Purpurediolin and Purpurenin, Two New Cytotoxic Adjacent Bis-tetrahydrofuran Annonaceous Acetogenins from the Seeds of *Annona purpurea*†

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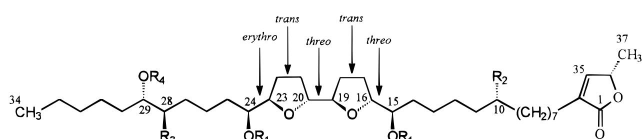
Two novel cytotoxic acetogenins, purpurediolin (**1**) and purpurenin (**2**), were isolated from the seeds of *Annona purpurea*. Their structures were elucidated by a combination of chemical and spectral methods including MS and NMR measurements. In addition, six known acetogenins were obtained, namely, bullatacin, squamocin (annonin I), motrilin (squamocin C), annoglaucin, xylomatenin, and annonacin A. Compounds **1** and **2** exhibited potent cytotoxic activity in vitro against six human solid tumor cell lines.

Annona purpurea Moc. & Sessé ex Dunal (Annonaceae) is a small tree up to 7 m high. The fruit of this plant, commonly known as " ilama " in Mexico, is edible. It is also used in folk medicine as remedy for fevers and colds.[1] From the leaves of this species, Hostettmann and co-workers obtained six acetogenins, namely, bullatacin, rolliniastatin 1, purpureacin 2, cherimoline, sylvaticin, and purpureacin.[2]

In our continued search for biologically active natural compounds from Mexican medicinal plants, we have investigated the seeds of *A. purpurea* and isolated the novel acetogenins **1** and **2**, which were given trivial names of purpurediolin and purpurenin, respectively. In addition, the six known acetogenins bullatacin,[3] squamocin[4] (annonin I[5]), motrilin[6] (squamocin C[4]), annoglaucin,[7] xylomatenin,[8] and annonacin A[9] have been isolated from the same source. In this paper, we describe the isolation, structure elucidation, and cytotoxic activity of compounds **1** and **2**.



	R1	R2	R3	R4
1	H	H	OH	H
1a	TMSi	H	TMSi	TMSi
1b	H	H	Acetonide	
1s	(*S*)-MTPA	H	(*S*)-MTPA	(*S*)-MTPA
1r	(*R*)-MTPA	H	(*R*)-MTPA	(*R*)-MTPA
2	H	OH	H	H
2a	TMSi	TMSi	H	TMSi
2s	(*S*)-MTPA	(*S*)-MTPA	H	(*S*)-MTPA
2r	(*R*)-MTPA	(*R*)-MTPA	H	(*R*)-MTPA

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Results and Discussion

The dried seeds of *A. purpurea* were extracted with $CHCl_3$–MeOH (1:1). As for most extracts from *Annona* species,[10–12] the crude extract of *A. purpurea* exhibited potent activity in the brine shrimp lethality test[13] and against human solid tumor cell lines. The active extract ($LC_{50} = 4.0$ μg/mL) was subjected to solvent partition with hexane and 10% H_2O in MeOH. The most active fraction as evaluated in the brine shrimp lethality test was the methanolic fraction (brine shrimp lethality test $LC_{50} = 0.11$ μg/mL). This fraction was further fractionated by open column chromatography using silica gel with increasing solvent polarities to yield 11 primary fractions (F_1–F_{11}, see the Experimental Section). Extensive HPLC separation of the most active fraction F_5 (brine shrimp lethality test $LC_{50} = 1.38 \times 10^{-2}$ μg/mL) yielded three known acetogenins, bullatacin, squamocin, and motrilin. Active fraction F_7 (brine shrimp lethality test $LC_{50} = 1.47 \times 10^{-2}$ μg/mL) was also chromatographed by HPLC to yield the known compounds annoglaucin, xylomatenin, and annonacin A as well as the novel acetogenins **1** and **2**. The spectral properties, including UV, IR, NMR, and MS of the known compounds, were identical to those previously described for bullatacin,[3] squamocin[4] (annonin I[5]), motrilin[6] (squamocin C[4]), annoglaucin,[7] xylomatenin,[8] and annonacin A,[9] respectively. All compounds except bullatacin have never been isolated from *A. purpurea* before.

