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Absolute stereochemistry of TT-1 (rasfonin), an α-pyrone-containing natural product from a fungus, *Trichurus terrophilus*

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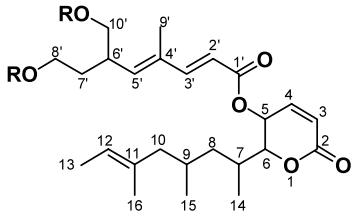
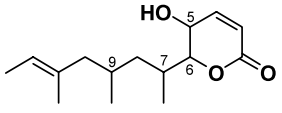
Abstract—The absolute stereochemistry of TT-1 (**1** = rasfonin), an α-pyrone-containing natural product from a Fungi Imperfecti, *Trichurus terrophilus*, was determined as 5*R*,6*R*,7*S*,9*R*, and 6′*S* on the basis of synthesis of diastereomers of two fragments of **1** in optically active forms, and comparison of their spectral and optical data with those of natural specimens.

1. Introduction

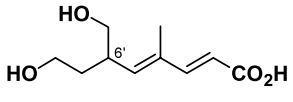
In 2000, an α-pyrone-containing natural product, TT-1, was isolated from the ethyl acetate extract of a Fungi Imperfecti *Trichurus terrophilus* culture by Fujimoto and co-workers in our laboratory.[1] TT-1 significantly suppressed proliferation (blastogenesis) of mouse splenic lymphocytes stimulated with mitogens, concanavalin A (Con A) and lipopolysaccharide (LPS), with IC_{50} values of 0.7 and 0.5 g/mL, respectively.[1] TT-1 was optically active and had a molecular formula of $C_{25}H_{38}O_6$, and its planar structure was elucidated as **1** consisting of two principal carbon-chains with an α-pyrone ring on the basis of the spectral data. Almost at the same time, Hayakawa and co-workers reported isolation of rasfonin, a new apoptosis inducer in *ras*-dependent cells from the fermented mycelia of an Ascomycete *Talaromyces* sp. 3656-A1.[2] The planar structure of rasfonin was identical with that of TT-1. However, the absolute stereochemistry of five chiral centers of **1** remained undetermined. Here we describe the determination of the absolute stereochemistry of five chiral centers of **1** as 5*R*,6*R*,7*S*,9*R*, and 6′*S* on the basis of synthesis of partial structural units (segments A and B) of **1** in optically active forms and comparison of their spectral and optical data with those of natural specimens.



1 R=H
9 R=(*R*)-MTPA
10 R=(*S*)-MTPA



segment A



segment B

2. Results and discussion

2.1. Segment B

We first studied the synthesis of segment B with 6′*S*-configuration as shown in Scheme 1. Triol (**2**), prepared from (*S*)-malic acid,[3] was partially protected as di-*tert*-butyldimethylsilyl (TBS) ether, followed by tosylation of the remaining secondary hydroxyl group to afford a tosylate (**3**), which was treated with sodium cyanide in

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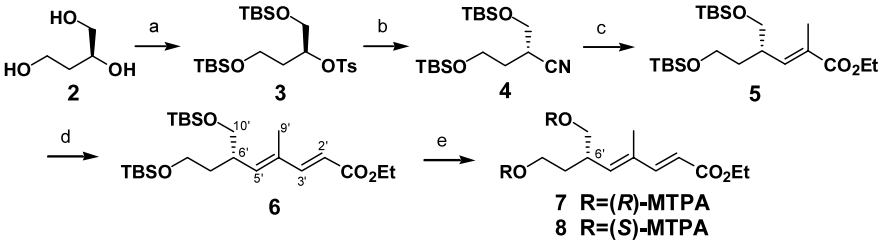


Scheme 1. *Reagents and conditions*: (a) (i) TBSCl, imidazole, DMF, 0°C, 2 h (73%); (ii) TsCl, pyridine, rt, 72 h (82%); (b) NaCN, DMSO, 90°C, 3 h (54%); (c) (i) DIBAL, CH_2Cl_2, −78°C, 2 h; (ii) $Ph_3P{=}C(CH_3)CO_2Et$, CH_2Cl_2, rt, 73 h (47% for 2 steps); (d) (i) DIBAL, CH_2Cl_2, −78°C, 1 h; (ii) MnO_2, CH_2Cl_2, rt, 14 h; (iii) $(EtO)_2P(O)CH_2CO_2Et$, NaH, DME, 0°C, 2 h (81% for 2 steps); (e) (i) TBAF, THF, rt, 2 h (99%); (ii) (*S*)- or (*R*)-MTPACl, pyridine, rt, 14 h (71–72%).

