, 0°C; (iv) H^+, 0°C

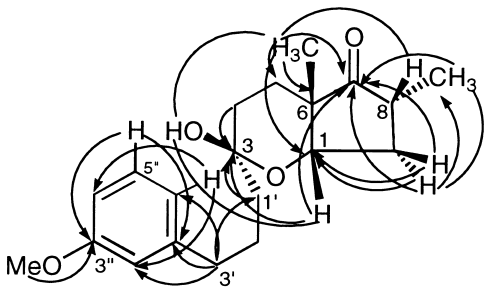


Figure 1. Selective NOESY (curves) and major HMBC correlation (arrows) for (+)-**11**

revealed a sole carbonyl carbon (δ 223.1, s), a dioxygenated carbon (δ 96.5, s), and an oxygenated methine (δ 75.2, d) which couples directly to a carbinoyl proton (δ 4.18), identified by an HMQC spectrum. These data indicate that (+)-**12** is structurally similar to (+)-**11**. An NOE study enhancing 3β-OH, 6-Me and H-9β upon irradiation of H-1, and another NOE study enhancing 3β-OH and 6-Me upon irradiation of 8β-Me showed the *cis*-relationship of H-1 to 3β-OH, 6-Me and 8β-Me (Fig. 2). Detailed ^{1}H and ^{13}C NMR assignments of (+)-**12** were made by analysis of the HMQC and HMBC spectra (Fig. 3). Based on these results, the structure of (+)-**12** was concluded to be (1*S*,3*R*,6*S*,8*S*)-3-hydroxy-3-(3-*m*-methoxyphenylpropyl)-6,8-dimethyl-2-oxabicyclo[4.3.0]nonan-7-one, which is the (8*S*)-methyl epimer of (+)-**11**. Detailed assignments of ^{1}H and ^{13}C NMR for (+)-**11** and (+)-**12** in comparison with (+)-**7** are given in Tables 1 and 2.

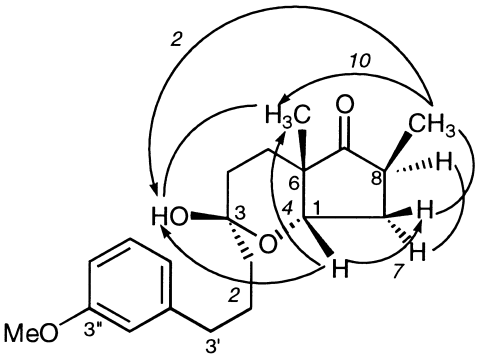


Figure 2. Selective NOESY (curves) and NOED (arrows, %) of (+)-**12**

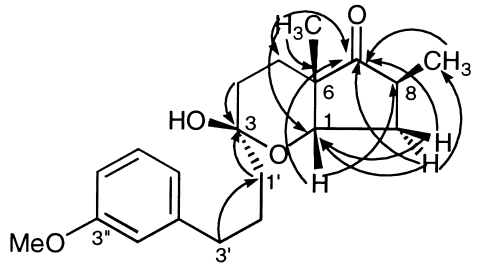


Figure 3. Major HMBC correlation for (+)-**12**

Compound (−)-**13** has a molecular formula $C_{20}H_{28}O_4$ as deduced from its HREIMS. Its IR absorption at 3480 cm^{-1} and a carbinoyl proton signal at δ 4.00 (dd) in the ^{1}H NMR spectrum support the presence of a secondary hydroxyl group on the five-membered ring: compound (−)-**13** is resistant to the Oppenauer oxidation[6] but oxidized by Jones' reagent[7] to (+)-**10a**, supporting this suggestion. In addition, the 2-Me carbon signal (δ 15.2) appearing more upfield than that of (+)-**10a** (δ 20.8), both identified by HETCO and HMQC spectra, respectively, suggested a further *r*-gauche effect by 1β-OH present in (−)-**13**. The ^{13}C NMR spectrum revealed the presence of two carbonyl carbons at δ 222.4 and 210.6, respectively, and the exact substitution pattern of the five-membered ring was established from the HMBC spectrum (Fig. 4). An NOESY study showed a *trans*-relationship between 2-Me and 4-Me, and a *cis*-relationship between H-1α and 4-Me.

Table 1
^{1}H NMR data for (+)-**7**, (+)-**11**, and (+)-**12** (δ)