Purpurediolin (**1**) was obtained as a white wax. Its molecular formula was established as $C_{37}H_{66}O_8$ by HRFABMS. The IR spectrum contained absorptions for hydroxyl (3418 cm^{-1}) and α,β-unsaturated methyl γ-lactone (1751 cm^{-1}) functionalities. Sequential losses of four molecules of H_2O from the MH^+ in the FABMS as well as the formation of the tetra-TMSi derivative **1a** confirmed the presence of four hydroxyl groups.

The NMR data of compound **1** (Tables1 and 2) clearly indicated that it was an adjacent bis-THF acetogenin very similar to bullatetrocin, a bullatacin-type of acetogenin possessing a 1,2-diol unit at C-31/32.[14] The resonances for the 4-deoxy-α,β-unsaturated methyl γ-lactone were observed at δ 6.99 (H-35), 5.00 (H-36),

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Table 1. ^{1}H NMR Data of Purpurediolin (**1**) and Purpurenin (**2**)[a]

position	1	2
3	2.26 tt (7.5, 2.0)	2.26 tt (7.0, 1.5)
4	1.54 m	1.55 m
5−8	1.20−1.60 m	1.20−1.60 m
9	1.20−1.60 m	1.42 m
10	1.20−1.60 m	3.58 m
11	1.20−1.60 m	1.42 m
12−13	1.20−1.60 m	1.20−1.62 m
14	1.37 m	1.40 m
15	3.40 m	3.40 m
16	3.83 m	3.83 m
17	1.63 m, 1.97 m	1.62 m, 1.97 m
18	1.63 m, 1.97 m	1.62 m, 1.97 m
19	3.85 m	3.85 m
20	3.93 m	3.93 m
21	1.59 m, 1.97 m	1.62 m, 1.97m
22	1.59 m, 1.90 m	1.60 m, 1.90m
23	3.93 m	3.93 m
24	3.86 m	3.85 m
25	1.42 m	1.41 m
26	1.44 m	1.20−1.62 m
27	1.47 m[b]	1.20−1.62 m
28	3.59 m	1.42 m
29	3.59 m	3.58 m
30	1.41 m[b]	1.42 m
31−33	1.20−1.60 m	1.20−1.62 m
34	0.89 t (7.0)	0.89 t (7.0)
35	6.99 ddd (2.0, 2.0, 2.0)	6.99 ddd (1.5, 1.5, 1.5)
36	5.00 qq (7.0, 2.0)	5.00 qq (7.0, 1.5)
37	1.40 d (7.0)	1.40 d (7.0)

[a] $CDCl_3$, 500 MHz (*J* in Hz). [b] Interchangeable assignments.

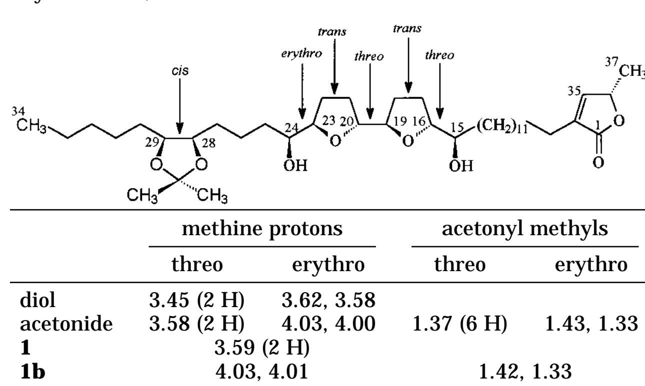
Table 2. ^{13}C NMR Data of Purpurediolin (**1**) and Purpurenin (**2**)[a]

position	1	2
1	173.9	173.8
2	134.4	134.4
3	25.1	25.1
4	27.4	27.4
5−13	28.3−29.7	28.3−29.6
9, 11	28.3−29.7	37.4[b]
10	28.3−29.7	71.8
12−13	28.3−29.7	28.3−29.6
14	33.2	33.3
15	74.1	74.0
16	83.3	83.2
17	28.4	28.4
18	28.9	28.8
19	82.5	82.4
20	82.2	82.2
21	29.1	29.2
22	25.1	24.7
23	82.9	82.8
24	71.6	71.5
25	32.5	32.4
26	22.3	28.3−29.6
27	30.8[b]	28.3−29.6
28	74.6[c]	37.3[b]
29	74.7[c]	71.8
30	31.4[b]	37.4[b]
31	28.3−29.7	28.3−29.6
32	31.8	31.9
33	22.5	22.6
34	13.9	14.0
35	148.9	148.8
36	77.4	77.3
37	19.1	19.2

[a] $CDCl_3$, 125 MHz. [b,c] Interchangeable assignments within same column.

