HETEROCYCLES, Vol. 93, No. 1, 2016, pp. 355 - 361. © 2016 The Japan Institute of Heterocyclic Chemistry
Received, 15th June, 2015, Accepted, 16th July, 2015, Published online, 5th August, 2015
DOI: 10.3987/COM-15-S(T)4

SYNTHESIS OF BENZOPHENONE AND PHENYLAZIDE DERIVATIVES OF SALICIN FOR FUNCTIONAL ANALYSIS OF THE BITTER TASTE RECEPTOR USING PHOTOAFFINITY LABELING

Takuma Yoshida, Yasuyuki Hashidoko, and Makoto Hashimoto*

Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kita-ku, Sapporo 060-8589, Japan, E-mail: hasimoto@abs.agr.hokudai.ac.jp

Abstract – Salicin is a substance that is well known for its bitter taste. Photoreactive benzophenone and phenylazide derivatives of salicin are for the functional analysis of interactions between the bitter taste receptor and salicin. First synthesis of these derivatives was prepared from acetobromo-α-D-glucose via glucosidation with salicylaldehyde derivatives followed by reduction of aldehyde to form salicin skeleton.

Salicin **1** is a well-known major constituent of phenyl glycosides in the family *Salicaceae*[1] and is a bitter anti-inflammatory compound.[2] Elucidation of the functions of its gustatory receptor through structure–activity relationships may reveal the mechanism of its homeostatic functions, which is of great scientific interest. A study using docking simulation predicted that the hydroxyl groups of carbohydrate and a benzyl alcohol in salicin played an important role to bind bitter taste receptor.[3] However, it did not clearly show whether aromatic ring interactions and substituent effects on the aromatic ring affect this receptor. These results indicate that five hydroxyl groups (i.e., at 2, 3, 4, 6, and 2' positions) of salicin play roles in binding to the bitter taste receptor and that all hydroxyl groups on the mother skeleton (salicin) must retain substituent effects on the aromatic ring. A comprehensive analysis of substituent effects on the aromatic ring, however, has not been performed because of the difficulty in glycosidation of salicyl alcohol derivatives.[4] Photoaffinity labeling is one of the most common techniques used in chemical biology.[5] In our previous studies, we reported the synthesis and properties of several photoaffinity labeling reagents for gustatory receptors.[6] Various photophores, benzophenone, phenylazide, and phenyldiazirine are used for the elucidation of ligand-receptor interactions. Selection of photophores for photoaffinity labeling is very critical in obtaining highly accurate data on the interactions. However,

This paper is dedicated to Prof. Dr. Lutz F. Tietze on the occasion of his 75th birthday.

there is no method for the universal selection of photophores. In a recent study, diazirine-based salicin derivative, which was used as photoaffinity labeling reagent for bitter taste receptor, were synthesized.[6e] In this paper, we report the synthesis of benzophenone- and phenylazide-based salicin derivatives for functional analysis of the salicin bitter taste receptor (Figure 1, **2** and **3**).

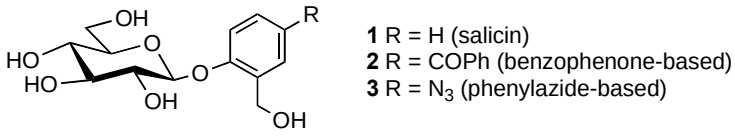
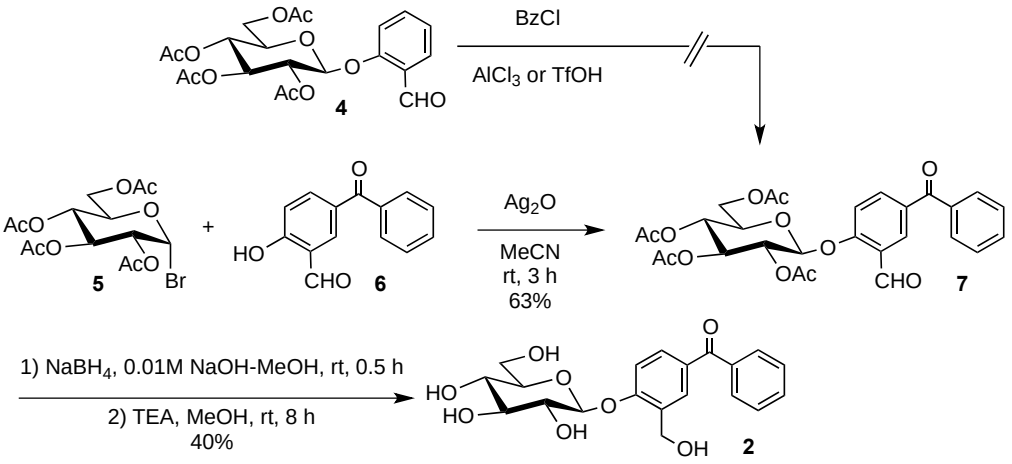