DMSO to give a nitrile (**4**) with inversion of configuration.[4,5] Reduction of the nitrile (**4**) with DIBAL followed by the Wittig reaction with (1-carbethoxyethylidene)triphenylphosphorane afforded the trisubstituted unsaturated ester (**5**). The ester (**5**) was reduced with DIBAL, followed by oxidation with manganese dioxide to give an aldehyde, which was immediately subjected to Horner–Emmons reaction with triethyl phosphonoacetate in the presence of sodium hydride to afford the di-TBS ether of segment B (**6**). The geometry of two double bonds contained in **6** was shown to be both *E* from the observation of NOE (H-2′ and H_3-9′; H_3-9′ and H_2-10′; H-3′ and H-5′), the coupling constant ($J_{2',3'}=15.6$ Hz), and high-field resonance of the C-9′ methyl carbon (δ_C 12.7). Deprotection of the TBS groups of **6** followed by treatment with (*S*)- and (*R*)-MTPA chloride in pyridine gave di-(*R*)- and di-(*S*)-MTPA esters of segment B (**7** and **8**), respectively. On the other hand, di-(*R*)- and di-(*S*)-MTPA esters (**9** and **10**, respectively) of natural product TT-1 (**1**) were also prepared by treatment with (*R*)- and (*S*)-MTPA acid with DCC and pyridine in CH_2Cl_2, respectively. Comparison of the ^{1}H NMR chemical shift data of the MTPA esters (**7**/**8** and **9**/**10**) is summarized in Table 1. Even though the alcohol moieties of the C-1′ ester group are different between **7**/**8** (=ethyl group) and **9**/**10** (=segment A), it was quite apparent that the ^{1}H NMR chemical shifts of synthetic and natural di-(*R*)-MTPA esters (**7** and **9**, respectively) coincided well with each other, and the ^{1}H NMR chemical shifts of synthetic and natural di-(*S*)-MTPA esters (**8** and **10**, respectively) were also parallel. Particularly, the methylene protons on the C-10′ position of di-(*R*)-MTPA esters (**7** and **9**) resonated equivalently at δ_H 4.20 (2H), while the C-10′ methylene protons of di-(*S*)-MTPA esters (**8** and **10**) were observed unequivalently [**8**: 4.12 (1H, dd, $J=10.6$ and 5.6 Hz) and 4.26 (1H, dd, $J=10.6$ and 6.8 Hz); **10**: 4.11 (1H, dd, $J=10.9$ and 5.6 Hz) and 4.25 (1H, dd, $J=10.9$ and 6.5 Hz)].[6] These results implied that the synthetic segment B derivative (**6**) had the same absolute configuration at the C-6′ position as that of natural product (**1**). If the synthetic compound had the opposite absolute configuration, ^{1}H NMR chemical shift data of synthetic di-(*R*)- and (*S*)-MTPA esters had to be identical with those of natural di-(*S*)- and (*R*)-MTPA esters, respectively. Since the synthetic compound (**6**) had the 6′*S*-configuration, the absolute configuration of C-6′ position of **1** was concluded as *S*.

2.2. Segment A

Compound **1** contained four chiral centers in segment A, and two of them (C-5 and C-6) are located vicinally on the α-pyrone ring. The coupling constant ($J_{5,6}=2.5$ Hz) and observation of NOE between H-5 and H-6 suggested that these two hydrogens (H-5 and H-6) were *cis*.[1]

The remaining two other chiral centers (C-7 and C-9) consisted in a 1,3-dimethyl system in an acyclic side chain. As a result of literature search[7,8] and analysis of synthetic compounds prepared by us[9] (Fig. 1), we proposed a hypothesis on the relative stereochemistry of acyclic 1,3-dimethyl systems as follows. The ^{1}H NMR

Table 1. Comparison of ^{1}H NMR data of segment B parts of synthetic and natural MTPA esters (**7**–**10**) in $CDCl_3$