compound / position	(+)-7	(+)-11	(+)-12
1 (1 H)	4.17 (t, J =4.4 Hz, H-1β)	4.18 (dd, J = 3.2, 1.3 Hz,H-1β)	4.18 (d, J = 3.4 Hz, H-1β)
4 (2 H)	1.51 (ddd, J = 13.4, 4.6, 2.8 Hz,H-4β) 1.22 (ddt, J = 13.4, 4.8, 2.0 Hz, H-4α)	1.49 (ddt, J = 13.6, 5.0, 1.9 Hz, H-4β) 1.26 (ddt, J = 13.6, 5.0, 1.9 Hz, H-4α)	1.48 (m, H-4β) 1.26 (m, H-4α)
5 (2 H)	1.97 (ddd, J = 13.4, 4.8, 2.8 Hz, H-5α) 1.70 (dt, J = 13.4, 4.6 Hz, H-5β)	1.94 (ddd, J = 13.6, 5.0, 2.5 Hz, H-5β) 1.74 (m, H-5α)	1.72 (dd, J = 13.4, 10.0 Hz, H-5β) 1.95 (ddd, J = 13.4, 4.3, 2.6 Hz, H-5α)
8 (1 H)	-	2.41(m, H-8β)	2.36 (m, H-8α)
9 (2 H)	2.08 (dd, J = 14.1, 4.4 Hz, H-9β) 1.86 (d, J =14.1 Hz, H-9α)	2.45 (m, H-9β) 1.60 (m, H-9α)	1.84 (m, H-9β) 2.14 (m, H-9α)
1′ (2 H)	1.55 (m)	1.53 (m)	1.53 (m)
2′ (2 H)	1.66 (m)	1.63 (m)	1.65 (m)
3′ (2 H)	2.54 (t, J = 7.7 Hz)	2.56 (t, J = 7.6 Hz)	2.55 (t, J = 7.6 Hz)
2″ (1 H)	6.68 (br s)	6.71 (m)	6.71 (m)
4″ (1 H)	6.70 (br d)	6.71 (m)	6.71 (m)
5″ (1 H)	7.16 (t, J = 7.8 Hz)	7.16 (dd, J = 7.8, 7.8 Hz)	7.17 (dd, J = 7.8, 7.8 Hz)
6″ (1 H)	6.72 (br d)	6.71 (m)	6.71 (m)
3″-OMe	3.76 (s)	3.77 (s)	3.77 (s)
6-Me	0.95 (s)	0.94 (s)	0.88 (s)
8-Me	1.13 (s) 1.10 (s)	1.15 (d, J = 7.4 Hz)	1.15 (d, J = 7.3 Hz)
3β-OH	1.93 (d, J = 2.0 Hz)	1.79 (d, J = 1.9 Hz)	1.85 (br s)

These data suggest that (−)-**13** is structurally similar to (−)-**5** which was obtained during the microbial reduction of (±)-**4** study. Based on these results, the structure of (−)-**13** was established as (1*S*,2*R*,4*S*)-1-hydroxy-2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-3-one.

Compound (−)-**14** has a molecular formula $C_{20}H_{28}O_4$ as deduced from its HREIMS. Its IR absorption at 3475 cm^{-1} and a carbinoyl proton signal at δ 3.93 (dd) in the ^{1}H NMR spectrum support the presence of a secondary hydroxyl group on the five-membered ring. Compound (−)-**14** also resisted the Oppenauer oxidation but oxidized to (−)-**10b** by Jones' reagent. It also showed an *r*-gauche effect by the 1β-OH on 2-Me carbon signal in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum also showed the presence of two carbonyl carbons at δ 221.4 and 211.3, respectively. These data suggest that the structure of (−)-**14** is similar to (−)-**13**. An NOE study enhancing 2-Me upon irradiation of 4-Me established the *cis*-relationship between 2-Me and 4-Me (Fig. 5). Based on these results, the structure of (−)-**14** was concluded as (1*S*,2*R*,4*R*)-1-hydroxy-2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-3-one, which is the (4*R*)-methyl epimer of (−)-**13**. Detailed assignments of ^{13}C and ^{1}H NMR data for (−)-**13** and (−)-**14** in comparison with (−)-**5** are given in Tables 3 and 4.

Chromic acid oxidation of (+)-**11**, (+)-**12**, (−)-**13**, and (−)-**14** yielded (−)-**10a**, (+)-**10b**, (+)-**10a**, and (−)-**10b**, respectively. Compound (+)-**10a**, $[\alpha]_D^{26}$ +43 (*c* 1.0, $CHCl_3$), has a molecular formula $C_{20}H_{26}O_4$ as deduced from its HREIMS. Its IR absorption and ^{13}C NMR spectrum

Table 2
^{13}C NMR data for (+)-**7**, (+)-**11**, and (−)-**12** (δ)

compound / position	(+)-7	(+)-11	(+)-12
1	75.5 (d)	76.4 (d)	75.2 (d)
3	96.4 (s)	96.6 (s)	96.5 (s)
4	30.0 (s)	29.6 (s)	29.8 (s)
5	24.9 (t)	24.1 (t)	24.98(t)
6	49.4 (s)	49.2 (s)	48.3 (s)
7	225.5 (s)	223.1 (s)	223.1 (s)
8	43.4 (s)	37.9 (d)	40.7 (d)
9	41.1 (t)	33.2 (t)	34.8 (t)
1′	42.6 (t)	42.6 (t)	42.5 (t)
2′	24.7 (t)	24.8 (t)	24.90(t)
3′	35.8 (t)	35.8 (t)	35.8 (t)
1″	143.6 (s)	143.6 (s)	143.7 (s)
2″	114.2 (d)	114.2 (d)	114.3 (d)
3″	159.6 (s)	159.6 (s)	159.6 (s)
4″	111.1 (d)	111.1 (d)	111.0 (d)
5″	129.3 (d)	129.3 (d)	129.3 (d)
6″	120.8 (d)	120.8 (d)	120.8 (d)
3″-OMe	55.1 (q)	55.1 (q)	55.1 (q)
6-Me	22.5 (q)	21.7 (q)	21.8 (q)
8-Me	28.4 (q) 27.1 (q)	17.9 (q)	16.1 (q)

