

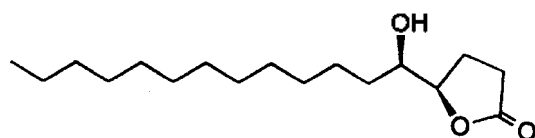
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## Synthesis and Cytotoxicity of (-)-(4*R*,5*R*)-5-*C*-(11-Methoxy)muricatacin

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Muricatacin (1), an acetogenin derivative, which is isolated from the seeds of the tropical fruit *Annona muricata* L., has received a great deal of attention because it shows some cytotoxicities against human tumor cell lines and its congeners show a wide range of biological activities.<sup>1</sup> The natural muricatacin is comprised of (-)-(4*R*,5*R*)-5-hydroxyheptadeca-4-nolide and its (+)-(4*S*,5*S*) enantiomer, with the former predominating. (+)-Muricatacin and/or (-)-muricatacin was recently synthesized from various starting materials.<sup>2</sup>



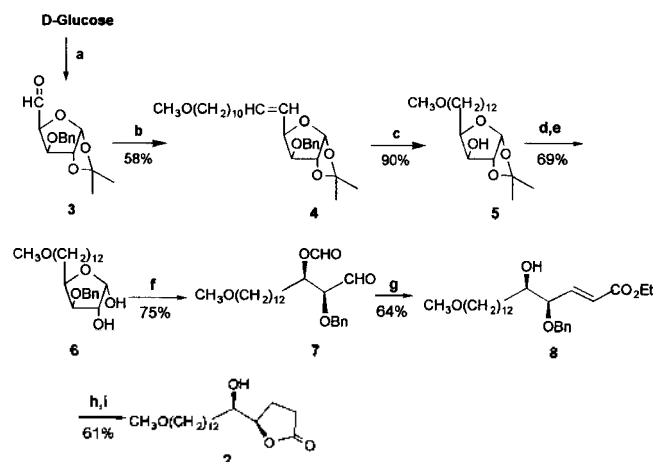
(-)-(4*R*,5*R*)-Muricatacin (1)

In connection with our projects to understand structure-activity relationship (SAR) of acetogenin derivatives, we recently reported that the stereochemistry at C<sub>4</sub> and C<sub>5</sub> position of muricatacin did not significantly affect the cytotoxicities.<sup>3</sup> In the continuous effort to obtain SAR, we were interested in evaluating the effect of the long alkyl chain in muricatacin on cytotoxicity. Thus, we substituted the hydrophobic methyl group in the long alkyl chain with the more hydrophilic methoxy group. Here, we report a stereocontrolled synthesis of pure (-)-(4*R*,5*R*)-5-*C*-(11-methoxy) muricatacin (2) from D-glucose, as well as its cytotoxicity.

### Results and Discussion

**Synthesis.** According to Scheme 1, the synthesis of (-)-(4*R*,5*R*)-5-*C*-(11-methoxy)muricatacin (2), (or (-)-(4*R*,5*R*)-5-(11-methoxy)-hydroxy-4-heptadecanolide) was started from 3-*O*-benzyl-1,2-*O*-isopropylidene- $\alpha$ -D-xylo-pentoaldo-1,4-

furanose (3) which was easily prepared from D-glucose in four steps.<sup>4-7</sup> Wittig reaction of 3 with *n*-BuLi and 11-methoxy-undecanyltriphenylphosphonium bromide (3a) gave a 3-*O*-benzyl-5,6-dideoxy-5-*C*-(11-methoxy)-undecyl-1,2-isopropylidene- $\alpha$ -D-xylo-dode-5-cenofuranose (4), which was hydrogenated in the presence of 10% Pd-C under 1 atm of hydrogen to give a 5,6-dideoxy-1,2-isopropylidene-5-*C*-(11-methoxy-*n*-undecanyl)- $\alpha$ -D-glucofuranose (5). Monoprotection of the hydroxy group at 3-position in 5 with benzyl chloride in tetrahydrofuran (THF) using NaH as a base followed by removal of the isopropylidene group with 9.6 N HCl afforded 1,2-diol compound 6. After (2*S*,3*R*)-*O*-protected 2,3-dihydroxy aldehyde 7 was obtained from the oxidative cleavage of 6 with sodium periodate, it was



