



Title	Screening and Identification of Disaccharides with Insulin Mimetic Activity against L6 Cells
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3 Screening and Identification of Disaccharides with Insulin Mimetic Activity against L6

4 Cells

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1      Running title  
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8      Kazuaki YAMASAKI, Ryogo HISHIKI, Eisuke KATO,<sup>†</sup> Jun KAWABATA  
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12  
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17

1      Abstract

2            Insulin mimetics are considered as prospecting anti-diabetic agent. Recently, a  
3            disaccharide neohesperidose was found to show insulin mimetic activity against L6  
4            cells. Here, several other disaccharides were screened for their insulin mimetic activity  
5            and resulted in an identification of three new insulin mimetic disaccharides.

6

7      Key words

8      Insulin mimetic; Disaccharide; Diabetes mellitus; Glucose uptake; L6 Cell

9

10            Glucose homeostasis is maintained by multiple hormones with adverse effect.  
11            Elevation of blood sugar level is handled together by several hormones, but lowering of  
12            the elevated blood sugar level is handled by the peptide hormone insulin alone.  
13            Defection in insulin secretion or decrease of its sensitivity easily leads to the impaired  
14            control of blood sugar level which status is called diabetes mellitus. In the medical  
15            treatment of diabetes mellitus, regulation of blood sugar level is the most important  
16            piece, as high level of blood sugar eventually leads to several symptoms like  
17            cardiovascular disease, renal failure, blindness and neurological disorders.<sup>1)</sup> Especially,  
18            maintenance after dining, the main factor that rapidly elevates blood-sugar, is important.  
19            For this purpose, exogenous insulin is currently employed as a medicine to maintain  
20            blood-sugar level of the patients.<sup>2, 3)</sup> However, insulin's physical instability due to its  
21            peptidic character, restricts the use of insulin only by injection. Development of an  
22            alternative agent with a stable character, which can be used orally, should give patients  
23            of diabetes mellitus a choice for maintaining their disease.

24            Natural products have been widely studied as a source of blood sugar  
25            controlling agent, and several plants are utilized as an easily applicable supplementary  
26            food. Several natural products like flavonoids are reported as insulin mimetic compound,  
27            except most of those compounds are known to work as a peroxisomal proliferator  
28            activated receptor (PPAR) agonist and does not exactly mimics the activity of insulin.<sup>4,</sup>

1       <sup>5)</sup> Other than those PPAR agonists, a complex of flavonoids and an inorganic metal has  
2       been reported to resemble the action of insulin.<sup>6-8)</sup> Recently, we have explored an  
3       organic compound that has insulin mimetic activity.<sup>9)</sup> The compound, neohesperidose  
4       (**1**), is a disaccharide composed of D-glucose and L-rhamnose and was found as an  
5       active principle of insulin mimetic flavonoid glycoside, Kaempferol  
6       3-O-neohesperidoside. By treating muscle model cell L6 with neohesperidose (**1**) at  
7       concentration of sub-nanomolar range, the stimulated cells rapidly uptake glucose just  
8       as it was stimulated by insulin. The simple and stable character of **1** gave an opportunity  
9       to utilize it as an insulin alternative, but the maximum uptake of glucose induced by **1**  
10      was relatively low compared to insulin. In this study, we presumed that several other  
11      disaccharides may exhibit the same activity. And to find a disaccharide with this unique  
12      activity, we chose several disaccharides and investigated for their insulin mimetic  
13      activity.

