Asitrocin, (2,4)-*cis*- and *trans*-Asitrocinones: Novel Bioactive Mono-tetrahydrofuran Acetogenins from *Asimina triloba* Seeds

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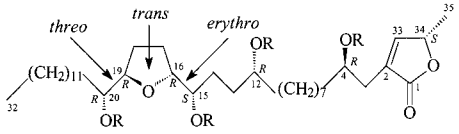
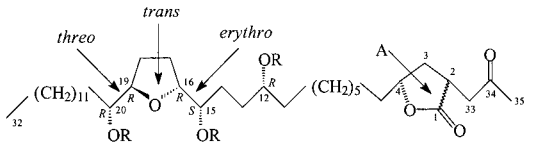
Asitrocin (**1**) and the mixture of (2,4)-*cis*- and *trans*-asitrocinones (**2** and **3**), new bioactive Annonaceous acetogenins, were isolated from the seeds of *Asimina triloba* by activity-directed fractionation using the brine shrimp lethality test. Asitrocin and the mixture of (2,4)-*cis*- and *trans*-asitrocinones have a configuration of *erythro*/*trans*/*threo* from C-15 to C-20, the mono-THF moiety with two flanking hydroxyl groups. The structures were determined by spectroscopic methods. These acetogenins showed potent bioactivities in the brine shrimp lethality test (BST) and among six human solid tumor cell lines with notable selectivity for the prostate (PC-3) and the pancreatic (MIA PaCa-2) cell lines at 10−100 times the potency of adriamycin.

The Annonaceous acetogenins are a well-established class of compounds with significant bioactivities. Their mechanism of action is via inhibition of NADH ubiquinone oxido-reductase (complex I) in mitochondrial electron transport systems and inhibition of NADH oxidase in the plasma membranes of tumor cells.[1] *Asimina triloba* (L.) Dunal (Annonaceae) is a native North American plant commonly named the paw paw tree.[2] Looking for natural antitumor compounds from the seeds of *Asimina triloba*, we have succeeded in the isolation and characterization of a series of acetogenins.[3−7] This paper includes three mono-THF acetogenins, asitrocin (**1**) and the mixture of (2,4)-*cis*- and *trans*-asitrocinones (**2** and **3**) (Chart 1). The absolute configurations of **1** and the mixture of **2** and **3** were determined through analyses of their respective per-Mosher esters. Compound **1** and the mixture of **2** and **3** showed significant cytotoxicities among human tumor cell lines with selectivities for the colon (HT-29), prostate (PC-3), and pancreatic (MIA PaCa-2) cell lines.

Chart 1



Compound	R
1	H
1a	TMSi
1r	(*R*)-MTPA
1s	(*S*)-MTPA



Compound	A	R	Compound	A	R
2	*cis*	H	**3**	*trans*	H
2a	*cis*	TMSi	**3a**	*trans*	TMSi
2r	*cis*	(*R*)-MTPA	**3r**	*trans*	(*R*)-MTPA
2s	*cis*	(*S*)-MTPA	**3s**	*trans*	(*S*)-MTPA

Results and Discussion

Compound **1**, $[\alpha]^{22}_D$ +5.2° (*c* 0.01, CH_2Cl_2), was obtained as a white powder. Its molecular weight was suggested by the mass peak $[M + Na]^+$ at *m*/*z* 619 in the FABMS. The HRFABMS gave *m*/*z* 619.4556 (calcd 619.4550) for the $[M + Na]^+$ ion corresponding to the molecular formula $C_{35}H_{64}O_7Na$. Compound **1** showed an IR carbonyl absorption at 1735 cm^{-1}, a UV (MeOH) λ_{max} at 215 nm (log ϵ, 3.43), proton resonances at δ 7.19 (q, H-33), 5.06 (dq, H-34), 1.44 (d, H-35), 2.40 (ddt, H-3a), 2.53 (dt, H-3b), and 3.83 (H-4), and carbon resonances at δ 174.61 (C-1), 151.84 (C-33), 131.14 (C-2), 77.98 (C-34), 19.10 (C-35), and 69.88 (C-4), all of which provided characteristic spectroscopic features for an α,β-unsaturated γ-lactone fragment with an OH-4 (Table 1).[8−10] The signals in the ^1H and ^{13}C NMR spectra of **1** at δ 3.59 and 71.75 are characteristic of a hydroxyl group in an alkyl chain.[11] The THF ring was located from C-16 to C-19 in the hydrocarbon chain on the basis of typical fragments observed in the EIMS spectrum of **1a** (Figure 1). Thus **1** was named asitrocin and is a new natural annonaceous acetogenin.