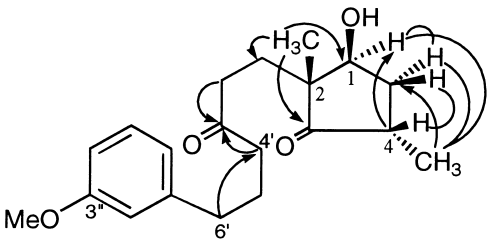


Figure 4. Selective NOESY (curves) and major HMBC correlation (arrows) for (−)-**13**

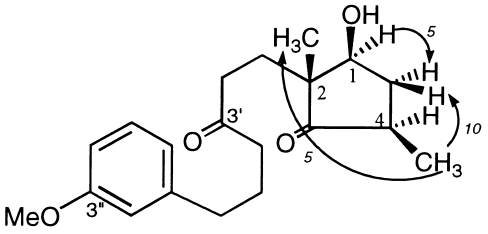


Figure 5. NOED (arrows, %) of (−)-**14**

Table 3
^{13}C NMR data for (−)-**5**, (−)-**13**, and (−)-**14** (δ)

compound / position	(-)-**5**	(-)-**13**	(-)-**14**
1	73.0 (d)	74.8 (d)	73.9 (d)
2	52.8 (s)	52.8 (s)	52.2 (s)
3	224.0 (s)	222.4 (s)	221.4 (s)
4	44.6 (s)	39.9 (d)	39.9 (d)
5	42.7 (t)	36.2 (t)	36.0 (t)
1′	28.6 (t)	28.3 (t)	28.6 (t)
2′	37.6 (t)	37.2 (t)	41.9 (t)
3′	211.2 (s)	210.6 (s)	211.3 (s)
4′	41.8 (t)	42.0 (t)	37.6 (t)
5′	24.9 (t)	25.0 (t)	25.0 (t)
6′	35.0 (t)	35.1 (t)	35.1 (t)
1″	143.0 (s)	143.1 (s)	143.1 (s)
2″	114.2 (d)	114.2 (d)	114.3 (d)
3″	159.6 (s)	160.0 (s)	159.7 (s)
4″	111.2 (d)	111.2 (d)	111.3 (d)
5″	129.3 (d)	129.3 (d)	129.4 (d)
6″	120.8 (d)	120.9 (d)	120.8 (d)
3″-OMe	55.0 (q)	55.1 (q)	55.2 (q)
2-Me	15.8 (q)	15.2 (q)	15.5 (q)
4-Me	26.0 (q) 25.2 (q)	15.7 (q)	15.3 (q)

Table 4
^{1}H NMR data for (−)-**5**, (−)-**13**, and (−)-**14** (δ)

compound / position	(-)-**5**	(-)-**13**	(-)-**14**
1 (1 H)	4.04 (dd, J = 8.9, 6.5 Hz)	4.00 (dd, J = 4.8, 4.8 Hz)	3.93 (dd, J = 9.5, 6.8 Hz)
4 (1 H)	-	2.49 (dq, J = 8.3, 7.6 Hz, H-4β)	2.11 (m, H-4α)
5 (2 H)	2.05 (dd, J = 12.8, 6.5 Hz, H-5α) 1.76 (dd, J = 12.8, 8.9 Hz, H-5β)	2.13 (ddd, J = 13.6, 8.8, 4.8 Hz, H-5α) 1.90 (m, H-5β)	2.42 (m, H-5α) 1.49 (ddd, J = 12.3, 11.1, 9.5 Hz, H-5β)
1′ (2 H)	1.74 (m)	1.67 (ddd, J = 14.8, 7.4, 7.4 Hz) 1.56 (ddd, J = 14.5, 7.4, 7.4 Hz)	1.73 (m)
2′ (2 H)	2.38 (t, J = 7.2 Hz)	2.38 (m)	2.39 (m)
4′ (2 H)	2.38 (t, J = 7.2 Hz)	2.38 (m)	2.39 (m)
5′ (2 H)	1.85 (q)	1.85 (tt, J = 14.8, 7.2 Hz)	1.87 (tt, J = 7.6, 7.4 Hz)
6′ (2 H)	2.54 (t, J = 7.5 Hz)	2.56 (t, J = 7.7 Hz)	2.56 (t, J = 7.6 Hz)
2″ (1 H)	6.68 (br s)	6.71 (m)	6.71 (m)
4″ (1 H)	6.70 (br d, J = 7.8 Hz)	6.71 (m)	6.71 (m)
5″ (1 H)	7.15 (t, J = 7.8 Hz)	7.16 (dd, J = 7.8, 7.8 Hz)	7.17 (dd, J = 7.8, 7.8 Hz)
6″ (1 H)	6.71 (br d, J = 7.8 Hz)	6.71 (m)	6.71 (m)
3"-OMe	3.75 (s)	3.77 (s)	3.76 (s)
2-Me	0.93 (s)	0.98 (s)	0.90 (s)
4-Me	1.12 (s) 0.94 (s)	1.06 (d, J =7.4 Hz)	1.14 (d, J = 7.2 Hz)

revealed the presence of three carbonyl carbons at δ 218.0. 215.2, and 209.6, respectively. An NOESY study showed the *trans*-relationship between 2-Me and 4-Me. An NOE study of no enhancements of 2-Me (δ, 1.10, s) upon irradiation of 4-Me (δ, 1.30, d) confirmed the *trans*-relationship between them. The ^{13}C and ^{1}H NMR assignments of (+)-**10a** were made by analysis of HMQC and HMBC spectra (Fig. 6). Based on these results, the structure of (+)-**10a** was concluded to be (2*R*,4*S*)-2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-1,3-dione. Compound (−)-**10a**, $[\alpha]_D^{26}$ −43 (*c* 1.0, $CHCl_3$), has a molecular formula $C_{20}H_{26}O_4$ as deduced from its HREIMS. The compound (−)-**10a** is identical in every respect (UV, IR, and NMR) to (+)-**10a**, and, therefore, they are enantiomeric isomers. Thus, the stereochemistry of (−)-**10a** can be assigned (2*S*,4*R*).