**Scheme 1.** a) See reference 8; b) 9.6 N *n*-BuLi, 11-methoxy-undecanyl-triphenylphosphonium bromide (3a), THF, -78 °C - rt; c) H<sub>2</sub>, Pd-C, EtOAc, d) NaH, BnCl, THF, rt, 5h; e) 9.6 N HCl/TFA, DME, rt, 48 h; f) NaIO<sub>4</sub>, MeOH, rt, 1 h; g) NaH, (EtO)<sub>2</sub>-POCH<sub>2</sub>CO<sub>2</sub>Et, THF, rt, 3 h; h) H<sub>2</sub>, Pd-C, EtOAc, rt, 24 h; i) TFA-H<sub>2</sub>O (4:1), rt, 3 h.

**Table 1.** *In vitro* inhibition of A-549 and MCF-7 cell

Compound	IC <sub>50</sub> (μg/ml)	
	A-549	MCF-7
(-)-Muricatacin	18.5	17.6
<b>2</b>	23.6	22.0

reacted with the anion of triethylphosphonoacetate to give (E)-unsaturated ester **8** in 64% yield. Finally, hydrogenation of **8** in the presence of 10% Pd-C under 1 atm of hydrogen followed by ring cyclization with treatment of trifluoroacetic acid afforded (-)-(4*R*,5*R*)-5-*C*-(11-methoxy)muricatacin (**2**) in 61% yield.<sup>8</sup>

**Cytotoxicity.** The cytotoxicities of **2** and muricatacin were tested against *in vitro* A-549 cell line as well as MCF-7 cell line by measuring the inhibition of cell growth.<sup>9</sup> As shown in Table 1, the methoxy compound **2** exhibited weaker cytotoxicities in both cells than parent compound.

### Experimental

**Instruments.** Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were determined at a sodium D line using a JASCO 370-DIP polarimeter and measured in chloroform. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on a Varian Gemini 200 spectrometer at 200 MHz and 50 MHz. Chemical shifts were given in relative tetramethylsilane. Infrared spectra were recorded on a Nicolet FT-IR 550 spectrometer. Flash column chromatography was done by using Merck silica gel 60 (15-40 μm).

**Preparation of 11-methoxyundecanyltriphenylphosphonium bromide (3a).** To a suspension of NaH (1.25 g, 95% powder, 52.1 mmol) in anhydrous THF (20 mL) was added 11-hydroxyundecanyl bromide (10.0 g, 39.8 mmol) in anhydrous THF (100 mL) under a nitrogen atmosphere. After the mixture was stirred for 30 min at room temperature, methyl iodide (7.4 g, 52.4 mmol) was added. The reaction mixture was stirred for 12 h at room temperature and then quenched with saturated aqueous NH<sub>4</sub>Cl solution (50 mL). The organic solvent was removed and the aqueous solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL × 3). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude product was chromatographed on a silica gel column (hexane:ethyl acetate = 1:1) to give 11-methoxyundecanyl bromide (10 g, 95%) as a slightly yellow oil. IR (neat) 2950, 2890, 1450, 1380 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.18-1.49 (s, 18H, -(CH<sub>2</sub>)<sub>8</sub>-), 1.58 (m, 4H, -2CH<sub>2</sub>-), 1.85 (s, 2H, -CH<sub>2</sub>-), 3.33 (s, 3H, -OCH<sub>3</sub>), 3.34 (t, 2H, *J* = 7.6 Hz, -OCH<sub>2</sub>), 3.62 (m, 2H, -CH<sub>2</sub>-), 7.78 (m, 15H, 3*x*-phenyl).