Position	Synthetic di-(*R*)-MTPA ester (**7**)	Natural di-(*R*)-MTPA ester (**9**)	Synthetic di-(*S*)-MTPA ester (**8**)	Natural di-(*S*)-MTPA ester (**10**)
2′	5.80 (d, $J=15.6$)	5.78 (d, $J=15.7$)	5.80 (d, $J=15.9$)	5.78 (d, $J=15.7$)
3′	7.20 (d, $J=15.6$)	7.23 (d, $J=15.7$)	7.20 (d, $J=15.9$)	7.23 (d, $J=15.7$)
5′	5.48 (d, $J=10.1$)	5.53 (d, $J=10.2$)	5.50 (d, $J=10.1$)	5.55 (d, $J=9.0$)
6′	2.95 (m)	2.95 (m)	2.91 (m)	2.90 (m)
7′	1.62 (m) and 1.93 (m)	1.62 (m) and 1.93 (m)	1.61 (m) and 1.91 (m)	1.60 (m) and 1.91 (m)
8′	4.11 (m)	4.09 (m)	4.06 (m)	4.04 (m)
	4.33 (quint, $J=5.4$)	4.33 (quint, $J=5.4$)	4.41 (quint, $J=5.4$)	4.40 (quint, $J=5.4$)
9′	1.58 (3H, s)	1.57 (3H, s)	1.58 (3H, s)	1.57 (3H, s)
10′	4.20 (2H, d, $J=6.1$)	4.20 (2H, m)	4.12 (dd, $J=10.6$, 5.6)	4.11 (dd, $J=10.9$, 5.6)
			4.26 (dd, $J=10.6$, 6.8)	4.25 (dd, $J=10.9$, 6.5)

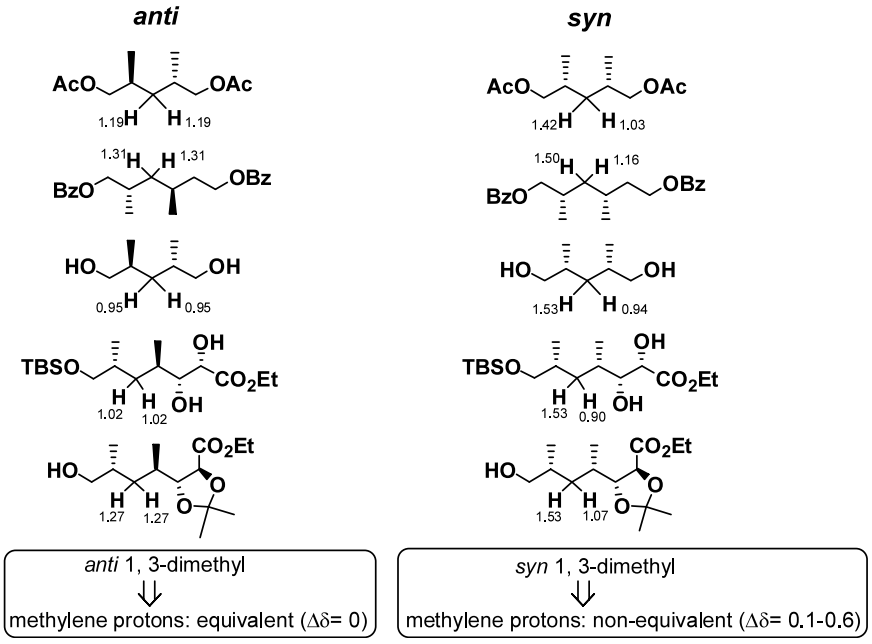
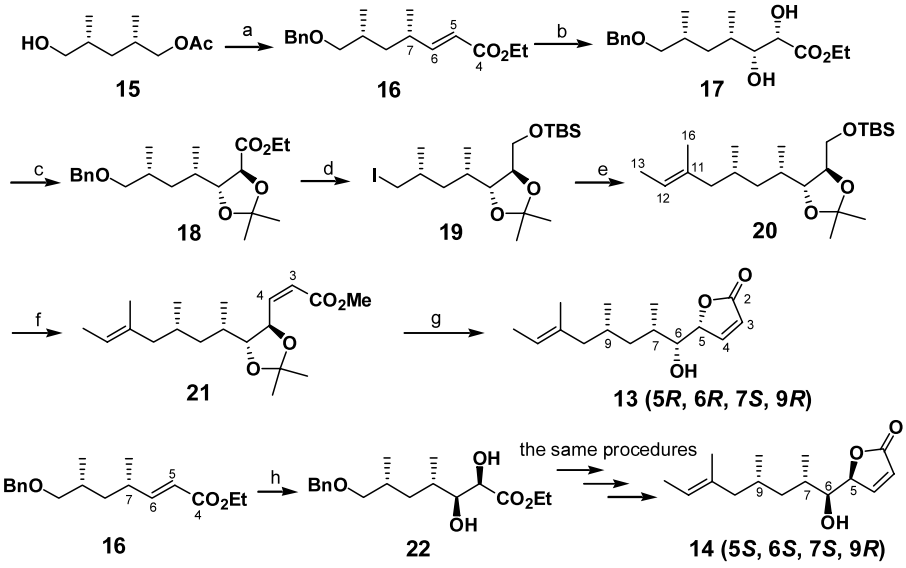