2.26 (H-3), and 1.40 (H-37) in the ^{1}H NMR spectrum (Table 1) and at δ 173.9 (C-1), 148.9 (C-35), 134.4 (C-2), 77.4 (C-36), 25.1 (C-3), and 19.1 (C-37) in the ^{13}C NMR spectrum (Table 2). The absorptions for the adjacent bis-THF α,α'-dihydroxylated portion appeared at δ 74.1 (C-15), 83.3 (C-16), 82.5 (C-19), 82.2 (C-20), 82.9 (C-23), and 71.6 (C-24) in the ^{13}C NMR spectrum. The signal attributable to C-15 showed a cross-peak with the signal at δ 3.40 (H-15) in the HETCOR spectrum. The remaining five carbon resonances correlated with two multiplets in the region at δ 3.81−3.96 (5H, H-16, H-19, H-20, H-23, and H-24).

Table 3. ^{1}H NMR Signals for the Protons of the Threo and Erythro Diols,[15] **1** and **1b**[a]



	methine protons		acetonyl methyls	
	threo	erythro	threo	erythro
diol	3.45 (2 H)	3.62, 3.58		
acetonide	3.58 (2 H)	4.03, 4.00	1.37 (6 H)	1.43, 1.33
1	3.59 (2 H)			
1b	4.03, 4.01		1.42, 1.33	

[a] $CDCl_3$, 500 MHz.

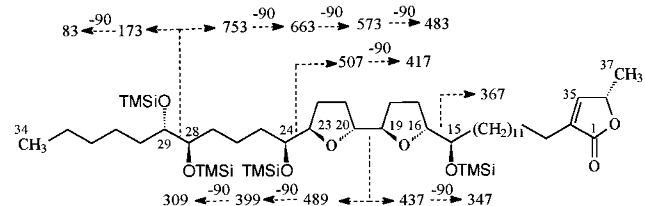


Figure 1. Diagnostic EIMS fragment ions of **1a** (shown as *m*/*z* values).

The presence of a vicinal diol moiety in the molecule was ascertained by the resonances at δ_H 3.59 (m, 2H) and δ_C 74.6 and 74.7 in the NMR spectra. Treatment of compound **1** with acetone/HCl afforded the acetonide derivative **1b**, which confirmed chemically the presence of the vicinal diol in **1**. The ^{1}H NMR data (Table 3) of this derivative substantiated an erythro configuration for the diol group. The appearance of the acetonyl methyl protons as two well-separated singlet peaks at δ 1.42 and 1.33 and the chemical shift values of the oximethine signals at δ 4.03 (H-28) and 4.01 (H-29) were consistent with this proposal.[15]

The disposition of the diol moiety and the adjacent bis-THF unit along the aliphatic chain were determined by analysis of the MS fragmentation pattern displayed by the tetra-TMSi derivative **1a** (Figure 1).[12] Thus, the intense fragment ion peak at *m*/*z* 367 was consistent with a cleavage at C-15/16; the fragment at *m*/*z* 507 (cleavage at C-23/24) placed the bis-THF rings with two flanking hydroxyls groups at C-15 to C-24. Finally, the presence of fragments ions at *m*/*z* 173 and 753 located the vicinal diol at C-28/29 rather than at C-31/32 as in the case of bullatetrocin.[14]

The threo/trans/threo/trans/erythro relative stereochemistry from C-15 to C-24 was assigned on the basis of the chemical shift values observed for these nuclei in the NMR spectra, which were very similar to those previously described for bullatacin and related compounds.[16−19]

Table 4. Partial ^{1}H NMR Data of the (*S*)- and (*R*)-Mosher Esters of **1** and **2**[a]