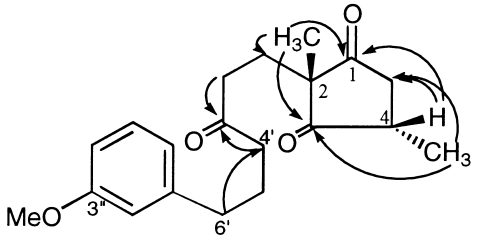


Figure 6. Major HMBC correlation for (+)-**10a**

Compound (−)-**10b**, $[\alpha]_D^{26}$ −48 (*c* 1.0, $CHCl_3$), has a molecular formula $C_{20}H_{26}O_4$ as deduced from its HREIMS. Its IR absorption and ^{13}C NMR revealed the presence of three carbonyl carbons at δ 218.1, 215.2, and 209.3, respectively. An NOE study enhancing 2-Me (δ 1.05, s) upon irradiation of 4-Me (δ 1.24, d) established the *cis*-relationship between these two methyl groups (Fig. 7). Thus, compound (−)-**10b** is the (4*R*)-methyl epimer of (+)-**10a** and the structure can be assigned as (2*R*,4*R*)-2-(6-*m*-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-1,3-dione. Compound (+)-**10b**, $[\alpha]_D^{26}$ +48 (*c* 1.0, $CHCl_3$), has a molecular formula $C_{20}H_{26}O_4$ as deduced from its HREIMS. Compound (+)-**10b** is identical in every respect (UV, IR, and NMR) to (−)-**10b**, and, therefore, they are enantiomeric isomers. Thus, the stereochemistry of (+)-**10b** can be assigned (2*S*,4*S*).

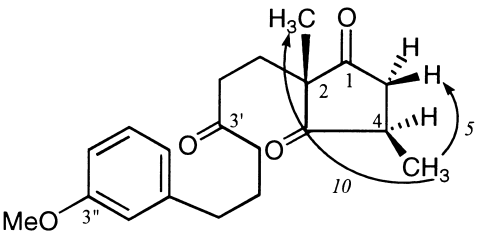


Figure 7. NOED (arrows, %) of (−)-**10b**

In this study, we succeeded in the separation of the four constituents of **10** when it was synthesized by condensation of **8** and **9**. The ^{13}C and ^{1}H NMR assignments of (+)-**10a** and (+)-**10b** in comparison with (±)-**4** are given in Tables 5 and 6.

Upon inspection of these four products isolated from microbial reduction of **10** with *S. pombe* (NRRL Y-164), we noticed that the less sterically hindered carbonyl groups on the cyclopentane

Table 5
^{13}C NMR data for (±)-**4**, (+)-**10a**, and (+)-**10b** (δ)

compound / position	(±)-**4**	(+)-**10a**	(+)-**10b**
1	215.8 (s)	215.2 (s)	215.2 (s)
2	54.5 (s)	55.1 (s)	55.12(s)
3	220.4 (s)	218.0 (s)	218.1 (s)
4	46.4 (s)	40.6 (d)	41.1 (d)
5	50.5 (t)	43.6 (t)	43.4 (t)
1′	28.5 (t)	27.5 (t)	28.7 (t)
2′	36.8 (t)	36.9 (t)	36.6 (t)
3′	208.9 (s)	209.6 (s)	209.3 (s)
4′	41.8 (t)	41.9 (t)	41.9 (t)
5′	24.9 (t)	24.9 (t)	24.9 (t)
6′	35.0 (t)	35.0 (t)	35.0 (t)
1″	143.2 (s)	143.2 (s)	143.1 (s)
2″	114.2 (d)	114.2 (d)	114.2 (d)
3″	159.7 (s)	159.7 (s)	159.7 (s)
4″	111.3 (d)	111.3 (d)	111.3 (d)
5″	129.3 (d)	129.3 (d)	129.3 (d)
6″	120.9 (d)	120.9 (d)	120.9 (d)
3″-OMe	55.1 (q)	55.1 (q)	55.06(q)
2-Me	19.6 (q)	20.8 (q)	18.1 (q)
4-Me	26.5 (q) 25.1 (q)	15.3 (q)	15.8 (q)