Compounds **2** and **3**, $[\alpha]^{23}_D$ +13.2° (*c* 0.005, CH_2Cl_2), were obtained in the mixture as a white powder. HRFABMS gave $[M + Na]^+$ ions at *m*/*z* 619.4546 (calcd 619.4550) corresponding to the formula $C_{35}H_{64}O_7Na$. Signals in the ^1H NMR of the mixture of **2** and **3** at δ 4.39 and 4.55 (see Experimental Section), with a combined integration for one proton, were assigned to H-4 and indicated the presence of a (2,4-*cis* and *trans*)-mixture at the ketolactone moiety, which is common for acetogenins of this type.[12,13] Resonances in the ^1H NMR of the mixture of **2** and **3** at δ 2.61 and 2.67 (H-33a), 3.10 and 3.05 (H-33b), and 2.20 (H-35) further substantiated this assignment. In the ^{13}C NMR of the mixture of **2** and **3**, signals at δ 205.61 and 205.57 (C-34), 178.27 and 178.87 (C-1), 35.40 and 33.27 (C-2), 79.29 and 78.84 (C-4), and 25.21 and 25.63 (C-35) (see

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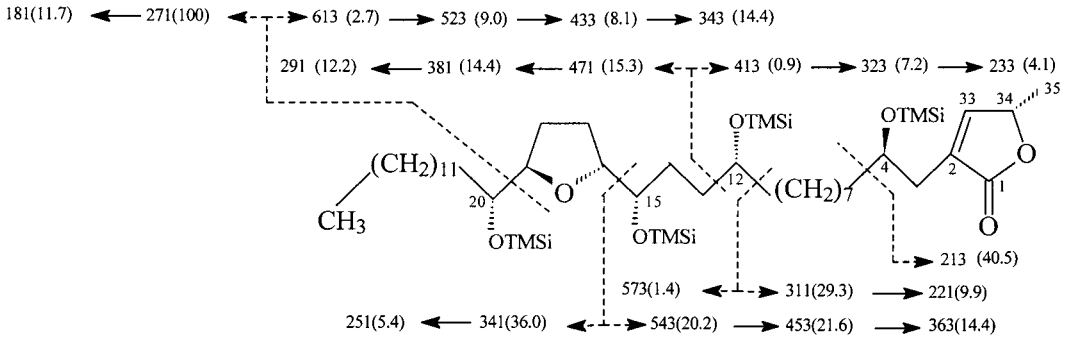

Figure 1. Diagnostic EIMS peaks for tetra-TMSi (**1a**) derivative (intensities are indicated in parentheses).

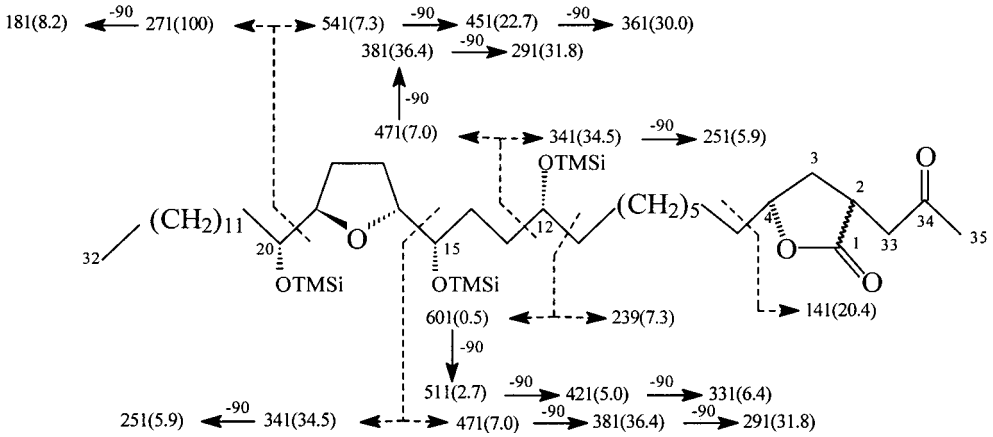

Figure 2. Diagnostic EIMS peaks for tri-TMSi (**2a** and **3a**) derivatives (intensities are indicated in parentheses).

