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# HETEROCYCLES, Vol. , No. , , pp. -. © The Japan Institute of Heterocyclic Chemistry Received, , Accepted, , Published online, . DOI: 10.3987/COM- (Please do not delete.) SYNTHESIS OF PHOTOREACTIVE DIAZIRINYL SALICIN DERIVATIVE TO ELUCIDATE FUNCTIONAL ANALYSIS OF THE BITTER TASTE RECEPTOR

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Abstract – Salicin (salicyl alcohol glucoside) is a substance well known for its bitter taste. A photoreactive diazirinyl derivative of salicin will be utilized for the functional analysis of interactions between the bitter taste receptor and salicin. Glucosides of salicyl derivatives are more difficult than phenol derivatives that are unsubstituted at the ortho-position. A diazirinyl salicin derivative was synthesized at moderate vields glucosidation by of 2,3,4,6-tetra-O-acetyl- $\alpha$ -D-glucopyranosyl bromide and 2-hydroxy-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]-benzaldehyde in the presence of a phase-transfer catalyst, nBuEt<sub>3</sub>NBr, followed by reduction and deprotection.

Salicin is long known as one of the major components of phenyl glycosides in the family *Salicaceae*<sup>1</sup> and is a bitter anti-inflammatory compound.<sup>2</sup> Elucidation of the functions of its gustatory receptor on the basis of structure–activity relationships may reveal the mechanism of homeostatic functions, which is of great interest to scientists. Photoaffinity labeling is one of the most common techniques used in the analysis of chemical biology.<sup>3</sup> To the best of our knowledge, there are no reports on the preparation of photoreactive salicin derivatives for the analysis of bitter taste receptors. Two modifications of phenyl glucoside derivatives using diazirine have been reported. The derivatives are not substituted at the o-position of phenol. One modification is glucosidation of *p*-diazirinyl phenol to synthesize  $\alpha$ -glucoside<sup>4</sup> and the other

This paper is dedicated to Prof. Dr. Isao Kuwajima on the occasion of his 77th birthday.

is construction of the diazirine moiety in the precursor of the phenyl glucoside derivative.<sup>5</sup> However, glucosides of salicyl alcohol derivatives are more complicated than glucosides of the o-position unsubstituted phenol derivatives.<sup>6</sup> Optimization of glucosidation of salicyl alcohol or salicyl aldehyde derivatives is essential to the synthesis of target diazirinyl derivatives of salicin. In this study, we report the synthesis of diazirinyl derivative and preliminary screening of glucosidations for salicyl alcohol and salicyl aldehyde derivatives.



Scheme 1. Retrosynthesis of diazirinyl salicin derivative (1)

We conducted retrosynthesis of the diazirinyl salicin derivative **1**. Salicyl alcohol derivatives (**4** and **5**) were subjected to glucosidation with a donor derivative (**2** or **3**), as shown in route a in Scheme 1. Although route a seems to be straightforward construction of the salicin skeleton, few studies have described glucosidations of salicyl alcohol derivatives.<sup>7</sup> Another route involves glucosidation with salicyl aldehyde derivatives (**7**) followed by reduction of the aldehyde group to primary alcohol (Scheme 1, route b). Although this route is an indirect method for constructing the target skeleton, others investigators used it in the glucosidation of salicyl aldehyde due to more effective glucoside formation than using salicyl alcohol derivatives.<sup>6</sup> But comprehensive comparison of glucosidation for salicyl alcohol and salicyl aldehyde derivatives were screened as model reactions to synthesize the diazirinyl salicin derivative.



Scheme 2. Glucosidation of  $\beta$ -pentaacetylglucose 2 with salicyl derivatives 8–10.



Scheme 3. Glucosidation of  $\alpha$ -bromotetraacetylglucose (3) with salicyl derivatives 8–10.

Glucosidation of 1,2,3,4,6-penta-O-acetyl- $\beta$ -D-glucopyranose (**2**) and 4-acetoxymethyl phenol with boron trifluoride etherate and triethylamine<sup>8</sup> has been reported to result in good isolation yield. However, all salicyl derivatives (**8–10**, 1 - 1.5 eq), even though 2-acetoxymethyl phenol (**9**), with boron trifluoride etherate (1.4 - 2.5 eq) and triethylamine (0.3 - 0.5 eq) did not yield a glucosidated product. These results indicated that the reactivity of 2-acetoxymethyl substituted phenol was different from that of 4-acetoxymethyl substituted phenol (Scheme 2). The *ortho*-substitution phenols inhibited the glucosidations of **2** with BF<sub>3</sub> etherate.