Figure 1. Salicin **1** and photoreactive derivatives **2** and **3**

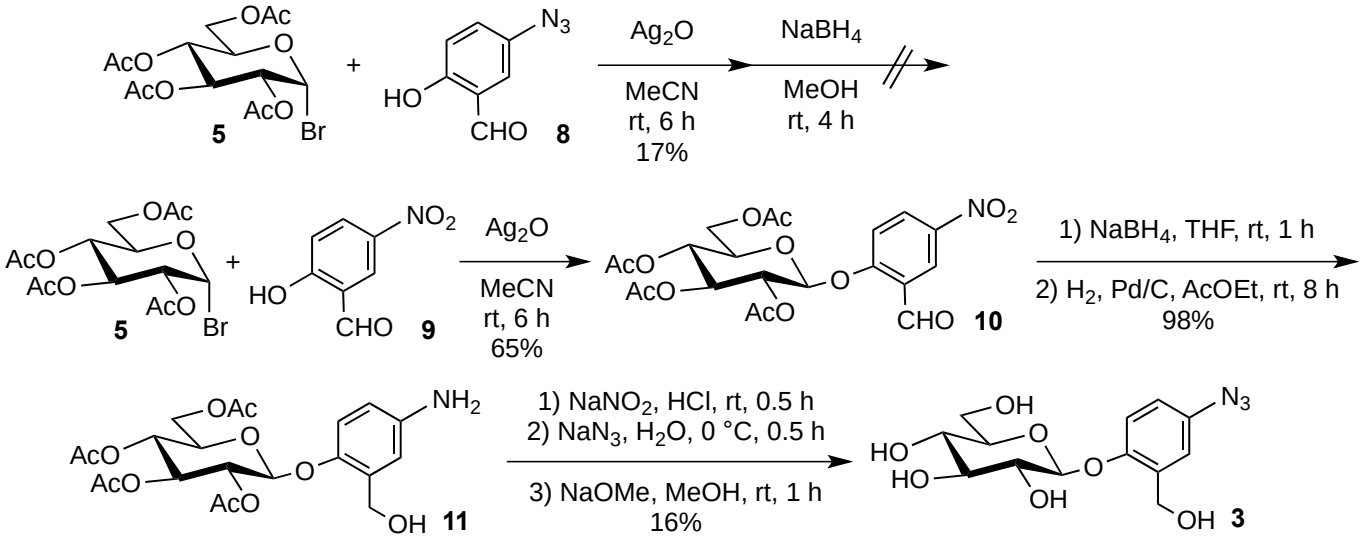
Glucosides of salicyl alcohol derivatives are more complicated than are glucosides of phenol derivatives without substitution at the ortho position.[4] Acetylated helicine **4** was subjected to Friedel–Crafts benzoylation with $AlCl_3$ or triflic acid[7] to construct the benzophenone moiety. However, the reaction afforded a very complex mixture (Scheme 1).



Scheme 1. Synthesis of benzophenone-based salicin derivative **2**

Glucosidation with salicylaldehyde derivatives followed by reduction of benzaldehyde to benzyl alcohol is one of the most effective methods for construction of the salicin skeleton.[6e] 5-Benzoyl-2-hydroxybenzaldehyde **6** synthesized from 2-hydroxybenzophenone[8] was used in glucosidation with acetobromo-α-D-glucose **5** with silver oxide in acetonitrile.[9] The reaction, which was conducted at room temperature, produced β-glucosides **7** in moderate yield. Selective reduction of aldehyde over ketone was achieved by using a limited amount of sodium borohydride in accordance with the literature.[10] The reaction mixture was directly subjected to deacetylation, as partial deacetylation was observed during the reduction. The compound **2** was deacetylated with TEA-MeOH[11] without decomposition of the ketone group (Scheme 1). The anomeric proton coupling constant of compound **7** and **2** (J = 6.3 and 7.3 Hz) define the β-configuration.