Table 1. Characteristic ^{1}H NMR Data of Mosher Esters of **1** and the Mixture of **2** and **3** for Determinations of Stereochemistries

position	**1s** $\delta(S)$	**1r** $\delta(R)$	$\Delta\delta_{S-R}$	position	**2s** $\delta(S)$ **3s** $\delta(S)$	**2r** $\delta(R)$ **3r** $\delta(R)$	$\Delta\delta_{S-R}$
5	1.60	1.57	+0.03,				
	1.69	1.65	+ 0.04				
4	5.30	5.35	*R*	4	4.54	4.52	+0.02
3	2.56	2.60	−0.04		4.37	4.36	+0.01
	2.58	2.65	−0.07	5	1.70	1.68	+0.02
33	6.73	6.96	−0.23	12	5.07	5.02	*R*
34	4.85	4.89	−0.04	14	1.56	1.60	−0.04
35	1.27	1.30	−0.03	15	4.98	5.28	*S*
12	5.04	4.90	*R*	16	3.93	3.98	−0.05
14	1.55	1.59	−0.04	17	1.66	1.70	−0.04
15	4.97	5.27	*S*		1.71	1.84	−0.13
16	3.92	3.98	−0.06	18	1.47	1.47	+0.00
17	1.65	1.70	−0.05		1.88	1.86	+0.02
	1.70	1.84	−0.14	19	3.93	3.75	+0.18
18	1.47	1.47	+0.00	20	5.23	4.96	R
	1.88	1.86	+0.02	21	1.47	1.40	+0.07
19	3.92	3.74	+0.18				
20	5.22	4.95	*R*				
21	1.47	1.40	+0.07				

Experimental Section) also confirmed that the mixture of **2** and **3** is a *cis*/*trans* mixture of ketolactone isomers. The stereochemistry at C-4 was assumed as *R*, on the basis of spectral comparison, with (2,4)-*cis*- and *trans*-bullatacinone, which has known chirality,[13] and the fact that all 4-oxygenated acetogenins known to date are 4-*R*. Like **1**, the mixture of **2** and **3** has one THF ring with two flanking hydroxyls and an additional hydroxyl group three methylene units away. Thus, the ring and flanking hydroxyls in the mixture of **2** and **3** begin at C-15, and the additional hydroxyl group is at C-12 (Figure 2). The bis-hydroxyl-flanked mono-THF ring subunit, located from C-15 to C-20, was proposed to have *erythro*/*trans*/*threo* relative stereochemistry by applying Born's rule[14] and by comparing the ^{13}C MNR data of **1** and the mixture of **2** and **3** with those of Fujimoto's model compounds.[15] The location of the *threo* assignment should be at C-19/20 rather than at the C-15/16 junction because the δ value of the *threo* carbinol methine in the ^{1}H NMR spectra of **1** and the mixture of **2** and **3** was 3.40; if the reverse were true, this value would have been 3.44.[10] Compounds **2** and **3** are, thus, the ketolactones of **1** and were named (2,4)-*cis*- and *trans*-asitrocinones. Hui *et al.*[12] and Duret *et al.*[13] have demonstrated that these ketolactones are formed by translactonization to the OH-4 from α,β-unsaturated γ-lactones. This translactonization from asitrocin to (2,4)-*cis*- and *trans*-asitrocinones is possible during our extraction. However, the lesser quantity isolated of the *trans*-isomer (compound **3**) suggests that translactonization in the plant may be stereospecific and, thus, a natural process.