	1				2			
protons	(*S*)-MTPA	(*R*)-MTPA	$\Delta\delta_{S-R}$	carbinol confign	(*S*)-MTPA	(*R*)-MTPA	$\Delta\delta_{S-R}$	carbinol confign
3					2.250	2.238	+0.012	10*R*
4					1.532	1.514	+0.018	
14	1.600	1.511	+0.089		1.63	1.482	+0.131	
16	3.922	3.921	+0.001	15*R*	3.976	3.944	+0.032	15*R*
17	1.976	1.982	−0.006		1.899	1.971	−0.072	
	1.528	1.559	−0.031		1.600	1.616	−0.016	
22	1.854	1.807	+0.047		1.824	1.798	+0.026	
	1.616	1.613	+0.003		1.664	1.606	+0.058	
23	3.906	3.905	+0.001	24*S*	3.944	3.930	+0.014	24*S*
25	1.556	1.597	−0.041		1.532	1.554	−0.022	
34	0.832	0.866	−0.034	29*S*	0.832	0.864	−0.032	29*S*

[a] $CDCl_3$, 500 MHz.

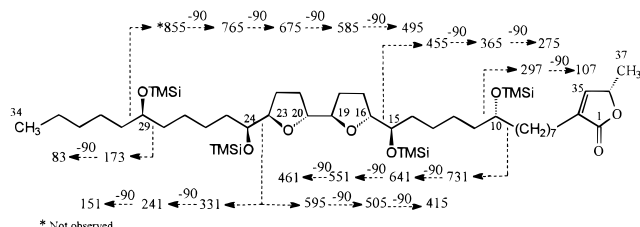


Figure 2. Diagnostic EIMS fragment ions of **2a** (shown as *m*/*z* values).

The *S* configuration at C-36 was established by the negative Cotton effect at 238 nm.[4,19] The absolute configuration of the stereogenic carbinol centers were established using Mosher ester methodology.[13] The analysis of the $\Delta\delta_H(S-R)$ data (Table 4) of the per-(*S*)- and per-(*R*)-MTPA Mosher ester derivatives **1s** and **1r** showed that the absolute stereochemistry of the chiral centers at C-15 and C-24 was *R* and *S*, respectively. On the other hand, the absolute configuration at C-29 was established as *S* considering the negative difference in chemical shift for the terminal methyl group with respect to the (*S*)- and (*R*)-Mosher ester derivatives.[19] Thereafter, the absolute configuration at C-28 was determined as *R* because of the *erythro* configuration of the vicinal diol at C-28/29.

Recently, a similar compound to purpurediolin (**1**), namely rollitacin, was isolated from *Rollinia mucosa*.[20] However, the absolute stereochemistry for the stereogenic carbinol centers was not described. Thus, purpurediolin (**1**) and rollitacin could be the same compound.

Purpurenin (**2**) was obtained as a pale yellow wax. The molecular formula $C_{37}H_{66}O_8$ was also deduced by HRFABMS. The NMR properties of compound **2** indicated that its structure was very similar to those of compound **1**. Once again, the existence of four hydroxyl groups was revealed by the analysis of the FABMS data and the formation of the tetra-TMSi derivative **2a**. The major differences observed between compounds **1** and **2** referred to the third and fourth hydroxyl groups along the aliphatic chain. According to an analysis of the EIMS of the tetra-TMSi derivative **2a** (Figure 2), these two groups were at C-10 and C-29. The presence of fragment ions at *m*/*z* 731 (cleavage between C-9/10), 297 (cleavage between C-10/11), and 173 (cleavage between C-28/29) was in agreement with this proposal. On the other hand, as in compound **1**, the adjacent bis-THF α,α′-dihydroxylated moiety was located between C-15 and C-24 due to the presence of fragment ions at *m*/*z* 455 and 595 (cleavages at C-15/16 and C-23/24, respectively).

The relative configuration of this system from C-15 to C-24 was deduced as threo/trans/threo/trans/erythro by considering the similarity of the ^{1}H and ^{13}C NMR data of **2** with those of **1** and other bullatacin-type acetogenins.[16−19] The absolute configuration of the carbinol centers was also determined by analysis of the ^{1}H NMR data of the per-(*S*)- and per-(*R*)-Mosher ester derivatives **2s** and **2r**. The negative value ($\Delta\delta_{S-R}$) obtained for H-34 (Table 4) was consistent with an *S* configuration at C-29. The absolute stereochemistry at C-10 was assigned as *R* because the difference values for H-3 and H-4 were positive. In the case of C-15 and C-24, the absolute stereochemistries turned out to be identical to those of compound **1**. Finally, the configuration at C-36 was determined to be *S* on the basis of CD measurements.[4,19]