5-Azidesalicylaldehyde **8**[12] and 5-nitrosalicylaldehyde **9** derivatives were subjected to glucosidation with acetobromo-α-D-glucose **5** and silver oxide in acetonitrile. Glucosidation of the azide derivative **8** had a very low yield, and subsequent reduction with sodium borohydride afforded a complex mixture (Scheme 2).



Scheme 2. Synthesis of phenylazide-based salicin derivative **3**

Glucosidation of the 5-nitrosalicylaldehyde **9** was more effective than that of the 5-azidesalicylaldehyde derivative **8**. The aldehyde group of compound **10** was reduced with 1.2 eq sodium borohydride. The excess sodium borohydride made the reaction mixture alkaline, thus promoting partial deacetylation. The reaction mixture was directly subjected to reduction of the nitro group to afford the 5'-amino-substituted salicin derivative **11**. Compound **11** was then subjected to diazotization followed by azidation to produce glucosylamine derivatives.[13] Isolation of the 5'-azide-tetraacetylsalicin derivative was very difficult because of partial deacetylation during the reaction. The reaction mixture was subjected to deacetylation without purification. The TEA-MeOH system could be used for synthesis ofbenzophenone derivatives, but not for azide derivatives. The use of sodium methoxide / methanol solution effectively produced the phenylazide-based salicin derivatives, **3**, the azide group of which was detected by IR spectrometry. The anomeric proton coupling constant of compound **10** and **3** (J = 6.9 and 7.6 Hz) define the β-configuration.

The photoreactive salicin derivatives **2** and **3** were subjected to photoirradiation experiments in MeOH and afforded MeOH adducts within 30 min. These results indicated that both compounds have enough reactivity for photoaffinity label.

We thus accomplished novel synthesis of benzophenone- and phenylazide-based salicin derivatives. Further functional analysis of the bitter taste receptor is underway.

EXPERIMENTALS

General 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. IR spectra were measured by JASCO FTIR-4100.

5-Benzoyl-2-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxy]-benzaldehyde 7

Acetobromo-α-D-glucose **5** (1.605 g, 3.90 mmol) and Ag_2O (3.093 g, 13.3 mmol) were suspended in MeCN (40 mL). 5-Benzoyl-2-hydroxybenzaldehyde **6**[8] (0.500 g, 2.21 mmol) was added dropwisely. After stirring at rt for 3 h, the reaction mixture was centrifuged, filtrated Celite, and the filtrate was concentrated. The residue was subjected to silica column chromatography (AcOEt / hexane = 1 / 9, then 1 / 0) to afford colorless oil (0.771 g, 63%). ^{1}H-NMR ($CDCl_3$) δ: 10.36 (1H, s), 8.25 (1H, d, J = 2.0 Hz), 8.12 (1H, dd, J = 8.6, 2.0 Hz), 7.75 (2H, d, J = 6.9 Hz), 7.62 (1H, d, J = 7.4 Hz), 7.50 (2H, d, J = 7.4 Hz), 7.25 (1H, d, J = 8.6 Hz), 5.42 (1H, d, J = 6.3 Hz), 5.37 (1H, d, J = 6.3 Hz), 5.34 (1H, d, J = 5.9 Hz), 5.24 (1H, d, J = 9.9 Hz), 4.32 (1H, dd, J = 12.5, 5.3 Hz), 4.21 (1H, dd, J = 12.5, 2.5 Hz), 4.00 (1H, d, J = 9.9 Hz), 2.09 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.04 (3H, s); ^{13}C-NMR ($CDCl_3$) δ: 194.4, 188.1, 170.3, 170.0, 169.3, 169.0, 161.0, 137.0, 136.9, 132.7, 132.6, 130.7, 129.7, 128.5, 125.1, 115.4, 98.3, 72.4, 72.2, 70.7, 68.0, 61.7, 20.5; $[\alpha]_D$ −42 (c 1, $CHCl_3$); HRMS-ESI (*m/z*) MH^+ calcd for $C_{28}H_{28}O_{12}$ 557.1659, found 557.1658.