14      Our first choices were disaccharides connected through  $\beta$ 1 $\rightarrow$ 4 bond as it is one  
15      of the most common glycoside bond seen in natural products. Cellobiose (**2**),  
16       $\beta$ -D-glucose-(1 $\rightarrow$ 4)-D-mannose (**3**),      $\beta$ -D-mannose-(1 $\rightarrow$ 4)-D-glucose (**4**),  
17       $\beta$ -D-mannose-(1 $\rightarrow$ 4)-D-mannose (**5**), lactose ( $\beta$ -D-galactose-(1 $\rightarrow$ 4)-D-glucose) (**6**) and  
18      epilactose ( $\beta$ -D-galactose-(1 $\rightarrow$ 4)-D-mannose) (**7**) were tested for their activity <sup>10)</sup> but all  
19      of these compounds failed to induce insulin mimetic activity (data not shown). We then  
20      turned our attention from testing various combinations of  $\beta$ 1 $\rightarrow$ 4 connected  
21      disaccharides to various types of glycoside bond. Di-glucose isomers (**2**, **8-15**)<sup>11)</sup> with  
22      ideally most of the possible structures were selected and tested. Fortunately, three of the  
23      tested disaccharides, trehalose (**8**), isomaltose (**12**) and gentiobiose (**15**) showed insulin  
24      mimetic activity against L6 cells inducing 130, 136 and 132% increase of  
25      2-deoxyglucose (2-DG) uptake compared to control (Fig. 1). The rest of disaccharides  
26      seem to slightly elevate 2-DG uptake, but there were no significant differences to the  
27      control. Structure of the active disaccharides varies at this moment. Some restriction to  
28      the position of glycoside bond is seen from the result, at least we can point out that

Structures

Fig. 1.

1 1,4-disaccharide have no activity recognizable from the results of compound **2-7**, **11**. It  
2 is fairly difficult to see other structural rules between the three active disaccharides or  
3 with the previously found insulin mimetic disaccharide neoheperidose (**1**) except for  
4 they are all a disaccharide. However, the results obtained here give an opportunity to  
5 find additional active disaccharides. And our first goal to show “Some disaccharides  
6 have insulin mimetic activity” has been accomplished.

7 In conclusion, we have explored three new insulin mimetic disaccharides.  
8 Although none of these compounds (**8**, **12**, **15**) induced higher activity compared to  
9 neohesperidose (**1**), and the common glycoside bond of these compound seems to have  
10 low tolerance under digestive conditions, the result obtained here showed the possibility  
11 of several disaccharides to work as an insulin mimetic. Further research about insulin  
12 mimetic disaccharides shall give an opportunity to use them as a chemical treatment  
13 against diabetes mellitus.

14

## 15 Acknowledgement

16 We thank Prof. Wataru Saburi and Prof. Hirokazu Matsui for kindly providing  
17 disaccharides **3-7**. This work was supported by Suntory Institute for Bioorganic  
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## 20 References

- 21 1. Coccheri S, *Drugs* , **67**, 997-1026 (2007).
- 22 2. Barag SH, *J. Am. Osteopath. Assoc.*, **111**, S13-S15 (2011).
- 23 3. Hirsch IB, Bergenstal RM, Parkin CG, Wright E, Buse JB, *Clin. Diabetes*, **23**, 78-86  
24 (2005).
- 25 4. Hsu FL, Liu IM, Kuo DH, Chen WC, Su HC, Cheng JT, *J. Nat. Prod.*, **66**, 788-792  
26 (2003).
- 27 5. Chacko BK, Chandler RT, D'Alessandro TL, Mundhekar A, Khoo NKH, Botting N,  
28 Barnes S, Patel RP, *J. Nutr.*, **137**, 351-356 (2007).

- 1    6. Sakurai H, Shimomura S, Fukuzawa K, Ishizu K, *Biochem. Biophys. Res. Commun.*,  
2    **96**, 293–298 (1980).
- 3    7. Cazarolli LH, Zanatta L, Jorge AP, Sousa E, Horst H, Woehl VM, Pizzolatti MG,  
4    Szpoganicz B, Silva FRMB, *Chem-Biol. Interact.*, **163**, 177–191 (2006).
- 5    8. Sakurai H, Adachi Y, *BioMetals*, **18**, 319–323 (2005).
- 6    9. Yamasaki K, Hishiki R, Kato E, Kawabata J, *ACS Med. Chem. Lett.*, **2**, 17-21 (2011).
- 7    10. Insulin mimetic activity assay was performed as written in reference 9.
- 8    11. Commercial products were used without further purification. Compounds **2**, **8-12**  
9    (>98%) were purchased from Wako Pure Chem. In, Ltd., **13** (>98%) was purchased  
10   from Carbosynth Ltd., **14** (>97%) was purchased from Seikagaku Biobusiness Co., **15**  
11   (>96%) was purchased from Tokyo Chem. Ind. Co., Ltd.
- 12