Acetogenins **1**, **2**, annoglaucin, and annonacin A were significantly bioactive in the brine shrimp lethality test and were also cytotoxic to six human solid tumor cell lines in a 7-day MTT test using adriamycin as the positive control (Table 5). Purpurediolin (**1**) and annoglaucin showed high selectivity against HT-29 (human colon adenocarcinoma). Purpurenin (**2**) was less potent than **1** and annoglaucin. The level of activity displayed by acetogenins **1** and **2** is comparable to that previously described for similar compounds.[14]

Experimental Section

General Experimental Procedures. Melting point determinations were performed on a Fisher-Johns apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-360 polarimeter. UV spectra were obtained on a Shimadzu 160 UV spectrometer in MeOH solution. CD spectra were performed on a JASCO 720 spectropolarimeter at 25 °C in MeOH solution. IR spectra (film) were measured on a Perkin-Elmer 599 spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra (all in $CDCl_3$) were obtained on a Varian Unity Plus 500 spectrometer. The FABMS data were recorded using a glycerol matrix on a JEOL DX300 mass spectrometer. HRFABMS were obtained in a JEOL JX102A mass spectrometer using an NBA matrix. EIMS for TMSi derivatives was performed on a JEOL JMS-AX505HA mass spectrometer. HPLC was carried out with a Waters HPLC instrument equipped with a Waters UV photodiode array detector (900) set at 209−220 nm, using a silica gel column (19 mm i.d. ×

Table 5. Brine Shrimp Lethality and Cytotoxicity Data for the Extract and Selected Compounds from *A. purpurea*

compound	BST[a] (μg/mL)	tumor cell line [ED_{50} (μg/mL)]					
		A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	PACA-2[g]
extract	1.1×10^{-1}	$<10^{-2}$	1.53	1.47	3.53	1.16	$<10^{-2}$
1	7.0×10^{-2}	4.43×10^{-1}	9.16×10^{-1}	$<10^{-7}$	1.36	3.53×10^{-1}	1.44
2	2.9×10^{-2}	1.29	1.67	3.16×10^{-1}	1.25	1.07	1.98
annoglaucin	2.2×10^{-2}	1.08	1.56	$<10^{-7}$	1.01	3.56×10^{-1}	1.45
annonacin A	4.6×10^{-1}	1.32	1.96	1.18	1.16	1.33	1.90
adriamycin		3.67×10^{-3}	2.12×10^{-1}	9.66×10^{-3}	1.98×10^{-3}	2.27×10^{-2}	1.45×10^{-3}

[a] Brine shrimp lethality test. [b] Human lung carcinoma. [c] Human breast carcinoma. [d] Human colon adenocarcinoma. [e] Human kidney carcinoma. [f] Human prostate adenocarcinoma. [g] Human pancreatic carcinoma.

300 mm). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 2000 software program (Waters).

Plant Material. The seeds of *A. purpurea* (Annonaceae) were collected in July 1994 in Catemaco, Veracruz, Mexico. A voucher specimen of the plant (no. CA94-1) is preserved in the Herbarium of the Instituto de Ecología (XAL), Xalapa, Veracruz.

Bioassays. Brine shrimp lethality test of the extract, fractions and isolated compounds was performed according to the standard procedure.[13] Cytotoxicity against human solid tumors cells was measured in a 7-day MTT assay at the Purdue Cell Culture Laboratory using adriamycin as a positive control.[18]