5-Benzoyl-2-[(β-D-glucopyranosyl)oxy]-benzyl alcohol 2

To a EtOH solution (120 mL) of compound **7** (0.062 g, 0.11 mmol), 0.63 mL (0.015 mmol) of 23.8 mM $NaBH_4$ in 0.01M NaOH-EtOH solution was added at rt. The reaction mixture was stirred at rt for 0.5 h and quenched with addition of 0.1M HCl (1 mL) followed by concentrated. The residue was dissolved in MeOH (2 mL). TEA (0.7 mL) was added and the reaction mixture was stirred at rt for 8 h and concentrated. The residue was subjected to column chromatography ($CHCl_3$ / MeCN = 6 / 1 then 5 / 1) afforded colorless amorphous mass (0.018 g, 39%). ^{1}H-NMR (CD_3OD) δ: 7.89 (1H, d, J = 2.0 Hz), 7.74 (3H, d, J = 7.3 Hz), 7.62 (1H, t, J = 7.3 Hz), 7.51 (2H, t, J = 7.3 Hz), 7.30 (1H, d, J = 8.6 Hz), 5.06 (1H, d, J = 7.3 Hz), 4.81 (1H, d, J = 13.8 Hz), 4.65 (1H, d, J = 13.8 Hz), 3.91 (1H, d, J = 12.0 Hz), 3.71 (1H, dd, J = 12.0, 5.1 Hz), 3.48 (4H, m); ^{13}C-NMR (CD_3OD) δ: 196.3, 158.7, 137.9, 132.0, 131.2, 130.1, 130.0, 129.3, 128.0, 114.1, 100.8, 76.9, 76.5, 73.4, 69.8, 61.0, 58.8; $[\alpha]_D$ −60 (c 2, MeOH); HRMS-ESI (*m/z*) MH^+ calcd for $C_{20}H_{22}O_8$ 391.1393, found 393.1395.

5-Nitro-2-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxy]-benzaldehyde 10

Acetobromo-α-D-glucose **5** (3.250 g, 7.90 mmol) and Ag_2O (6.036 g, 26.0 mmol) were suspended in MeCN (80 mL). 2-Hydroxy-5-nitrobenzaldehyde **9**[12] (0.786 g, 4.71 mmol) was added dropwisely. After stirring at rt for 6 h, the reaction mixture was centrifuged and the supernatant was concentrated. The

residue was subjected to silica column chromatography (AcOEt / hexane = 1 / 2, then 1 / 0) followed by reprecipitation from EtOH to afford 1.514 g (65%) as colorless amorphous mass. ^{1}H-NMR ($CDCl_3$) δ: 6.84 (1H, d, *J* = 8.6 Hz), 6.67 (1H, d, *J* = 3.0 Hz), 6.56 (1H, dd, *J* = 8.6, 3.0 Hz), 5.27 (2H, m), 5.14 (1H, t, *J* = 9.4 Hz), 4.93 (1H, d, *J* = 6.9 Hz), 4.63 (1H, d, *J* = 12.5 Hz), 4.38 (1H, d, *J* = 12.5 Hz), 4.25 (1H, dd, *J* = 12.5, 5.3 Hz), 4.15 (1H, dd, *J* = 12.0, 2.5 Hz), 3.77 (1H, dq, *J* = 9.8, 2.5 Hz), 2.11 (3H, s) , 2.07 (3H, s), 2.04 (6H, s); ^{13}C-NMR ($CDCl_3$) δ: 170.5, 170.2, 169.5, 169.3, 147.3, 143.0, 133.1, 118.5, 116.0, 114.9, 101.1, 72.6, 71.2, 68.3, 60.7, 20.6 (2C), 20.5 (2C); $[\alpha]_D$ -50 (c 1, $CHCl_3$); HRMS-ESI (*m/z*) MH^+ calcd for $C_{21}H_{23}NO_{13}$ 498.1241, found 498.1241.