Glucosidation of 2,3,4,6-tetra-O-acetyl- $\alpha$ -D-glucopyranosyl bromide (**3**) and phenol derivatives in the presence of phase-transfer catalysts is one of the mild reactions used for the construction of  $\beta$ -glucoside. The phase-transfer catalysts dimethylaminopyridine<sup>9</sup> and benzyltriethylammonium bromide<sup>10</sup> did not yield any glucosidation products (Scheme 3 entries 1 to 6). However, n-butyltriethylammonium bromide<sup>11</sup> improved glucosidation with salicyl derivatives. Although salicyl alcohol derivatives (**8** and **9**) did not undergo glucosidation, salicyl aldehyde (**10**) produced the glucoside, **11**, at moderate yield (Scheme 3 entries 7 to 9). The anomeric proton coupling constant of compound **11** (*J* = 7.6 Hz) define the  $\beta$ -configuration. Hence, diazirinyl salicin derivatives were synthesized with diazirinyl salicyl aldehyde derivative in the presence of the phase-transfer catalyst, n-BuEt<sub>3</sub>NBr.

Diazirinyl salicyl aldehyde derivative  $7^{12}$  was subjected to glucosidation with an equivalent of 2,3,4,6-tetra-O-acetyl- $\alpha$ -D-glucopyranosyl bromide (3) and nBuEt<sub>3</sub>NBr at rt to afford **12** at low yield (9%,

Scheme 4 entry 1). 2,3,4,6-Tetra-O-acetyl- $\alpha$ -D-glucopyranosyl bromide (**3**) was completely consumed under these conditions. The isolated yield of **12** improved with 2 equivalents of 2,3,4,6-tetra-O-acetyl- $\alpha$ -D-glucopyranosyl bromide (**3**) and 2.5 equivalents of nBuEt<sub>3</sub>NBr (Scheme 4 entries 2–4). The anomeric proton coupling constant of compound **12** ( $\delta$  5.18 ppm, J = 7.6 Hz) define the  $\beta$ -configuration.



**Scheme 4.** Glucosidation of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (**3**) with diazirinyl salicylaldehyde (**7**).

The aldehyde in compound 12 was subjected to reduction to primary alcohol with  $NaBH_4$  (84 %), followed by deacetylation with sodium methoxide (36%) to produce the diazirinyl salicin derivative, 1 (Scheme 5).



Scheme 5. Synthesis of the diazirinyl salicin derivative, 1.

Compound **1** was subjected to a photoirradiation experiment with black light (100 W) to ensure its photoreactivity.<sup>13</sup> Decay at approximately 360 nm was measured in a time-dependent manner (Scheme 6). Although higher reactivity of generated carbene afforded complex photolysis mixture, main product was identified as methanol adduct of **1** (FD-MS, m/z 398).<sup>14</sup> The half-life of diazirinyl moiety on compound **1** was found to be 0.78 min by using a semilogarithmic plot. These results indicated that **1** had sufficient reactivity for photoaffinity labeling. Hence, further functional analysis of the bitter receptor is underway.



Scheme 6. Photolysis of diazirinyl salicin (1) in methanol (1 mM) under black light (100W). UV spectra of the photolysis were recorded every minute for 15 min.

### **EXPERIMANTALS**

Genaral methods. NMR spectra were measured by JEOL EX-270 and ECA-500 spectrometers. ESI-TOF-MS data were obtained with a Waters UPLC ESI-TOF mass spectrometer. Optical rotation data were obtained with a JASCO DIP-370 polarimeter at 23°C.