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2     Figure Legend

3

4     Fig. 1. Insulin mimetic activity of di-glucoses.

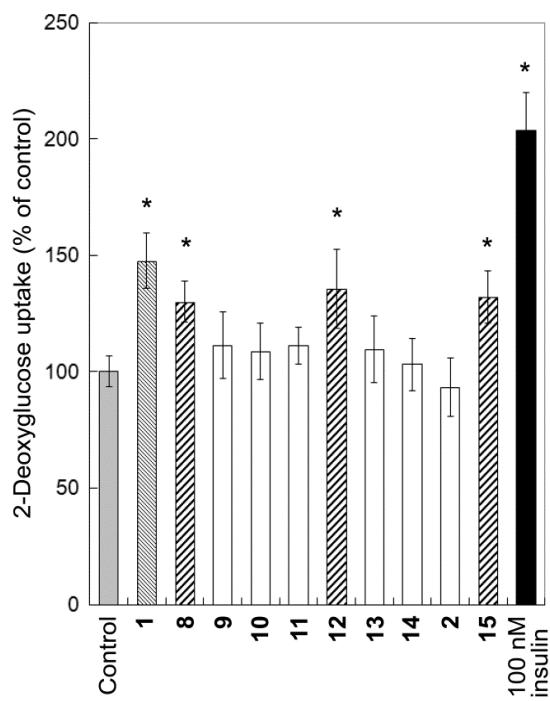
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6              Differentiated L6 cells were treated with 1 nM of each sample for 4 hours  
7       followed by 2-DG treatment for 30 min. Insulin-mimetic activity (i.e., 2-DG uptake) is  
8       expressed as a percentage of 2-DG uptake in control cells. Data are means  $\pm$  SEMs from  
9       five independent repeats of the experiment. \* denotes a statistically significant  
10      difference vs control ( $p \leq 0.05$ ).

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2



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4 Fig 1.

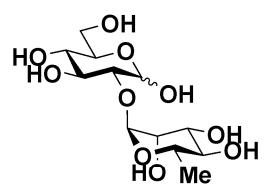
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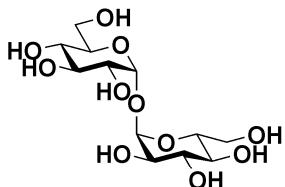
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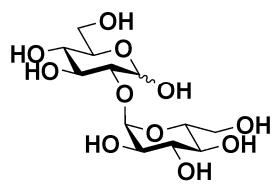
2 Structures



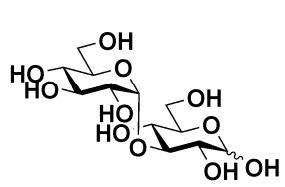
neohesperidose (1)



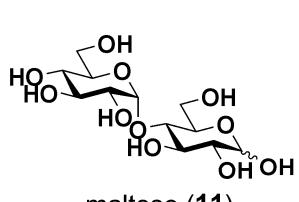
trehalose (8)



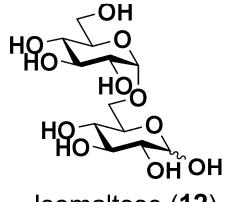
kojibiose (9)



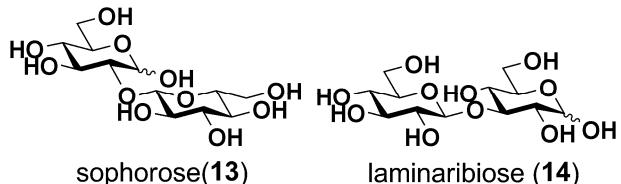
nigerose (10)



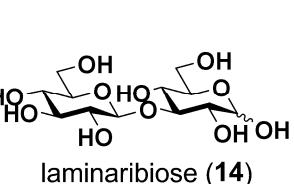
maltose (11)



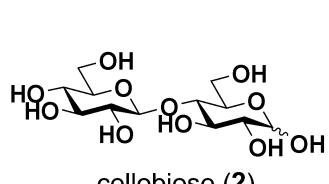
Isomaltose (12)



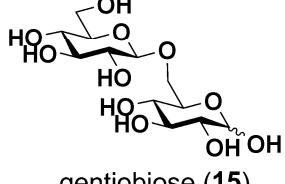
sophorose(13)



laminaribiose (14)



cellobiose (2)



gentiobiose (15)

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