5-Amino-2-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxy]-benzyl alcohol 11

Compound **10** (0.751 g, 1.51 mmol) was dissolved in THF (80 mL). Sodium borohydride (0.070 g, 1.84 mmol) was added dropweise at 0 °C. The reaction mixture was stirred at rt for 1 h. Several drops of 6 M HCl was added to quench the reaction and the reaction mixture was partioned between H_2O (50 mL) and AcOEt (100 mL). The organic layer was washed with 0.5% $NaHCO_3$, brine, dried over $MgSO_4$, filtrated and concentrated to afford crude residue. No more further purification was performed for next step. The residue was dissolved in AcOEt (50 mL). Pd/C (10%, 0.131 g) was suspended and the reaction mixture was stirred virgously under H_2 atmosphere over night. Pd/C was filtered off with Celite pad and the filtrate was concentrated to afford colorless amorphous mass (0.693 g, 98% for 2 steps) . ^{1}H-NMR ($CDCl_3$) δ: 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), 5.23-5.13 (1H, m), 5.18 (1H, d, *J* = 7.6 Hz), 4.27 (1H, dd, *J* = 5.3, 12.5 Hz), 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); ^{13}C-NMR ($CDCl_3$) δ: 170.5, 170.1, 169.5, 169.3, 147.3, 143.0, 133.1, 118.5, 116.0, 114.9, 101.1, 72.6, 71.9, 71.2, 68.3, 61.7, 60.7, 20.6 (2C), 20.5 (2C); $[\alpha]_D$ −9.0 (c 2, $CHCl_3$); HRMS-ESI (*m/z*) MH^+ calcd for $C_{21}H_{27}NO_{11}$ 470.1662, found 470.1665.

5-Azido-2-[(β-D-glucopyranosyl)oxy]-benzyl alcohol 3

Compound **11** (0.693 g, 1.48 mmol) was dissolved in 3.6 M HCl (6 mL) and cooled on ice bath. Sodium nitrite (0.122 g, 1.77 mmol) in H_2O (1 mL) was added dropweise and the reaction mixture was stirred at rt for 0.5 h. Sodium azide (0.127 g, 1.95 mmol) in H_2O (0.2 mL) was added dropweise and stirred virgously at 0 °C for 1 h. The precipitates were corrected with centrifugation and dissolved in AcOEt. The organic layers dried over $MgSO_4$, filtrated and concentrated to afford yellow residue. No more further purification was performed for next step. The residue was dissolved in MeOH (50 mL). Sodium methoxide solution (28%, 0.1 mL) was added at 0 °C and the reaction mixture was stirred at rt for 1 h, followed by concentrated. The residue was subjected to silica column chromatography (prewashed with MeOH, CH_2Cl_2 / MeOH = 2 / 1) to afford pale yellow amorphous mass (0.089 g, 71%). ^{1}H-NMR (D_2O) δ: 7.23 (1H, d, *J* = 8.6 Hz), 7.13 (1H, d, *J* = 2.6 Hz), 7.06 (1H, dd, *J* = 8.6, 2.6 Hz), 5.07 (1H, d, *J* = 7.6 Hz), 4.69

(2H, s), 3.93 (1H, dd, *J* = 12.4, 1.8 Hz), 3.75 (1H, dd, *J* = 12.4, 5.2 Hz), 3.59 (3H, m), 3.50 (1H, m); ^{13}C-NMR (D_2O) δ: 152.5, 135.6, 132.3, 120.3, 117.9, 101.8, 77.0, 76.5, 73.8, 70.3, 61.4, 59.8, 49.7. IR (neat) ν (cm^{-1}): 2110; $[\alpha]_D$ −26 (c 1, MeOH); HRMS-ESI (*m/z*) MH^+ calcd for $C_{13}H_{17}N_3O_7$ 328.1145 found 328.1144.

ACKNOWLEDGEMENTS

MH thanks the Suhara Memorial Foundation and Mishima Kaiun Memorial Foundation for financial support. Part of this work was performed under the Cooperative Research Program of "Network Joint Research Center for Materials and Devices"