Figure 1. ^{1}H NMR chemical shifts of methylene protons located between two methyl-bearing methine carbons in acyclic 1,3-dimethyl systems (in $CDCl_3$).[7–9]



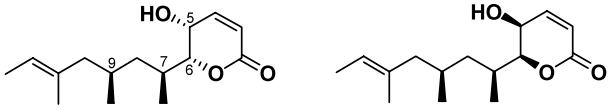
Scheme 2. *Reagents and conditions*: (a) (i) benzyl 2,2,2-trichloroacetimidate, TfOH, cyclohexane/CH_2Cl_2 (1:1), rt, 4 h; (ii) NaOHaq, MeOH, rt, 1 h (68% for 2 steps); (iii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C, 2 h; (iv) $(EtO)_2P(O)CH_2CO_2Et$, NaH, DME, 0°, 2h (91% for 2 steps); (b) AD-mix-β, $CH_3SO_2NH_2$, *t*-BuOH/H_2O (1:1), rt, 40 h (78%); (c) $(CH_3)_2C(OCH_3)_2$, TsOH, acetone, rt, 1 h (96%); (d) (i) $LiAlH_4$, THF, rt, 0.5 h; (ii) TBSCl, imidaole, DMF, rt, 2 h (94% for 2 steps); (iii) H_2, Pd/C, EtOH, rt, 18 h (91%); (iv) I_2, Ph_3P, imidazole, benzene, rt, 2 h (97%); (e) 2-bromo-*cis*-2-butene, Li, THF, 0°C to rt, 18 h (58%); (f) (i) TBAF, THF, rt, 1 h; (ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C, 2 h; (iii) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, KHMDS, 18-crown-6, THF, −78°C, 2 h (75% for 3 steps); (g) TsOH, MeOH (87%); (h) AD-mix-α, $CH_3SO_2NH_2$, *t*-BuOH/H_2O (1:1), rt, 43 h (82%).

chemical shifts of the methylene protons located between the two methyl-bearing methine carbons in acyclic 1,3-dimethyl systems may be diagnostic for the relative stereochemistry (*syn* or *anti*) of 1,3-dimethyl systems: 'the two methylene protons of *syn* diastereomers were magnetically non-equivalent ($\Delta\delta =$ 0.1–0.6), while those of *anti* diastereomers resonated equivalently ($\Delta\delta = 0$).' Similar arguments were previously described for 1,3-diol or 1,3-dimethoxy systems.[10,11] In the case of natural product (**1**), the corresponding two methylene protons on C-8 were observed non-equivalently (δ_H 1.04 and 1.20; $\Delta\delta =$ 0.16), suggesting that the relative stereochemistry of the 1,3-dimethyl groups on C-7 and C-9 was *syn*. From the discussions described above, 5,6-*cis* and 7,9-*syn* were proposed for the relative stereochemistry of segment A.

Table 2. Comparison of ^1H and ^{13}C NMR data of natural and synthetic 5-membered lactones (**13** and **14**) in $CDCl_3$