**Preparation of 3-*O*-benzyl-1,2-*O*-isopropylidene- $\alpha$ -D-xylo-pentaoido-1,4-furanose (3).** This was prepared from D-glucose according to the procedure reported by S-K Kang *et al.*<sup>8</sup> Slightly yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.33 (s, 3H, acetonide), 1.61 (s, 3H, acetonide), 4.15 (m, 1H, C<sub>4</sub>-H), 4.42 (m, 2H, C<sub>2</sub>-H, C<sub>3</sub>-H), 4.48-4.76 (dd, 2H, -OCH<sub>2</sub>), 5.91 (d, 1H, *J* = 3.9 Hz, C<sub>1</sub>-H), 6.13 (d, 1H, *J* = 3.9 Hz, C<sub>1</sub>-H), 7.34 (m, 5H, -phenyl), 9.67 (d, 1H, *J* = 1.5 Hz, -CHO).

**Preparation of 3-*O*-benzyl-5,6-dideoxy-5-*C*-(11-methoxy)-undecyl-1,2-isopropylidene- $\alpha$ -D-xylo-dode-**

**5-cenofuranose (4).** To a solution of 11-methoxyundecanyltriphenylphosphonium bromide **3a** (7.16 g, 15.0 mmol) in anhydrous THF (100 mL) was carefully added 9.6 N *n*-BuLi (10 mL, 16 mmol) under a nitrogen atmosphere at -78 °C. After the mixture was stirred for 40 min at -78 °C, 3-*O*-benzyl-1,2-*O*-isopropylidene- $\alpha$ -D-xylo-pentaoido-1,4-furanose (2.8 g, 10.1 mmol) in anhydrous THF (10 mL) was added. The reaction mixture was stirred for 24 h at room temperature and then quenched with saturated aqueous NH<sub>4</sub>Cl solution (50 mL). The organic solvent was removed and the aqueous solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL × 3). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude product was chromatographed on a silica gel column (hexane:ethyl acetate = 1:1) to give **4** (2.6 g, 58%) as a slightly yellow oil. IR (neat) 3040, 3020, 2970, 2890 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.25 (s, 16H, -(CH<sub>2</sub>)<sub>8</sub>-), 1.32 (s, 3H, acetonide), 1.63 (s, 3H, acetonide), 2.11 (m, 2H, -CH<sub>2</sub>-), 3.31 (s, 3H, -OCH<sub>3</sub>), 3.31 (t, 2H, *J* = 7.6 Hz, -OCH<sub>2</sub>), 3.81 (d, 1H, *J* = 3.0 Hz, C<sub>3</sub>-H), 4.51-4.78 (m, 3H, -OCH<sub>2</sub> & C<sub>2</sub>-H), 4.96 (m, 1H, C<sub>4</sub>-H), 5.68 (m, 2H, vinyl-H), 5.95 (d, 1H, *J* = 3.7 Hz, C<sub>1</sub>-H), 7.33 (m, 5H, -phenyl).

**Preparation of 5,6-dideoxy-1,2-isopropylidene-5-*C*-(11-methoxy-*n*-decanyl)- $\alpha$ -D-glucofuranose (5).** In the presence of 10% Pd-C (500 mg), a solution of **4** (2.1 g, 4.5 mmol) in ethyl acetate (150 mL) was hydrogenated under an atmosphere of hydrogen for 24 h at room temperature. After the catalyst was filtered, the filtrate was concentrated to give **5** (1.9 g, 90%) as a white solid. mp 60-60.5 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -13.30 (c 5.0, CHCl<sub>3</sub>); IR (KBr) 3410, 2980, 2920, 2890, 1380 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.25 (m, 20H, -(CH<sub>2</sub>)<sub>10</sub>-), 1.33 (s, 3H, acetonide), 1.62 (s, 3H, acetonide), 1.65 (m, 2H, -CH<sub>2</sub>-), 3.31 (s, 3H, -OCH<sub>3</sub>), 3.35 (t, 2H, *J* = 7.7 Hz, -OCH<sub>2</sub>), 4.08 (m, 2H, C<sub>3</sub>-H & C<sub>4</sub>-H), 4.52 (d, 1H, *J* = 4.0 Hz, C<sub>2</sub>-H), 5.91 (d, 1H, *J* = 3.9 Hz, C<sub>1</sub>-H).