REFERENCES

1. G. A. Boeckler, J. Gershenzon, and S. B. Unsicker, *Phytochemistry*, 2011, **72**, 1497.
2. B. Bufe, T. Hofmann, D. Krautwurst, J.-D. Raguse, and W. Meyerhof, *Nat. Genet.*, 2002, **32**, 397.
3. T. Sakurai, T. Misaka, M. Ishiguro, K. Masuda, T. Sugawara, K. Ito, T. Kobayashi, S. Matsuo, Y. Ishimaru, T. Asakura, and K. Abe, *J. Biol. Chem.*, 2010, **285**, 28373.
4. M. L. Belyanin, E. V. Stepanova, and V. D. Ogorodnikov, *Carbohydr. Res.*, 2012, **363**, 66.
5. a) J. Brunner, *Annu. Rev. Biochem.*, 1993, **62**, 483; b) Y. Hatanaka, H. Nakayama, and Y. Kanaoka, *Rev. Heteroat. Chem.*, 1996, **14**, 213; c) G. Dormán and G. D. Prestwich, *Trends Biotechnol.*, 2000, **18**, 64; d) T. Tomohiro, M. Hashimoto, and Y. Hatanaka, *Chem. Record*, 2005, **5**, 385; e) M. Hashimoto and Y. Hatanaka, *Eur. J. Org. Chem.*, 2008, 2513; f) L. Dubinsky, B. P. Krom, and M. M. Meijler, *Bioorg. Med. Chem.*, 2012, **20**, 554; g) T. Tomohiro and Y. Hatanaka, *Heterocycles*, 2014, **89**, 2697; g) Y. Hatanaka, *Chem. Pharm. Bull.*, 2015, **63**, 1.
6. a) K. Masuda, A. Koizumi, T. Misaka, Y. Hatanaka, K. Abe, T. Tanaka, M. Ishiguro, and M. Hashimoto, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1081; b) Y. Tsunekawa, K. Masuda, M. Muto, Y. Muto, Y. Murai, Y. Hashidoko, Y. Orikasa, Y. Oda, Y. Hatanaka, and M. Hashimoto, *Heterocycles*, 2012, **84**, 283; c) Y. Murai, K. Masuda, Y. Sakihama, Y. Hashidoko, Y. Hatanaka, and M. Hashimoto, *J. Org. Chem.*, 2012, **77**, 8581; d) M. Sakurai, K. Masuda, L. Wang, Y. Murai, Y. Sakihama, Y. Hashidoko, Y. Hatanaka, and M. Hashimoto, *Heterocycles*, 2014, **88**, 629; e) M. Sakurai, T. Yoshida, L. Wang, Y. Murai, K. Masuda, Y. Sakihama, Y. Hashidoko, Y. Hatanaka, and M. Hashimoto, *Heterocycles*, 2015, **90**, 698; f) L. Wang, Y. Murai, T. Yoshida, A. Ishida, K. Masuda, Y. Sakihama, Y. Hashidoko, Y. Hatanaka, and M. Hashimoto, *Org. Lett.*, 2015, **17**, 616; g) L. Wang, T. Yoshida, Y. Muto, Y. Murai, Z. P. Tachrim, A. Ishida, S. Nakagawa, Y. Sakihama, Y. Hashidoko, K. Masuda, Y. Hatanaka, and M. Hashimoto, *Eur. J. Org. Chem.*, 2015, 3129.
7. M. Hashimoto and M. Takahashi, *Heterocycles*, 2009, **77**, 227.

8. L. E. Elizalde, G. de los Santos, A. E. García, D. I. Medellín, and R. Acosta, *Synth. Commun.*, 2005, **35**, 3087.
9. J.-C. Florent, X. Dong, G. Gaudel, S. Mitaku, C. Monneret, J.-P Gesson, J.-C. Jacquesy, M. Mondon, B. Renoux, S. Andrianomenjanahary, S. Michel, M. Koch, F. Tillequin, M. Gerken, J. Czech, R. Straub, and K. Bosslet, *J. Med. Chem.*, 1998, **41**, 3572.
10. H.-K. Lee, P. Denner-Ancona, J. Sakakibara, T. Ono, and G. D. Prestwich, *Arch. Biochem. Biophys.*, 2000, **381**, 43.
11. P. Hendra, Y. Fukushi, and Y. Hashidoko, *Biosci. Biotech. Biochem.*, 2009, **73**, 2172.
12. H. Kai, H. Hinou, and S.-I. Nishimura, *Bioorg. Med. Chem.*, 2012, **20**, 2739.
13. E. Saman, M. Claeyssens, H. Kefsters-Hilderson, and C. K. De Bruyne, *Carbohydr. Res.*, 1973, **30**, 207.