Position	Synthetic **13** δ_H	Natural **13** δ_H	Synthetic **14** δ_H	Synthetic **13** δ_C	Natural **13** δ_C	Synthetic **14** δ_C
2	–	–	–	172.7	172.7	172.9
3	6.19 (dd, J=5.8, 2.1)	6.19 (dd, J=5.8, 2.1)	6.18 (dd, J=5.8, 2.1)	122.9	122.9	122.5
4	7.43 (dd, J=5.8, 1.5)	7.43 (dd, J=5.8, 1.4)	7.46 (dd, J=5.8, 2.1)	153.3	153.4	154.2
5	5.11 (dt, J=5.8, 1.8)	5.11 (dt, J=5.8, 1.6)	5.16 (quint, J=2.1)	85.5	85.6	84.1
6	3.50 (brs)	3.50 (brs)	3.47 (brs)	75.1	75.1	76.2
7	1.82 (m)	1.82 (m)	1.86 (m)	33.4	33.5	34.7
8	1.06 (m) 1.47 (m)	1.06 (m) 1.47 (m)	1.14 (m) 1.50 (m)	41.1	41.2	40.1
9	1.65 (m)	1.66 (m)	1.66 (m)	27.8	27.9	28.5
10	1.69 (m) 2.00 (dd, J=12.8, 4.1)	1.70 (m) 2.00 (dd, J=12.9, 4.1)	1.69 (m) 2.06 (dd, J=12.7, 4.2)	47.5	47.5	47.0
11	–	–	–	134.2	134.3	134.4
12	5.18 (q, J=6.7)	5.18 (q, J=6.7)	5.18 (d, J=6.7)	120.2	120.2	120.2
13	1.57 (d, J=6.7)	1.57 (d, J=6.7)	1.58 (d, J=6.7)	13.3	13.4	13.3
14	1.03 (d, J=6.7)	1.03 (d, J=6.6)	1.05 (d, J=6.6)	14.7	14.8	16.6
15	0.79 (d, J=6.3)	0.79 (d, J=6.3)	0.85 (d, J=6.3)	20.0	20.1	20.9
16	1.55 (s)	1.55 (s)	1.56 (s)	15.5	15.6	15.6

Thus, four diastereomers (**11** and **12**, and their enantiomers) remain to be likely out of sixteen possibilities for the absolute configuration of segment A. We then studied the synthesis of two possible diastereomers (**11** and **12**) as optically active forms to determine the absolute configurations by comparison of their spectral and optical data with those of the natural specimen. In our synthetic studies as shown in Scheme 2, the diastereomers (**13** and **14**) with a 5-membered lactone were eventually obtained in place of **11** and **12** with a 6-membered lactone. On the other hand, the 5-membered lactone (**13**) was also obtained by hydrolysis of natural product (**1**). We therefore were able to complete our purpose of determination of the stereochemistry of segment A by using the data of these 5-membered lactones.



11

12

The diastereomer **13** with 5*R*,6*R*,7*S*,9*R*-configurations was prepared as shown in Scheme 2. The known monoacetate (**15**), which was prepared from methyl dimethylmalonate[12,13] through enantioselective acetylation with lipase AK,[7,14] was converted into an *E*-unsaturated ester (**16**, $J_{4,5}$=15.6 Hz)[15] through four-step reactions [(1) benzylation, (2) hydrolysis, (3) Swern oxidation, and (4) Horner–Emmons reaction]. The asymmetric dihydroxylation of ester (**16**) with AD-mix-β[16] led to the α,β-dihydroxy ester (**17**), which was protected with an acetonide to give **18**. The $LiAlH_4$ reduction of **18**, followed by protection with TBS ether, hydrogenolysis of benzyl ether, and treatment with iodine and triphenylphosphine afforded the iodide (**19**). The iodide (**19**) was treated with the alkenyllithium reagent[17] derived from 2-bromo-*cis*-2-butene to give the *E*-alkene [**20**, δ_C 13.4 (C-13) and 15.5 (C-16)[18]]. Deprotection of the TBS group of **20** followed by Swern oxidation and Still's variant of the Horner–Emmons reaction[19] afforded the *Z*-unsaturated ester (**21**, $J_{3,4}$= 11.7 Hz). Treatment of **21** with TsOH in methanol afforded the 5-membered lactone (**13**) and no 6-membered lactone (**11**) was obtained.[20,21]

The other diastereomer **14** with 5*S*,6*S*,7*S*,9*R*-configuration was also prepared by similar procedures from the diastereomeric diol (**22**) which was obtained through asymmetric dihydroxylation of the unsaturated ester (**16**) using AD-mix-α.[16]

On the other hand, alkaline hydrolysis of natural product (**1**) was carried out [(i) 0.1 N KOH–Et_2O (1:4), rt, 28 h; (ii) HCl/MeOH (pH 3.5), rt, 18 h], and it was found that a 5-membered lactone was obtained in 44% yield for the segment A part.[22] The ^1H NMR chemical shift data of the 5-membered lactone obtained from the natural specimen were compared with those of the two diastereomers (**13** and **14**) to reveal that the relative stereochemistry of 5-membered lactone obtained from the natural specimen was identical with the diastereomer (**13**) (Table 2). The optical rotations of natural-**13** and synthetic-**13** were found to be both dextrorotatory, and both of them showed positive Cotton effects in their CD spectra,[23] indicating that natural-**13** and synthetic-**13** were optically identical (not enantiomers). Thus, the absolute configurations of TT-1 (**1**=rasfonin) were established as 5*R*,6*R*,7*S*,9*R*, and 6′*S* (Fig. 2).