**Preparation of 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-dihydroxy-5-*C*-(11-methoxy-*n*-decanyl)- $\alpha$ -D-glucofuranose (6).** To a suspension of NaH (450 mg of 95% powder, 18.9 mmol) in anhydrous DMSO (20 mL) was added **5** (3.4 g, 9.9 mmol) in anhydrous THF (100 mL) under a nitrogen atmosphere. After the mixture was stirred for 30 min at room temperature, benzyl chloride (1.6 g, 12.6 mmol) was added. The reaction mixture was stirred for 5 h at room temperature and then quenched with saturated aqueous NH<sub>4</sub>Cl solution (50 mL). The organic solvent was removed and the aqueous solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL × 3). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude product was chromatographed on a silica gel column (hexane:ethyl acetate = 1:1) to give 3-*O*-benzyl-5,6-dideoxy-1,2-isopropylidene-5-*C*-(11-methoxy-*n*-decanyl)-D-glucofuranose (4.0 g, 96%) as slightly yellow oil. IR (neat) 3040, 3020, 2940, 2890 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.25 (s, 20H, -(CH<sub>2</sub>)<sub>10</sub>-), 1.32 (s, 3H, acetonide), 1.63 (s, 3H, acetonide), 1.72 (m, 2H, -CH<sub>2</sub>-), 3.31 (s, 3H, -OCH<sub>3</sub>), 3.31 (t, 2H, *J* = 7.6 Hz, -OCH<sub>2</sub>), 3.78 (d, 1H, *J* = 2.9 Hz, C<sub>3</sub>-H), 4.11 (m, 1H, C<sub>4</sub>-H), 4.45-4.78 (m, 3H, -OCH<sub>2</sub> & C<sub>2</sub>-H), 5.91 (d, 1H, *J* = 3.9 Hz, C<sub>1</sub>-H), 7.33 (m, 5H, -phenyl). To a solution of 3-*O*-benzylated compound (3.5 g, 8.4 mmol) in DME (15 mL) was slowly added dropwise 9.6 N HCl (5 mL). The reaction mixture was stirred for 48 h at room temperature and then neutralized with saturated aqueous NaHCO<sub>3</sub> solution. After

the evaporation of DME, the aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  (30 mL $\times$ 3). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The residue was chromatographed on a silica gel column (hexane:ethyl acetate = 1:2) to give **6** (2.3 g, 69%) as a yellow oil. IR (neat) 3490, 3090, 2850, 1100  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.27 (s, 20H,  $-(\text{CH}_2)_{10}-$ ), 1.61 (m, 2H,  $-\text{CH}_2-$ ), 3.31 (s, 3H,  $-\text{OCH}_3$ ), 3.35 (t, 2H,  $J=7.6$  Hz,  $-\text{OCH}_2$ ), 3.86 (m, 1H,  $\text{C}_7\text{-H}$ ), 4.18 (m, 1H,  $\text{C}_4\text{-H}$ ), 4.48-4.76 (m, 3H,  $-\text{OCH}_2$  &  $\text{C}_2\text{-H}$ ), 5.48 (d, 1H,  $J=3.9$  Hz,  $\text{C}_1\text{-H}$ ), 7.34 (m, 5H, -phenyl).