The absolute stereochemistries of the chiral centers in **1** were determined by preparing the tetra-*S* and tetra-*R* Mosher ester derivatives (**1r** and **1s**, respectively). Analysis of the differences between the (*S*)- and (*R*)-Mosher derivatives allowed the absolute stereochemical assignments of the carbinol centers as C-4*R*, C-15*S*, C-20*R*, and C-34*S*. The $\Delta\delta_{H}(S-R)$ values for H-33 and H-34 in **1r** and **1s**, at −0.23 and −0.04, suggested that **1** has the C-4*R*, C-34*S* configuration, as is usual.[16,17]

The absolute stereochemistry of the mixture of **2** and **3** was also established using advanced Mosher ester methodology.[16,17] The Mosher ester data (Table 1) allowed the absolute stereochemical assignment of the carbinol centers adjacent to the mono-THF ring in the mixture of **2** and **3** as C-15*S* and C-20*R*. Because the chemical shifts of H-11 and H-13 were virtually indistinguishable by ^{1}H NMR, the absolute stereochemistry of carbinol center at C-12 was not solvable by spectral analysis of the per-Mosher ester derivatives. The resulting per-Mosher esters **2r**, **2s**, **3r**, and **3s** indicated that the absolute configuration at C-12 was

Table 2. Brine Shrimp Lethality Test (BST) and Cytotoxicity against Six Human Solid Tumor Cell Lines for **1** and the Mixture of **2** and **3**

		human cancer cell line (ED_{50}, μg/mL)					
compound	BST[a] LC_{50}(μg/mL) (95% confidence interval)	A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	MIA PaCa-2[g]
1	6.3×10^{-2} (3.03×10^{-2} to 1.50×10^{-1})	1.18×10^{-1}	3.28	2.99×10^{-3}	7.92×10^{-2}	1.22×10^{-3}	1.48×10^{-5}
2 and **3**	6.5×10^{-2} (2.27×10^{-2} to 1.99×10^{-1})	6.05×10^{-2}	>10	2.33×10^{-2}	1.46×10^{-1}	5.88×10^{-3}	1.01×10^{-3}
adriamycin[h]	not tested	6.22×10^{-3}	9.53×10^{-1}	2.87×10^{-2}	2.86×10^{-3}	5.77×10^{-2}	6.10×10^{-3}

[a] Brine shrimp test.[20,21] [b] Lung carcinoma.[25] [c] Breast carcinoma.[26] [d] Colon adenocarcinoma.[23] [e] Kidney carcinoma.[25] [f] Prostate adenocarcinoma.[24] [g] Pancreatic carcinoma.[27] [h] Positive control standard.

R because positive values (+0.02 and +0.01) of $\Delta\delta_H(S-R)$ for H-4 were observed.[18,19]

The biological activities of **1** and the mixture of **2** and **3** are summarized in Table 2. These compounds were quite active in the BST.[20,21] Compound **1** and the mixture of **2** and **3** also showed significant and selective cytotoxicities among the six human tumor cell lines in our 7-day MTT human solid tumor panel. Cytotoxic selectivities in **1** and the mixture of **2** and **3**, e.g., with activities against the prostate (PC-3) and the pancreatic (MIA PaCa-2) cell lines of 10−100 times that of adriamycin, were exhibited. Compound **1**, with a γ-lactone moiety, showed more active cytotoxicity than the mixture of **2** and **3**, having ketolactone, as is usual.[22] The mechanism of action of the acetogenins has been determined; they are powerful inhibitors of complex I in mitochondrial electron transport systems.[28,29]

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micro melting point apparatus and were uncorrected. Optical rotations were taken on a JASCO DIP-370 digital polarimeter. IR spectra were measured on a JASCO FT/IR 300E spectrophotometer. UV spectra were obtained on a Shimadzu UV-1601PC spectrophotometer. ^{1}H, ^{13}C, and COSY NMR spectra were recorded on a Varian VXR500S spectrometer in $CDCl_3$ using TMS as an internal standard. Low- and high-resolution FABMS data were collected on a JEOL JMS-HX110 spectrometer. EIMS was recorded on a Quattro II spectrometer. For TLC, silica gel 60 F-254 (EM 5717) glass plates (0.25 mm) were used and visualized by spraying with 5% phosphomolybdic acid in MeOH and heating. HPLC was performed on a Waters 600 apparatus equipped with a Waters 486 UV detector at 225 nm using the Autochrowin software system (Young Su Scientific Co., Seoul, Korea). A μ-Bondapak C_{18} column (19 × 300 mm and 7.8 × 300 mm) was used for preparative purpose.

Plant Material. The seeds of *Asimina triloba* were collected in the fall of 1993, from plantations of paw paw tree grown at the University of Maryland and were purchased from the Paw Paw Foundation. The identification was confirmed by R. Neal Peterson. A voucher specimen of the seeds is preserved at the Department of Pharmacy, Catholic University of Ðaegu, Korea.