Table 6
^{1}H NMR data for (±)-**4**, (+)-**10a**, and (+)-**10b** (δ)

compound / position	(±)-**4**	(+)-**10a**	(+)-**10b**
4 (1 H)		2.85 (m)	2.96 (m)
5 (2 H)	2.57 (d, J = 18 Hz) 2.69 (d, J = 18 Hz)	2.98 (dd, J = 18.2, 10.4 Hz, H-5α) 2.41 (dd, J = 18.3, 9.1 Hz, H-5β)	3.05 (dd, J = 18.0, 10.6 Hz, H-5β) 2.29 (dd, J = 18.0, 8.0 Hz, H-5α)
1′ (2 H)	1.85 (m)	1.86 (m)	1.84 (m)
2′ (2 H)	2.37 (m)	2.33 (m)	2.35 (m)
4′ (2 H)	2.37 (m)	2.33 (m)	2.35 (m)
5′ (2 H)	1.85 (m)	1.86 (m)	1.84 (m)
6′ (2 H)	2.55 (t, J = 7.4 Hz)	2.54 (t, J = 7.4 Hz)	2.54 (t, J = 7.7 Hz)
2″ (1 H)	6.70 (m)	6.71 (m)	6.71 (m)
4″ (1 H)	6.70 (m)	6.71 (m)	6.71 (m)
5″ (1 H)	7.17 (t, J = 7.8 Hz)	7.17 (dd, J = 7.8, 7.8 Hz)	7.17 (dd, J = 7.8, 7.8 Hz)
6″ (1 H)	6.70 (m)	6.71 (m)	6.71 (m)
3″-OMe	3.77 (s)	3.77 (s)	3.77 (s)
2-Me	1.11 (s)	1.10 (s)	1.05 (s)
4-Me	1.19 (s) 1.26 (s)	1.30 (d, J = 7.0 Hz)	1.24 (d, J = 7.0 Hz)

ring were preferentially reduced to give (*S*)-monoalcohols regardless of the stereochemistry of the methyl substituent. In (−)-**10a** and (+)-**10b**, the less sterically hindered carbonyls were reduced to alcohols so that the hydroxyl groups were *trans*-oriented in regard to the C-2 methyl, therefore forming a pyrane ring involving the C-3′ side-chain carbonyl which became a hemiketal carbon, while in (+)-**10a** and (−)-**10b** the hydroxyl groups were *cis*-oriented in regard to the C-2 methyl, and remained unchanged. In the present, as well as in our two previous studies, the reduction proceeded in a highly enantiofacially selective manner but not in a kinetically resolving manner between enantiomers or diastereomers. This phenomenon is similar to the results reported by Prelog and Acklin when (±)-Δ^4-9-methyloctalindion-(3,8) was reduced with *Curvularia falcata*, in which the C-8 carbonyl group on both enantiomers was reduced to give optically active products.[8] In our earlier studies with *Schizosaccharomyces pombe* strains, racemic 2-methyl-2-(3′-oxobutyl)-4,5-ethylenecyclopentan-1,3-dione[9] and 6,7,8,8a-tetrahydro-8a-methyl-6,9-dioxofluorene[10] were both resolved by reduction of only the carbonyl group on the cyclopentane ring of the (+)-isomers. One possible explanation for the reduction of all four components present in **10** might be that when the (−)-isomers were represented, e.g. (−)-**10a** and (−)-**10b** as shown in Scheme 2, they become identical to the usual representation of the D-ring portion in a (+)-steroid molecule and suitable for enzymatic reduction.

In this study, although the yields of the four isolated products range between 13 and 22%, a total of 70% of the theoretical yield was achieved. Thus, we have further demonstrated the applicability of microbial regio- and stereospecific reduction for the separation of racemic, as well as structurally related, steroid intermediates such as these cyclopentane ring methylated triones. These chiral synthons will serve as key intermediates in the subsequent synthesis of D-ring monomethylated steroid analogues.

3. Experimental

3.1. General

Melting points measured on a Buchi 510 melting point apparatus are uncorrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on a Hitachi 150-20 double beam spectrometer. IR spectra were recorded on a Jasco A-100 infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DPX-200 or a Bruker AMX-400 spectrophotometer in $CDCl_3$ or CD_3OD using the solvent peaks as reference standards. 2D NMR spectra were recorded by using Bruker's standard program: in the HMQC and HMBC experiments, $\Delta = 1$ s and $J = 140$, 8 Hz, respectively, the correlation maps consisted of 512×1 k data points per spectrum, each composed of 16 to 64 transients. EIMS were recorded on a Finnigan Mat 4500 GC/MS and HREIMS on a JEOL JMX-HX-100 mass spectrometer at 70 eV. The determination of enantiomeric excesses was carried out by HPLC using CHIRALPAK AS (Daicel Chemical Industries, Ltd.) column (eluent: hexane:isopropanol = 94:6, 90:10, and 75:25). Normal phase silica gel for column chromatography was purchased from Merck, 7734 (70–230 mesh) and 9385 (230–400 mesh); TLC plates of Merck 573 (Si60 with F_{254}, 0.25 mm) were purchased from E. Merck, A.G., Darmstadt, Germany.

All of the solvents and inorganic chemicals were reagent grade. Methyl succinic acid, 3-methoxy phenylacetic acid, and chlorotrimethylsilane were purchased from the Aldrich Chemical Company, Inc., Milwaukee, WI, USA. Nutrient broth, maltose, proteose peptone No. 3, and

bacto-agar were from Difco Laboratories, Detroit, MI. Dextrose was obtained from the Wako Pure Chemical Industries, Ltd., Osaka, Japan.

The microorganism, *Schizosaccharomyces pombe* (NRRL Y-164), was maintained on an Mp No. 3 agar (maltose 4%, proteose peptone No. 3 1.5%, and agar 3%) at 26°C for 11 days then transferred and grown in a nutrient broth–dextrose medium (nutrient broth 1.6% and dextrose 4%) at 24–26°C on a rotary shaker (250 rpm, 1 in. stroke). Transformations was carried out in 2-L Erlenmeyer flasks containing 400 mL of the same medium.