Extraction and Isolation. The air-dried seeds of *A. purpurea* (4.9 kg) were pulverized in a Wiley 4 mill. The pulverized seeds were extracted with $CHCl_3$–MeOH (1:1). The dried extract (1,500 g, brine shrimp lethality test $LC_{50} = 4.0$ μg/mL) was partitioned between hexane–MeOH (10% water). The methanolic fraction (378 g, brine shrimp lethality test $LC_{50} = 1.14 \times 10^{-1}$ μg/mL) was subjected to open column chromatography (1 kg of Kieselgel 60 Merck, 0.063 mm, 230 mesh ASTM) and eluted with a gradient of increasing polarity with hexane/$CHCl_3$/MeOH. Altogether, 89 fractions were collected and then combined according to their TLC patterns to yield 11 primary fractions (F_1–F_{11}). Bioactivities in the brine shrimp lethality test showed six active pools (F_4–F_9). HPLC purification of the active fraction F_7 (500 mg, brine shrimp lethality test $LC_{50} = 1.47 \times 10^{-2}$ ppm) on a normal-phase silica column [8.3 mL/min, hexane–*i*-PrOH–MeOH (85:7.5:7.5)] yielded **1** (22 mg), **2** (44 mg), and the known compounds annoglaucin, xylomatenin, and annonacin A (46, 30, and 10 mg, respectively). The retention times were 34.6, 50.0, 42.0, 31.5, and 33.0 min, respectively. HPLC purification of the most active pool F_5 (400 mg, brine shrimp lethality test $LC_{50} = 1.38 \times 10^{-2}$ ppm) on a normal-phase silica column [7.9 mL/min, hexane-*i*–PrOH–MeOH, (90:5:5)] afforded the known compounds bullatacin (20 mg), squamocin (70 mg), and motrilin (110 mg); retention times: 44.0, 45.2, and 47.0 min, respectively).

Purpurediolin (1): whitish wax; mp 35–39 °C; $[\alpha]_D$ +20° (*c* 1.3 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.89) nm; CD (MeOH) $\Delta\epsilon$ (nm) -3.77×10^3 (238); IR ν max (film) 3100–3650, 3023, 2928, 1751, 1641, 1423, 1215, 1028, 930 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); FABMS (glycerol) *m*/*z* $[MH]^+$ 639; HRFABMS (NBA) *m*/*z* 639.4837 $[MH]^+$, calcd for $C_{37}H_{66}O_8$, 639.4835.

Purpurenin (2): pale yellow wax; mp 36–38 °C; $[\alpha]_D$ +27 (*c* 1.0 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.09) nm; CD (MeOH) $\Delta\epsilon$ (nm) -2.66×10^3 (238); IR ν max (film) 3100–3700, 3021, 2940, 2859, 1751, 1642, 1428, 1215, 1074, 927 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); FABMS (glycerol) *m*/*z* $[MH]^+$ 639; HRFABMS (NBA) *m*/*z* 639.4837 $[MH]^+$, calcd for $C_{37}H_{66}O_8$, 639.4835.

TMSi Derivatizations. Small amounts (1.0 mg) of compounds **1** and **2** were treated with 100 μL of Sigma-Sil-A (trimethylchlorosilane–hexadimethylsilane–pyridine 1:3:9) and heated at 60 °C for 10 min to yield the respective TMSi derivatives **1a** and **2a**. **1a**: EIMS *m*/*z* 753 (17), 663 (10), 573 (5), 507 (11), 489 (5), 483 (5), 437 (10), 417 (10), 399 (10), 367 (80), 347 (6), 309 (2), 173 (33), 111 (14), 83 (25), 73 (100). **2a**: EIMS *m*/*z* 765 (5), 731 (6), 675 (2), 641 (7), 595 (31), 585 (3), 551 (7), 505 (27), 495 (7), 455 (97), 461 (4), 415 (32), 365 (63), 331 (11), 297 (83), 275 (22), 241 (9), 173 (21), 151 (15), 111 (12), 107 (14), 83 (18), 73 (100).

Preparation of Acetonide Derivative 1b. To a solution of 3 mg of **1** in 5 mL of acetone was added 1.5 μg of HCl (concentrated). The mixture was allowed to react for 48 h and monitored by TLC until the reaction was completed. The mixture was dried in vacuo to give the acetonide derivative **1b** (partial ^1H NMR spectrum shown in Table 3).

Preparation of Per-(*S*)- and Per-(*R*)-MTPA Ester Derivatives. To a solution of **1** or **2** (2 mg in 0.8 mL of $CDCl_3$ in a NMR tube) were sequentially added pyridine-d_5, (20 μL), 4-(dimethylamino)pyridine (0.5 mg), and (*R*)-(–)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (25 mg). The mixture was heated at 50 °C for 4 h under an N_2 atmosphere to give the (*S*)-Mosher esters **1s** and **2s** (^1H NMR data, Table 4). Treatment of **1** (2 mg) or **2** (2 mg) with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride as described above yielded the (*R*)-Mosher esters **1r** and **2r**, respectively (^1H NMR data, Table 4).

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