Bioassays. The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST). Seven-day *in vitro* MTT cytotoxicity tests against human tumor cell lines were carried out at the Cell Culture Laboratory, Purdue Cancer Center, using standard protocols for A-549 (human lung carcinoma),[25] MCF-7 (human breast carcinoma),[26] HT-29 (human colon adenocarcinoma),[23] A-498 (human kidney carcinoma),[25] PC-3 (human prostate adenocarcinoma),[24] and MIA PaCa-2 (human pancreatic carcinoma)[27] with adriamycin as a positive control.

Extraction and Isolation. Steps for extraction and chromatographic fractionation were identical to those reported previously.[4] The BST active fractions F (BST, $LC_{50} = 1.31 \times 10^{-1}$ μg/mL) and H (BST, $LC_{50} = 4.20 \times 10^{-3}$ μg/mL) were further subjected to repeated open column chromatography and HPLC to yield pure compounds **1** and the mixture of **2** and **3**.

Asitrocin (1): white powder (10 mg); mp 66.5−67.2 °C; $[\alpha]^{22}_D$ +5.2° (*c* 0.01, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 215 (3.4) nm; IR (film) ν_{max} 3421, 2920, 2850, 1735, 1466, 1375, 1321, 1074 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.19 (1H, q, J = 1.5 Hz, H-33), 5.06 (1H, dq, J = 6.8, 1.5 Hz, H-34), 3.84 (2H, m, H-15, H-16), 3.83 (2H, m, H-4, H-19), 3.59 (1H, m, H-12), 3.40 (1H, m, H-20), 2.53 (1H, dt, J = 15.0, 1.5 Hz, H-3a), 2.40 (1H, ddt, J = 15.0, 8.5, 1.5 Hz, H-3b), 2.00 (1H, m, H-18a), 1.90 (1H, m, H-17a), 1.86 (1H, m, H-17b), 1.63 (1H, m, H-18b), 1.44 (1H, d, J = 7.0 Hz, H-35), 0.88 (1H, t, J = 7.0, H-32); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.61 (s, C-1), 151.84 (d, C-33), 131.14 (s, C-2), 83.20 (d, C-19), 82.17 (d, C-16), 77.98 (d, C-34), 74.27 (d, C-20), 71.75 (d, C-12), 71.52 (d, C-15), 69.88 (d, C-4), 33.37 (t, C-3), 28.58 (t, C-18), 25.22 (t, C-17), 19.10 (q, C-35), 14.11 (q, C-32); FABMS *m/z* 619 $[M + Na]^+$, 601 $[MNa - H_2O]^+$, 583 $[MNa - 2H_2O]^+$, 565 $[MNa - 3H_2O]^+$, 547 $[MNa - 4H_2O]^+$; HRFABMS *m/z* $[M + Na]^+$ 619.4556 for $C_{37}H_{68}O_7Na$ (calcd 619.4550).

Asitrocin Tetra-TMSi Derivative (1a). Approximately 10 μg of compound **1** was treated with 0.2 μL of pyridine and 2 μL of *N*,*O*-bis(trimethylsilyl)acetamide for 5 h to give a **1a**: EIMS *m/z*, see Figure 1.

(2,4)-*cis*-Asitrocinone (2): white powder (10 mg); mp 69.2−69.9°; $[\alpha]^{22}_D$ +13.2° (*c* 0.005, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 205.40 (3.6) nm; IR (film) ν_{max} 3421, 2922, 2852, 1751, 1716, 1466, 1361, 1319, 1074 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.39 (1H, dddd, J = 10.9, 7.4, 5.6, 5.4 Hz, H-4), 3.87 (1H, m, H-16), 3.83 (2H, m, H-15, H-19), 3.59 (1H, m, H-12), 3.40 (1H, m, H-20), 3.10 (1H, dd, J = 18.5, 3.5 Hz, H-33a), 3.03 (1H, m, H-2), 2.61 (1H, dd, J = 18.5, 9.0 Hz, H-33b), 2.60 (1H, m, H-3a), 2.20 (1H, s, H-35), 2.00 (1H, m, H-18a), 1.90 (1H, m, H-17a), 1.86 (1H, m, H-17b), 1.76 (1H, m, H-5a), 1.63 (1H, m, H-18b), 1.60 (1H, m, H-5b), 1.48 (1H, m, 3b), 0.88 (1H, t, J = 7.0 Hz, H-32); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 205.61 (s, C-34), 178.27 (s, C-1), 83.17 (d, C-19), 82.15 (d, C-16), 79.29 (d, C-4), 74.26 (d, C-20), 71.78 (d, C-12), 71.54 (d, C-15), 43.79 (t, C-33), 35.40 (d, C-2), 28.57 (t, C-18), 25.22 (t, C-17), 25.21 (q, C-35), 14.10 (q, C-32); FABMS *m/z* 619 $[M + Na]^+$, 601 $[MNa - H_2O]^+$, 583 $[MNa - 2H_2O]^+$, 565 $[MH - 3H_2O]^+$; HRFABMS *m/z* $[M + Na]^+$ 619.4546 for $C_{35}H_{64}O_7Na$ (calcd 619.4550).