3.2. Microbial reduction of **10**

The microorganism was grown in 14.4 L of nutrient broth–dextrose medium contained in thirty-six 2-L Erlenmeyer flasks. Compound **10** (3.6 g), dissolved in 84 mL DMF, was added and the incubation was continued for 96 h. Extraction of the incubation mixture with $CHCl_3$ (4.8 L×3) and subsequent work-up gave an oily yellow residue (5.1 g). The residue was chromatographed over a silica gel column (1000 g) and eluted with a $CHCl_3$:Me_2CO mixture: the fractions (0.621 g) obtained from $CHCl_3$:Me_2CO (99:1) were further purified over a silica gel column to give pure (+)-**12** (0.468 g, 13%); the fractions (1.047 g) obtained from $CHCl_3$:Me_2CO (98:2) were further purified over a silica gel column to give pure (+)-**11** (0.684 g, 19%); and the fractions (2.04 g) obtained from $CHCl_3$:Me_2CO (95:5) were further purified over a silica gel column to give a mixture containing (−)-**13** and (−)-**14** (1.54 g, 43%).

3.3. Separation of (−)-**13** and (−)-**14**

A solution containing a mixture of (−)-**13** and (−)-**14** (1.54 g, 4.7 mmol), dry THF (10 mL), and triethylamine (0.5 mL, 5.0 mmol) was cooled to 0°C. Five milliliters of chlorotrimethylsilane (5.0 mmol) were added dropwise with stirring to the solution and the reaction continued for 1 h. Then H_2O (2 mL) was added to terminate the reaction. Extraction of the mixture with $CHCl_3$ (20 mL×3) and subsequent work-up gave an oily yellow residue (2.07 g). The residue was chromatographed over a silica gel column (100 g) and eluted with a C_6H_{12}:EtOAc mixture; the fractions (1.08 g) obtained from C_6H_{12}:EtOAc (95:5) were further purified over a silica gel column to give pure (+)-**15** (0.98 g); and the fractions (0.76 g) obtained from C_6H_{12}:EtOAc (92:8) were further purified over a silica gel column to give pure (−)-**16** (0.71 g). The (+)-**15** (0.98 g) obtained from the above separation was dissolved in $CHCl_3$ (25 mL) and cooled to 0°C, then 1N HCl (3 mL) was added and the reaction mixture was stirred for 10 min. The reaction mixture was then washed with ice–water (5 mL×3) and saturated NaCl solution, dried over Na_2SO_4, and evaporation of the solvent under reduced pressure gave a pale oil residue. Purification of the residue over a small silica gel column gave (−)-**13** (0.792 g, 22%). The same treatment for (−)-**16** (0.71 g) gave pure (−)-**14** (0.576 g, 16%).

3.4. (1S,3R,6S,8R)-3-Hydroxy-3-(3-m-methoxyphenylpropyl)-6,8-dimethyl-2-oxabicyclo[4.3.0]-nonan-7-one (+)-**11**

Colorless liquid; $[\alpha]_D^{25}$ +16 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3455 (OH), 2950, 2875, 1730 (C=O), 1600, 1580, 1485, 1455, 1380, 1315, 1260, 1190, 1150, 1125, 1100, 1050, 1020 cm^{-1}; UV (MeOH) λ_{max} (log ε) 272.4 (3.23), 278.8 (3.20) nm; HREIMS *m*/*z* $[M]^+$ 332.1979 (calcd for $C_{20}H_{28}O_4$: 332.1988); EIMS *m*/*z* $[M]^+$ 332 (7), 313 (100), 296 (5), 270 (2), 244 (2), 225 (2), 204 (5), 198 (10), 193 (35), 187 (6), 180 (25), 177 (18), 152 (5), 134 (100), 128 (30), 111 (5).

*3.5. (1S,3R,6S,8S)-3-Hydroxy-3-(3-m-methoxyphenylpropyl)-6,8-dimethyl-2-oxabicyclo[4.3.0]-nonan-7-one (+)-**12***

Colorless liquid; $[\alpha]_D^{25}$ +42 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3455 (OH), 2870, 1725 (C=O), 1600, 1580, 1485, 1455, 1375, 1310, 1260, 1220, 1150, 1050 cm^{-1}; UV (MeOH) λ_{max} (log ε) 272.4 (3.23), 278.8 (3.20) nm; HREIMS *m*/*z* $[M]^+$ 332.1992 (calcd for $C_{20}H_{28}O_4$: 332.1988); EIMS *m*/*z* $[M]^+$ 332 (9), 331 (44), 313 (100), 296 (6), 278 (2), 243 (3), 225 (3), 204 (6), 198 (22), 193 (23), 180 (39), 177 (25), 165 (10), 135 (100), 128 (37), 122 (16), 110 (19).