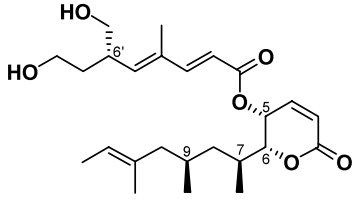
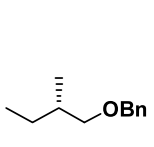


Figure 2. Structure of **1** including absolute stereochemistry.



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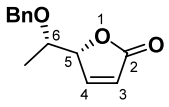

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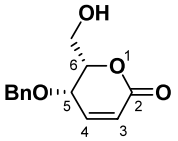

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Acknowledgements

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4. The nitrile (**4**) was transformed into (2*S*)-1-benzyloxy-2-methylbutane (**23**) by 6 steps [(1) DIBAL, CH_2Cl_2, −78°C, 2 h, (2) $NaBH_4$, EtOH, rt, 1 h (67% in 2 steps), (3) NaH, BnBr, DMF, 0°C, 3 h (66%), (4) TBAF, THF, rt, 1 h (98%), (5) MsCl, pyridine, rt, 4 h (72%), (6) $LiAlH_4$, THF, rt, 1 h (87%)]. (2*S*)-1-benzyloxy-2-methylbutane (**23**) was also prepared from commercially available (2*S*)-2-methyl-1-butanol by 1 step [NaH, BnBr, DMF, 0°C, 3 h (72%)]. The optical rotation data were as follows: **23** from nitrile (**4**): $[\alpha]_D^{27}$ +4.2 (*c* 0.21, $CHCl_3$); **23** from commercially available (2*S*)-2-methyl-1-butanol: $[\alpha]_D^{27}$ +4.3 (*c* 2.1, $CHCl_3$); literature value of the enantiomer, (2*R*)-1-benzyloxy-2-methylbutane (*ent*-**23**): $[\alpha]_D^{27}$ −4.5 (*c* 3.23, $CHCl_3$).[5] Thus, the absolute configuration of the nitrile (**4**) generated through inversion of configuration was established.
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18. The ^{13}C NMR chemical shifts for the corresponding methyl groups of 3-methyl-2*Z*-hexene [δ_C 12.0 (C-1) and 22.1 (3-Me)] and 3-methyl-2*E*-hexene [δ_C 12.0 (C-1) and 14.2 (3-Me)]: Dorman, D. E.; Jautelat, M.; Roberts, J. D. *J. Org. Chem*. **1971**, *36*, 2757–2766.
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20. The ^{13}C NMR of the 5-membered lactone (**13**) showed signals due to the 5-membered lactone moiety at δ_C 172.7 (C-2), 122.9 (C-3), 153.4 (C-4), 85.5 (C-5), and 75.0 (C-6). The ^{13}C NMR data in the literature[21] for the corresponding positions of the 5-membered and 6-membered lactones (**24** and **25**) are as follows: **24** (5-membered): δ_C 172.8 (C-2), 122.3 (C-3), 153.8 (C-4), 85.7 (C-5), and 74.7 (C-6); **25** (6-membered): δ_C 162.9 (C-2), 123.8 (C-3), 142.6 (C-4), 66.2 (C-5), and 80.6 (C-6).
21. Sánchez-Sancho, F.; Valverde, S.; Herradón, B. *Tetrahedron: Asymmetry* **1996**, *7*, 3209–3246.
22. The product corresponding to segment B was not isolated at this time from the hydrolysis products of **1**.
23. Synthetic **13**: $[\alpha]_D^{24}$ +31 (*c* 0.30, $CHCl_3$); CD (MeOH) Δε +5.2 (214 nm); Natural **13**: $[\alpha]_D^{24.5}$ +87 (*c* 0.76, $CHCl_3$); CD (MeOH) Δε +16.2 (215 nm).