**Preparation of (2S,3R)-2-benzyloxy-3-formyloxy-5-C-(11-methoxy)-1-pentadecanal (7).** To a solution of **6** (2.1 g, 5.3 mmol) in MeOH (160 mL) was added 0.6 N  $\text{NaIO}_4$  solution (200 mL). The reaction mixture was stirred for 1 h at room temperature and then concentrated. After the reaction mixture was diluted with  $\text{H}_2\text{O}$  (30 mL), it was extracted with  $\text{CH}_2\text{Cl}_2$  (30 mL $\times$ 3). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The residue was chromatographed on a silica gel column (hexane:ethyl acetate = 1:2) to give **5** (1.6 g, 75%) as a slightly yellow oil:  $[\alpha]_D^{20}$  1.16 (c 9.5,  $\text{CHCl}_3$ ); IR (neat) 3080, 2935, 2850, 1737, 1713, 1173  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.29 (s, 18H,  $-(\text{CH}_2)_8-$ ), 1.65-1.78 (m, 4H,  $-(\text{CH}_2)_2-$ ), 3.31 (s, 3H,  $-\text{OCH}_3$ ), 3.34 (t, 2H,  $J=7.6$  Hz,  $-\text{OCH}_2$ ), 3.81 (d, 1H,  $J=3.1$  Hz,  $\text{C}_2\text{-H}$ ), 4.586-4.86 (dd, 2H,  $J=11.8, 49.6$  Hz,  $-\text{OCH}_2$ ), 5.28 (m, 1H,  $\text{C}_7\text{-H}$ ), 7.34 (s, 5H, -phenyl), 8.04 (s, 1H,  $-\text{OCHO}$ ), 9.66 (s, 1H,  $-\text{CHO}$ ).

**Preparation of ethyl (4R,5R)-4-benzyloxy-5-C-(11-methoxy)-hydroxy-(2E)-heptadecanoate (8).** To a suspension of NaH (48 mg of 95% powder, 2.0 mmol) in anhydrous THF (10 mL) was slowly added a solution of triethyl phosphonoacetate (0.3 mL, 1.5 mmol) in anhydrous THF (5 mL). The reaction mixture was stirred for 30 min at 0  $^\circ\text{C}$ , and compound **6** (500 mg, 1.3 mmol) in THF (10 mL) was slowly added to this mixture. The reaction mixture was stirred for 3 h at room temperature under a nitrogen atmosphere and then quenched with  $\text{NH}_4\text{Cl}$  solution (30 mL). After the organic solvent was removed, the aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  (50 mL $\times$ 3). The combined organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The residue was chromatographed on a silica gel column (hexane:ethyl acetate = 1:1) to give **8** (350 mg, 64%) as a slightly yellow oil. IR (neat) 3440 ( $-\text{OH}$ ), 3040, 2930, 2860, 1730 ( $\text{C}=\text{O}$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.27 (s, 20H,  $-(\text{CH}_2)_{10}-$ ), 1.32 (t, 3H,  $-\text{CH}_3$ ), 1.58 (m, 2H,  $-\text{CH}_2-$ ), 2.57 (d, 1H,  $J=3.1$  Hz,  $-\text{OH}$ ), 3.31 (s, 3H,  $-\text{OCH}_3$ ), 3.37 (t, 2H,  $J=7.6$  Hz,  $-\text{OCH}_2$ ), 3.57 (m, 1H,  $\text{C}_5\text{-H}$ ), 3.81 (m, 1H,  $\text{C}_4\text{-H}$ ), 4.23 (q, 2H,  $J=4.8$  Hz,  $-\text{CO}_2\text{CH}_2-$ ), 4.52-4.79 (dd, 2H,  $J=11.8, 49.9$  Hz,  $-\text{OCH}_2$ ), 6.09 (d, 1H,  $J=14.6$  Hz,  $\text{C}_2\text{-H}$ ), 6.85 (dd, 1H,  $J=14.6, 6.7$  Hz,  $\text{C}_3\text{-H}$ ), 7.33 (s, 5H, -phenyl).