(2,4)-*trans*-Asitrocinone (3): white powder (10 mg): mp 69.2−69.9°; $[\alpha]^{22}_D$ +13.2° (*c* 0.005, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 205.40 (3.6) nm; IR (film) ν_{max} 3421, 2922, 2852, 1751, 1716, 1466, 1361, 1319, 1074 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.55 (1H, dddd, J = 8.3, 8.2, 5.7, 3.2 Hz, H-4), 3.87 (1H, m, H-16), 3.83 (2H, m, H-15, H-19), 3.59 (1H, m, H-12), 3.40 (1H, m, H-20), 3.05 (1H, dd, J = 9.3, 3.0 Hz, H-33a), 3.02 (1H, m, H-2), 2.67 (1H, dd, J = 18.5, 9.0 Hz, H-33b), 2.22 (1H, ddd, J = 12.9, 9.6, 3.4, H-3a), 2.20 (1H, s, H-35), 2.00 (1H, m, H-18a), 1.99 (1H, m, 3b) 1.90 (1H, m, H-17a), 1.86 (1H, m, H-17b), 1.63 (1H, m, H-18b), 1.56 (1H, m, H-5a), 1.48 (1H, m, H-5b), 0.88 (1H, t, J = 7.0 Hz, H-32); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 205.57 (s, C-34), 178.87 (s, C-1), 83.17 (d, C-19), 82.15 (d, C-16), 78.84 (d, C-4), 74.26 (d, C-20), 71.78 (d, C-12), 71.54 (d, C-15),

44.24 (t, C-33), 33.27 (d, C-2), 28.57 (t, C-18), 25.63 (q, C-35), 25.22 (t, C-17), 14.10 (q, C-32); FABMS *m/z* 619 $[M + Na]^+$, 601 $[MNa - H_2O]^+$, 583 $[MNa - 2H_2O]^+$, 565 $[MH - 3H_2O]^+$; HRFABMS *m/z* $[M + Na]^+$ 619.4546 for $C_{35}H_{64}O_7Na$ (calcd 619.4550).

(2,4)-*cis*- and *trans*-Asitrocinones Tri-TMSi Derivatives (2a and 3a). Approximately 10 μg of the mixture of **2** and **3** was treated with 0.2 μL of pyridine and 2 μL of *N*,*O*-bis(trimethylsilyl)acetamide for 5 h to give the mixture of **2a** and **3a**: EIMS, see Figure 2.

Preparation of Mosher Esters. A previously described method was used.[3] To each of 1 mg of **1** and the mixture of **2** and **3** in 0.5 mL of CH_2Cl_2 were added sequentially 0.2 mL of pyridine, 0.5 mg of 4-(dimethylamino)pyridine, and 12 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride, separately. The mixture was left at room temperature overnight and purified over a micro-column (0.6 × 6 cm) of silica gel (230−400 mesh) eluted with 3−4 mL of hexane−CH_2Cl_2 (1:2). The eluate was dried, CH_2Cl_2 (5 mL) was added, and the CH_2Cl_2 was washed using 1% $NaHCO_3$ (5 mL × 3) and H_2O (5 mL × 2). The washed eluate was dried in vacuo to give the *S* Mosher esters of **1** and the mixture of **2** and **3**, respectively. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride afforded the *R* Mosher esters. Their pertinent ^{1}H NMR chemical shifts are given in Table 1.

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