# **2-**[(2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl)oxy]-benzaldehyde (11)<sup>15</sup>

Benzaldehyde **10** (0.1287 g, 1.05 mmol), 2,3,4,6-tetra-O-acetyl- $\alpha$ -D-glucopyranosyl bromide **3** (0.4811 g, 1.17 mmol) and n-BuEt<sub>3</sub>NBr (0.3888 g, 1.17 mmol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL). A solution of sodium hydroxide (5%, 4 mL) was added to the above solution. The reaction mixture was stirred at rt for 20 h virgously and diluted with CHCl<sub>3</sub>. The diluted solution was washed with cold 1 M HCl solution and brine, dried over MgSO<sub>4</sub>, and concentrated. The residue was purified by silica gel column chromatography (hexane / AcOEt = 3 / 1) to afford **11** (0.2185 g, 46%) as colorless solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 10.35 (1H, s), 7.87 (1H, dd, J = 7.7, 1.8 Hz), 7.56 (1H, ddd, J = 8.7, 7.0, 1.4 Hz), 7.19 (1H, t, J = 7.6 Hz), 7.12 (1H, d, J = 8.2 Hz), 5.41 - 5.30 (2H, m), 5.23 - 5.16 (1H, m), 5.19 (1H, d, J = 7.6 Hz), 4.30 (1H, dd, J = 12.5, 5.3 Hz), 4.18 (1H, dd, J = 12.4, 2.5 Hz), 3.90 (1H, ddd, J = 10.1, 5.2, 2.5 Hz), 2.08 (1H, s), 2.07 (1H, s), 2.06 (1H, s), 2.05 (1H, s); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ : 189.06, 170.43, 170.12, 169.30, 169.14, 158.74, 135.64, 128.31, 126.28, 123.61, 116.00, 99.07, 72.23, 70.92, 68.18, 61.76, 20.53; [  $\alpha$  ]<sub>D</sub> -29.0 (c 1.0, CHCl<sub>3</sub>).

# 2-[(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)oxy]-4-[3-(trifluoromethyl)-3*H*-diazirine-3-yl]benzaldehyde (12)

Diazirinylbenzaldehyde **7** (0.0553 g, 0.24 mmol), 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide **3** (0.2862 g, 0.48 mmol), and n-BuEt<sub>3</sub>NBr (0.1938 g, 0.60 mmol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4 mL). A solution of sodium hydroxide (5%, 4 mL) was added. The reaction mixture was stirred at rt for 21 h and diluted with CHCl<sub>3</sub>. The diluted solution was washed with cold 1 M HCl solution and brine, dried over MgSO<sub>4</sub>, and concentrated. The residue was purified by silica gel column chromatography (hexane / AcOEt = 3 / 1 to hexane / AcOEt = 1 / 1) to afford **12** (0.0875 g, 65%) as colrless solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 10.30 (1H, s), 7.88 (1H, d, *J* = 8.2 Hz), 6.97 (1H, d, *J* = 8.9 Hz), 6.94 (1H, s), 5.41-5.30 (2H, m), <del>5.37,</del> 5.23-5.13 (1H, m), 5.18 (1H, d, *J* = 7.6 Hz), 4.27 (1H, dd, *J* = 5.3, 12.5Hz), 4.20 (1H, dd, *J* = 2.6, 12.5 Hz), 3.95 (1H, ddd, *J* = 2.6, 5.3, 9.9 Hz), 2.13 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.05 (3H, s). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ : 187.99, 170.59, 170.07, 169.33, 169.13, 158.41, 136.54, 128.96, 126.55, 121.68 (q, <sup>*I*</sup>*J*<sub>CF</sub> = 274.9 Hz), 121.24, 113.92, 98.88, 72.59, 72.17, 70.68, 67.92, 61.87, 28.45 (q, <sup>2</sup>*J*<sub>CF</sub> = 39.1 Hz), 20.55. <sup>19</sup>F-NMR(CDCl<sub>3</sub>)  $\delta$ : -64.57. [α]<sub>D</sub> +7.3 (c 1.0, MeOH). HRMS-FD (m/z) M<sup>+</sup> calcd for C<sub>23</sub>H<sub>23</sub>F<sub>3</sub>N<sub>2</sub>O<sub>11</sub> 560.1254, found 560.1238.