**Preparation of (-)-(4R,5R)-5-C-(11-methoxy)-hydroxy-heptadeca-4-nolide (2).** In the presence of 10% Pd-C (300 mg), a solution of **8** (320 mg, 0.71 mmol) in ethyl acetate (50 mL) was hydrogenated under an atmosphere of hydrogen for 24 h at room temperature. The catalyst was filtered and the filtrate was concentrated to give the corresponding saturated ester as a white solid. The ester was dissolved in  $\text{H}_2\text{O}$ -trifluoroacetic acid (4:1, 50 mL) and the resulting solution was stirred for 3 h at room temperature. After the solution was neutralized with saturated aqueous  $\text{NaHCO}_3$  solution, the neutralized aqueous

layer was extracted with chloroform (30 mL $\times$ 3). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The residue was chromatographed on a silica gel column (hexane:ethyl acetate = 1:2) to give the product, which was recrystallized from hexane-ethyl acetate (10:1) to give **2** (500 mg, 61%) as a white solid: mp 46-47  $^\circ\text{C}$ ,  $[\alpha]_D^{20} -10.5$  (c 10.3,  $\text{CHCl}_3$ ); IR (KBr) 3450 ( $-\text{OH}$ ), 2950, 2850, 1767 ( $\text{C}=\text{O}$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.25 (s, 20H,  $-(\text{CH}_2)_{10}-$ ), 1.58 (m, 2H,  $-\text{CH}_2-$ ), 2.36 (m, 2H,  $\text{C}_3\text{-H}$ ), 2.55 (m, 2H,  $\text{C}_2\text{-H}$ ), 3.31 (s, 3H,  $-\text{OCH}_3$ ), 3.38 (t, 2H,  $J=7.6$  Hz,  $-\text{OCH}_2$ ), 3.63 (m, 1H,  $\text{C}_5\text{-H}$ ), 4.45 (m, 1H,  $\text{C}_4\text{-H}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.4, 25.2, 26.07, 28.6, 28.9, 29.3, 32.4, 35.5, 58.4 ( $-\text{OCH}_3$ ), 72.9 ( $-\text{OCH}_2-$ ), 73.6 ( $\text{C}_3$ ), 81.0 ( $\text{C}_4$ ), 178.8 ( $\text{C}_1$ ).

**Cytotoxicity assay.** Cytotoxicities (SRB assay) against *in vitro* A-549 and MCF-7 cell lines were determined at the Choong-Wae Pharmaceutical Company, LTD., Korea, according to the procedure described by P. Skehan *et al.*<sup>9</sup>

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## ERRATUM

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The previous table 2 on the page 502 is corrected as shown below.

**Table 2.** Product Distribution (%) for Photoreaction of **1** in Zeolites X and Y<sup>a</sup>

medium	from intermediate A		from intermediate B			2+3:4+5+6
	2	3	4	5	6	
LiX	9.8	17.6	51.7	4.6	16.3	27.4:72.6
NaX	15.6	14.6	49.6	3.1	17.1	30.2:69.8
KX	15.9	21.3	46.1	3.2	13.5	37.2:62.8
RbX	17.8	29.4	40.3	2.6	9.9	47.2:52.8
CsX	13.3	45.8	28.1	2.1	10.7	59.1:40.9
LiY	9.0	18.3	54.2	12.3	6.2	27.3:72.7
NaY	13.2	11.3	57.1	7.0	11.4	24.5:75.5
KY	17.1	21.1	47.4	3.2	11.2	38.2:61.8
RbY	17.3	26.3	38.8	3.0	14.6	43.6:56.4
CsY	16.5	37.4	32.5	-	13.6	53.9:46.1

<sup>a</sup> Numbers reported are the average of at least two measurements. Error limit of the analysis is  $\pm 3\%$ .