*3.6. (1S,2R,4S)-1-Trimethylsilyloxy-2-(6-m-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-3-one (+)-**15***

Colorless liquid; $[\alpha]_D^{27}$ +23 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 2950, 1730, 1710, 1600, 1580, 1490, 1450, 1370, 1250, 1150, 1080, 1045 cm^{-1}; UV (MeOH) λ_{max} (log ε) 272.4 (3.23), 278.8 (3.20) nm; ^1H NMR δ ($CDCl_3$) 7.16 (1H, dd, *J* = 7.8, 7.8 Hz, H-5″), 6.71 (3H, m, H-6″, H-4″, and H-2″), 3.95 (1H, dd, *J* = 3.7, 3.2 Hz, H-1), 3.77 (3H, s, –OCH_3), 2.55 (2H, t, *J* = 7.7 Hz, H-6′), 2.48 (1H, m, H-4β), 2.35 (4H, m, H-4′, and H-2′), 2.04 (1H, ddd, *J* = 13.2, 8.8, 2.6 Hz, H-5α), 1.85 (1H, m, H-5β), 1.82 (2H, m, H-5′), 1.63 (1H, ddd, *J* = 14.6, 9.9, 5.9 Hz, H-1′β), 1.47 (1H, ddd, *J* = 14.6, 9.9, 5.6 Hz, H-1′α), 1.06 (3H, d, *J* = 7.3 Hz, 4-CH_3), 0.92 (3H, s, 2-CH_3), 0.07 (s, 9H, –$Si(CH_3)_3$); ^{13}C NMR δ ($CDCl_3$) 222.9 (s, C-3), 209.9 (s, C-3′), 159.6 (s, C-3″), 143.2 (s, C-1″), 129.3 (d, C-5″), 120.8 (d, C-6″), 114.2 (d, C-2″), 111.2 (d, C-4″), 75.8 (d, C-1), 55.1 (q, –OCH_3), 52.9 (s, C-2), 42.0 (t, C-5), 39.9 (d, C-4), 37.4 (t, C-2′), 37.1 (t, C-4′), 35.1 (t, C-6′), 28.8 (t, C-1′), 25.0 (t, C-5′), 15.6 (q, 4-CH_3), 15.4 (q, 2-CH_3), 0.01 (q, –Si–CH_3), −0.03 (q, –Si–CH_3), −0.07 (q, –Si–CH_3); HREIMS *m*/*z* $[M]^+$ 404.2379 (calcd for $C_{23}H_{36}O_4Si$: 404.2383); EIMS *m*/*z* $[M]^+$ 404 (1), 389 (28), 371 (13), 355 (1), 314 (3), 296 (6), 270 (12), 255 (100), 243 (40), 213 (18), 199 (100), 187 (45), 177 (90), 165 (42), 143 (20), 134 (100), 123 (51), 111 (48).

*3.7. (1S,2R,4R)-1-Trimethylsilyloxy-2-(6-m-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-3-one (+)-**16***

Colorless liquid; $[\alpha]_D^{27}$ −16.0 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 2950, 1740, 1715, 1600, 1580, 1485, 1450, 1410, 1370, 1250, 1150, 1110 cm^{-1}; UV (MeOH) λ_{max} (log ε) 272.4 (3.21), 278.8 (3.18) nm; ^1H NMR δ ($CDCl_3$) 7.17 (1H, dd, *J* = 7.8, 7.8 Hz, H-5″), 6.72 (3H, m, H-6″, H-4″, and H-2″), 3.98 (1H, dd, *J* = 8.2, 6.0 Hz, H-1α), 3.77 (3H, s, –OCH_3), 2.56 (2H, t, *J* = 7.7 Hz, H-6′), 2.38 (5H, m, H-5α, H-4′, and H-2′), 2.14 (1H, m, H-4α), 1.86 (2H, tt, *J* = 7.5, 7.5, H-5′), 1.68 (2H, m, H-1′), 1.47 (1H, ddd, 12.5, 10.1, 8.2, H-5β), 1.12 (3H, d, *J* = 7.1 Hz, 4-CH_3), 0.87 (3H, s, 2-CH_3), 0.098 (9H, s, –$Si(CH_3)_3$); ^{13}C NMR δ ($CDCl_3$) 221.9 (s, C-3), 210.1 (s, C-3′), 159.7 (s, C-3″), 143.2 (s, C-1″), 129.3 (d, C-5″), 120.9 (d, C-6″), 114.2 (d, C-2″), 111.3 (d, C-4″), 75.0 (d, C-1), 55.1 (q, –OCH_3), 52.1 (s, C-2), 41.9 (d, C-4), 41.6 (t, C-2′), 37.3 (t, C-4′), 37.2 (t, C-5), 35.1 (t, C-6′), 29.3 (t, C-1′), 25.1 (t, C-5′), 15.8 (q, 2-CH_3), 15.4 (q, 4-CH_3), 0.22 (q, –Si–$(CH_3)_3$); HREIMS *m*/*z* $[M]^+$ 404.2379 (calcd for $C_{23}H_{36}O_4Si$: 404.2383); EIMS *m*/*z* $[M]^+$ 404 (4), 389 (20), 371 (10), 314 (5), 296 (10), 270 (20), 255 (90), 241 (5), 227 (5), 213 (20), 199 (100), 187 (60), 177 (100), 165 (37), 143 (20), 134 (100), 122 (76), 111 (42).

*3.8. (1S,2R,4S)-1-Hydroxy-2-(6-m-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-3-one (+)-**13***

Colorless liquid; $[\alpha]_D^{25}$ −7 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3480 (OH), 2930, 1725 (C=O), 1710 (C=O), 1600, 1580, 1490, 1455, 1410, 1370, 1260, 1155 cm^{-1}; UV (MeOH) λ_{max} (log ε) 272.4

(3.23), 278.8 (3.20) nm; HREIMS m/z $[M]^+$ 332.1988 (calcd for $C_{20}H_{28}O_4$: 332.1988); EIMS m/z $[M]^+$ 332 (9), 314 (65), 299 (15), 286 (3), 270 (4), 255 (3), 227 (4), 211 (7), 198 (11), 183 (30), 174 (20), 161 (42), 147 (6), 134 (100), 127 (22), 121 (22).

