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# Design and synthesis of new sweeteners*

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*Abstract*: Sweet taste induction by alkyl 2,3-di-*O*-(L-aminoacyloxy)-α-D-glucopyranosides requires a combination of hydrophobic α-alkoxy and hydrophilic vicinal, diequatorially oriented, L-aminoacyloxy units. Pyranoside chair conformations afford the preferred stereochemical arrangements of these residues for optimum interaction with the receptor. For the design of new sweeteners based on sweetness inhibitors, the introduction of a di-*O*-aminoacyloxy unit as the hydrogen-bonding component was applied to effect their intertransformation. Thus, the known sweetness inhibitor, methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside, was successfully transformed into sweet-tasting 2,3-di-*O*-(L-aminoacyl) derivatives. The inhibition of the 4,6-dichloro derivative is therefore competitive. Amongst the related amino-chloro-deoxysugars, methyl 6-chloro-6-deoxy-2,3-di-*O*-(L-alanyl)-α-D-glucopyranoside was found to be a full agonist. Our studies were then extended to disaccharide derivatives based on trehalose. This approach led to new highly intense sweeteners, as dimeric forms of the full agonist 2,3,2′,3′-tetra-*O*-(L-alanyl)-6,6′-dichloro-6,6′-dideoxytrehalose. The derivatives with effective hydrophobic groups on the C-6 and C-6′ positions, were found to be up to 800–1000 times sweeter than sucrose.

## INTRODUCTION

A bifunctional entity of $AH_s$ and $B_s$ as a proton-donating group and a proton-accepting group, respectively, act in conjunction with a hydrophobic site ($X_s$) as an essential molecular feature in sweet-tasting organic compounds, mostly discovered by serendipity. A clockwise arrangement of the $AH_s/B_s/X_s$ triad is required to allow a completion of three-point coupling with a reciprocal $AH_r/B_r/X_r$ function by stereospecific interaction with the receptor [1]. As an extension of this theory in the design of new sweeteners, we have investigated the structural requirement for sweet taste induction in 2,3-di-*O*-(L-aminoacyl) derivatives of methyl α-D-glucopyranoside (Fig. 1), whose substantial sweetness was found by Okai et al. [2]. They suggested that the sweet taste induction by these compounds depends upon the nature of the amino acid residues. Hence, only L-aminoacyloxy units, not their D-isomers, with small alkyl side-chains, were substantially sweet. One essential structural feature present in such sweet compounds is considered to be a combination of hydrogen-bonding components ($AH_s$) in the amino acid residues. This feature utilizes the sugar skeleton for a favorable stereochemical arrangement of hydrophilic amino acid residues for interaction with the receptor.

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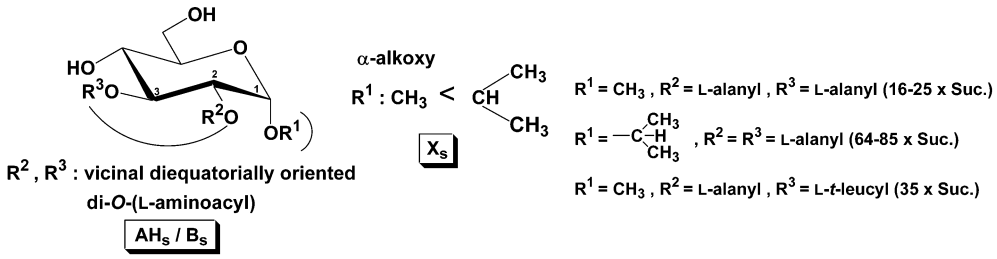


**Fig. 1** Alykyl 2,3-di-*O*-(L-aminoacyl)-α-D-glucopyranosides.

## STRUCTURAL FEATURES OF THE SWEET-TASTING AMINOACYLOXY PYRANOSIDES

In order to design new sweeteners, especially their stereochemical requirements, we have examined the structure–sweet taste relationships of a variety of aminoacyloxy sugars using the following guidelines. First, the vicinal di-*O*-(L-aminoacyloxy) unit was a fundamental structural requirement, since substantial sweetness disappeared when one of the two aminoacyloxy residues was either eliminated or substituted by a D-aminoacyloxy unit. Second, the β-anomer of the sweet-tasting glycosides was not sweet, showing the requirement for the α-anomer [3]. Finally, regiospecific sweet taste induction was only observed in the 2,3-di-*O*-aminoacyl derivatives, and not in the 3,4- and 4,6-di-*O*-aminoacyloxy derivatives. This requirement is even more stereospecific since sweet taste induction was detected only in diequatorial 2,3-di-*O*-(L-aminoacyl)-α-D-pyranoside structures, whereas 2,3-di-*O*-(L-aminoacyl) derivatives with an axial aminoacyloxy group resulted in the loss of sweetness. Sweet taste enhancement was observed by the introduction of moderately small hydrophobic alkyl groups into α-alkoxy anomers and also the side-chain of the amino acid moieties. Hence, minimum structural requirements in sweet-tasting aminoacyloxy pyranosides were determined as the α-alkoxy anomer and vicinal diequatorially orientated 2,3-di-*O*-L-aminoacyl groups (Fig. 1). This putative model was then applied to the design of new sweeteners from known sweetness inhibitors (Fig. 2).