# 2-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)oxy]-4-[3-(trifluoromethyl)-3*H*-diazirine-3-yl]-benzyl alcohol (13)

The diazirinyl tetraacetyl phenylglucoside derivative **12** (0.0673 g, 0.12 mmol) was dissolved in methaol and cooled to 0 °C and NaBH<sub>4</sub> (0.0054 g, 0.14 mmol) was added to the reaction mixture. The reaction mixture was stirred at rt for 4 h. After acidification of the solution with 1 M HCl at 0 °C the reaction mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic solution was dried over MgSO<sub>4</sub>, filtrated, and concentrated. The residue was purified by silica gel column chromatography (hexane / AcOEt = 1 / 1) to afford diazirinyl tetraacetyl salicin derivative **13** (0.0567 g, 84%) as colorless solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 7.40 (1H, d, *J* = 7.9 Hz), 6.92 (1H, d, J = 7.9 Hz), 6.84 (1H, s), 5.34 (1H, t, *J* = 5.9 Hz), 5.33 (1H, dd, *J* = 13.4, 17.3 Hz), 5.13 (2H, dd, *J* = 8.2, 17.8 Hz), 4.65 (1H, d, *J* = 13.5 Hz), 4.56 (1H, d, *J* = 13.2 Hz), 4.23 (1H, d, *J* = 5.6 Hz), 4.21 (1H, d, *J* = 2.6 Hz), 3.91 (1H, dq, *J* = 2.6, 9.7 Hz), 2.37 (1H, br s), 2.11 (3H, s), 2.10 (3H, s), 2.07 (3H, s), 2.05 (3H, s). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ : 170.66, 170.06, 169.68, 169.39, 154.58, 132.94, 129.88, 129.72, 121.99(q, <sup>*I*</sup>*J*<sub>CF</sub> = 274.3 Hz), 121.71, 113.26, 99.16, 72.41, 72.21, 70.97, 68.10, 61.90, 60.36, 28.30 (q, <sup>2</sup>*J*<sub>CF</sub> = 40.2 Hz), 20.63, 20.55. <sup>19</sup>F-NMR(CDCl<sub>3</sub>)  $\delta$ : -65.06. [ $\alpha$ ]<sub>D</sub> -2.4 (c 1.0, MeOH). HRMS-FD (m/z) M<sup>+</sup> calcd for C<sub>23</sub>H<sub>25</sub>F<sub>3</sub>N<sub>2</sub>O<sub>11</sub> 562.1410, found 562.1410.

## 2-Hydroxymethyl-5-[3-(trifluoromethyl)-3*H*-diazirine-3-yl]phenyl-β-D-glucopyranoside (1)

To a solution of diazirinyl pentaacetyl salicin derivative **13** (47.5 mg, 0.084 mmol) in MeOH (2 mL), a solution of NaOMe in MeOH (28%, 25  $\mu$  L) was added at 0 °C, and the reaction mixture was stirred at rt for 1 h. After the reaction, the mixture was concentrated in vacuo. The residue was purified by silica gel

column chromatography (CH<sub>2</sub>Cl<sub>2</sub> / MeOH = 4 / 1) to afford diazirinyl salicin **1** (0.0120 g, 36%) as colorless solid. <sup>1</sup>H-NMR (CD<sub>3</sub>OD)  $\delta$ : 7.38 (1H, d, J = 8.2 Hz), 6.95 (1H, s), 6.84 (1H, d, J = 7.9 Hz), 4.76-4.73 (1H, d), 4.67 (1H, d, J = 14.2 Hz), 4.52 (1H, d, J = 13.8 Hz), 3.79 (1H, d, J = 11.9 Hz), 3.62 (1H, dd, J = 3.1, 12.4 Hz), 3.43-3.33 (4H, m), 1.19 (1 H, br s). <sup>13</sup>C-NMR (CD<sub>3</sub>OD),  $\delta$ : 156.86, 134.82, 130.25, 129.92, 123.59 (q,  ${}^{1}J_{CF} = 274.9$  Hz), 121.77, 115.01, 103.26, 78.32, 77.96, 74.86, 71.19, 62.30, 60.10, 29.45 (q,  ${}^{2}J_{CF} = 40.2$  Hz). <sup>19</sup>F-NMR(CD<sub>3</sub>OD)  $\delta$ : -66.89. [ $\alpha$ ]<sub>D</sub> -23.1 (c 1.0, MeOH). HRMS-FD (m/z) M<sup>+</sup> calcd for C<sub>15</sub>H<sub>17</sub>F<sub>3</sub>N<sub>2</sub>O<sub>7</sub> 394.0988, found 394.1002.

### Photolysis of compound 1 in methanol

A 1 mM degassed methanolic solution of the diazirinyl salicin **1** was placed in a quartz cuvette. Photolysis was carried out with 100 W black-light at a distance of 5cm from surface of light source. Spectra were measured after each minute, and then the half-life was caluculated from the decrements of the absorbance around 360 nm.

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