3.9. (1S,2R,4R)-1-Hydroxy-2-(6-m-methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-3-one (+)-**14**

Colorless liquid; $[\alpha]_D^{25}$ −44 (c 1.0, $CHCl_3$); IR (film) ν_{max} 3475 (OH) 2925, 1730 (C=O), 1715 (C=O), 1600, 1585, 1485, 1455, 1410, 1370, 1310, 1260, 1150, 1050 cm^{-1}; UV (MeOH) λ_{max} (log ε) 272.4 (3.23), 278.8 (3.20) nm; HREIMS m/z $[M]^+$ 332.1987 (calcd for $C_{20}H_{28}O_4$: 332.1988); EIMS m/z $[M]^+$ 332 (8), 314 (58), 296 (15), 286 (3), 274 (4), 256 (2), 227 (3), 211 (5), 198 (10), 187 (28), 161 (45), 147 (7), 134 (100), 127 (23), 104 (17).

3.10. Chromic acid oxidation of carbinols[7]

In a typical Jones oxidation procedure, a solution of Me_2CO (10 mL) containing the carbinol (100 mg, 0.301 mmol) was cooled to 0–5°C, then 0.38 mL (0.91 mmol) of a CrO_3–H_2SO_4 solution (prepared by dissolving 26.8 g of CrO_3 in 23 mL of conc. H_2SO_4 and H_2O added to make 100 mL) was added dropwise with stirring and the reaction mixture was stirred at room temperature for 30 min. Then EtOH (0.8 mL) and H_2O (15 mL) were added to terminate the reaction. The mixture was extracted with $CHCl_3$ (15 mL×3), and the combined $CHCl_3$ extract was washed with a saturated NaCl solution and dried over Na_2SO_4. Evaporation of the solution under reduced pressure and purification of the product residue over a silica gel column gave an analytical sample.

3.11. (2R,4S)-2-(6-m-Methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-1,3-dione (+)-**10a**

Oxidation of (−)-**13** (100 mg) gave (+)-**10a** (78 mg, 78%). Compound (+)-**10a**, a colorless liquid; $[\alpha]_D^{26}$ +43 (c 1.0, $CHCl_3$); >99% *e.e.*; IR (film) ν_{max} 2940, 1760 and 1720 (C=O), 1600, 1580, 1485, 1450, 1415, 1370, 1315, 1295, 1260, 1150, 1050 cm^{-1}; UV (MeOH) λ_{max} (log ε) 272.4 (3.21), 278.8 (3.18) nm; HREIMS m/z $[M]^+$ 330.1830 (calcd for $C_{20}H_{26}O_4$: 330.1831); EIMS m/z $[M]^+$ 330 (15), 295 (1), 279 (2), 256 (1), 223 (1), 205 (1), 196 (3), 177 (5), 149 (12), 134 (100), 121 (10).

3.12. (2S,4R)-2-(6-m-Methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-1,3-dione (−)-**10a**

Oxidation of (−)-**11** (100 mg) gave (−)-**10a** (80 mg, 80%). Compound (−)-**10a**, a colorless liquid; $[\alpha]_D^{26}$ –43 (c 1.0, $CHCl_3$); >99% *e.e.*; HREIMS m/z $[M]^+$ 330.1830 (calcd for $C_{20}H_{26}O_4$: 330.1831); EIMS m/z $[M]^+$ 330 (13), 314 (1), 279 (2), 256 (2), 223 (4), 205 (3), 196 (2), 177 (4), 167 (3), 149 (51), 134 (100), 121 (11).

3.13. (2S,4S)-2-(6-m-Methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-1,3-dione (+)-**10b**

Oxidation of (+)-**12** (100 mg) gave (+)-**10b** (85 mg, 85%). Compound (+)-**10b**, a colorless liquid; $[\alpha]_D^{26}$ +48 (c 1.0, $CHCl_3$); >99% *e.e.*; IR (film) ν_{max} 2925, 1760 and 1720 (C=O), 1600, 1580, 1485, 1450, 1415, 1370, 1315, 1295, 1260, 1150, 1050 cm^{-1}; UV (MeOH) λ_{max} (log ε) 272.4 (3.20), 278.8 (3.17) nm; HREIMS m/z $[M]^+$ 330.1832 (calcd for $C_{20}H_{26}O_4$: 330.1831); EIMS m/z $[M]^+$ 330 (15), 312 (1), 279 (2), 260 (2), 243 (2), 227 (2), 188 (5), 177 (5), 161 (2), 134 (100), 121 (11).

3.14. (2R,4R)-2-(6-m-Methoxyphenyl-3-oxohexyl)-2,4-dimethylcyclopenta-1,3-dione (−)-10b

Oxidation of (−)-**14** (100 mg) gave (−)-**10b** (82 mg, 82%). Compound (−)-**10b**, colorless liquid; $[\alpha]_D^{26}$ −48 (*c* 1.0, $CHCl_3$); >99% *e.e.* HREIMS *m/z* $[M]^+$ 330.1831 (calcd for $C_{20}H_{26}O_4$: 330.1831); EIMS *m/z* $[M]^+$ 330 (14), 316 (2), 295 (1), 279 (1), 196 (3), 177 (4), 161 (2), 149 (2), 134 (100), 121 (12).

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