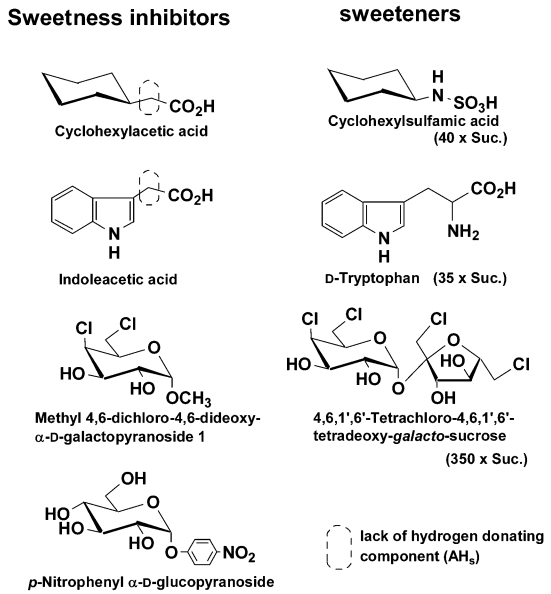


**Fig. 2** Sweetness inhibitors and related sweet compounds.

## SWEETNESS INHIBITORS

The existence of compounds capable of inhibiting the perception of sweetness has been known for many years, but they have not been the subjects of intense study. Lindley [4] suggested structural analogies between some sweetness inhibitors and sweeteners, with ligand–receptor interaction operating to induce their effects by a similar mechanism. Each retains the hydrophobic component ($X_S$). Sweetness inhibitors are generally considered to be structurally deficient, either lacking hydrogen-donating components ($AH_s$) or unable to make contact with the hydrogen-accepting component of the receptor ($AH_r$) for steric reasons [4]. The complete or partial inhibition by a chiral substance is highly dependent on the configurational structure of the inhibitor. This is best exemplified by the inhibitory activity of (*S*)-2-(4-methoxy)phenoxypropionic acid and the complete lack of inhibition by its (*R*)-isomer. If the sweetness-inhibiting (*S*)-isomer lacks a putative $AH_s$ component on the site where $CH_3$ is positioned, stereochemical arrangements of $B_s$ (COOH) and $X_s$ (phenyl) in the (*S*)-isomer are consistent with those in the sweet-tasting D-phenylalanine, whose L-isomer is not sweet (Fig. 3).

Amongst the inhibitors, methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside (**1**) [5], a component part of 4,6,1′,6′-tetrachloro-4,6,1′,6′-tetra-deoxy-*galacto*-sucrose (Fig. 2, –350 times sweeter than sucrose), was reported to decrease the physiological response against sucrose on gerbils [6] by competitive inhibition, due to lack of the hydrophilic interaction $AH_s$, although an allosteric effect was not excluded. The degree of inhibition was also established for humans, not only with sucrose but also with the sodium salt of saccharin, with acesulfame-K and with stevioside [7].

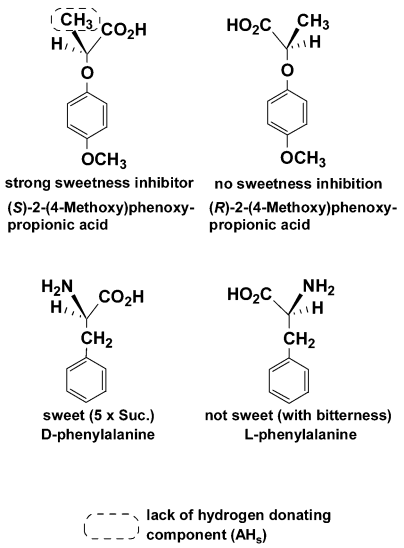


**Fig. 3** Stereochemical requirements for sweetness inhibition and sweet taste induction.

## TRANSFORMATION OF SWEETNESS INHIBITORS INTO SWEETENERS

If a sweetness inhibitor lacks the putative hydrogen-bonding component ($AH_s$), thus acting as an antagonist, structurally similar compounds having a suitable hydrogen-bonding unit ($AH_s$) should act as the agonist at the same receptor site. To test this theory and obtain further evidence for the inhibition mode of **1**, the introduction of vicinal diequatorial-di-*O*-(L-aminoacyl) groups into the sweetness inhibitor **1** was attempted.

In this way, the inhibitor **1** was successfully transformed into its sweet-tasting aminoacyloxy derivative **3**, which was up to 100 times sweeter than sucrose (Fig. 4). Similarly, another inhibitor *p*-nitrophenyl α-D-glucopyranoside [8], was also transformed into its sweet-tasting 2,3-di-*O*-(L-aminoacyl) derivative. 2,3-Di-*O*-(L-alanyl) derivatives of related chloro-deoxy-glycosides were synthesized, and their structure–sweet taste relationships investigated. The conversion of the α-anomer inhibitor **1** gave a sweet-tasting derivative **3**, whereas the weakly inhibiting β-anomer **2** yielded a less-potent sweetener **4**. The parallel relationships between sweetness inhibition and sweet taste induction suggested that these compounds acted as antagonists and agonists at the same receptor site. Moreover, in this series of compounds the highest potency of sweetness, 150 times sweeter than sucrose, was observed in methyl 2,3-di-*O*-(L-alanyl)-6-chloro-6-deoxy-α-D-glucopyranoside (**5**), which is structurally related to the transformed sweetener **3** [9], but lacks an axial 4-chloro group (Fig. 4). The structural analogies and taste characteristic relationships amongst the inhibitor **1** and its related *O*-aminoacyl-chloro-deoxypyranosides **3** and **5**, suggested that these compounds acted as the antagonist and the agonists, respectively, and further emphasized that the mode of sweetness inhibition by the inhibitor **1** was competitive.

X-ray crystallographic analysis of the putative partial agonist **3** as its dihydrochloride dihydrate showed the same dihedral angles between the 1-*O*-methyl carbon to O-5 through the anomeric carbon and between 6-chloro to O-5 through C-6 with those reported for the antagonist **1** (Fig. 5) [10]. Introduction of 2,3-di-*O*-(L-alanyl) groups into the antagonist did not alter the conformations of 6-*O*-methyl and 6-chloro derivatives, suggesting that the stereospecificities of the hydrophobic components are similar in both the antagonist and the agonist.

The two amino groups in the agonist **3** were closely positioned (4.7 Å between the two amino nitrogen atoms). This also suggested that the diequatorially-oriented 2,3-di-*O*-(L-aminoacyl) groups enabled both of the amino groups to be in close proximity and provided an excellent spatial arrangement of hydrogen-bonding components facing the receptor. The increased number of hydrophilic interactions presumably provides a more stable interaction between the sweetener and the receptor resulting in the high potency of sweetness. These successful transformations prompted us to design and search for other sources of new high-intensity sweeteners.

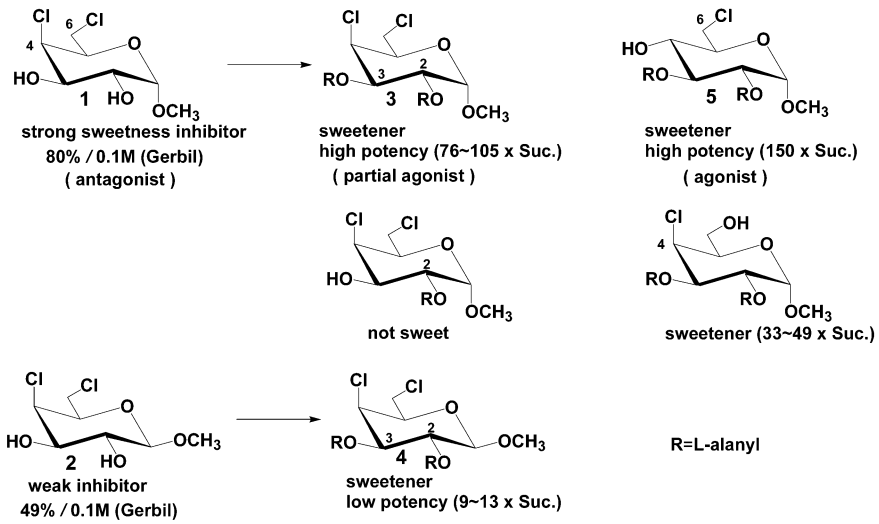


**Fig. 4** Synthesis of sweet compounds from reported inhibitors.

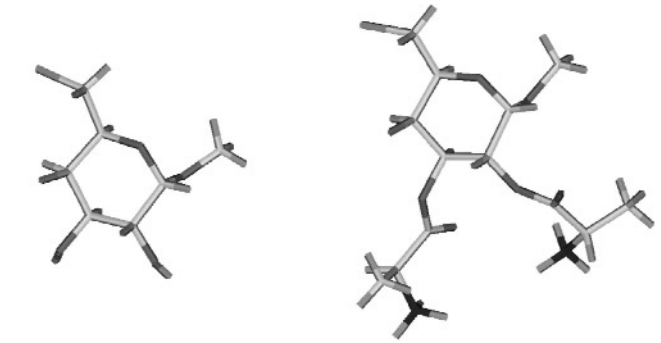


**Fig. 5** Conformations of inhibitor **1** and sweetener **3**.

## DESIGN AND SYNTHESIS OF AMINOACYL-DISACCHARIDE SWEETENERS

As one of the structural features in free disaccharides for sweet taste induction, α-linked maltose and α-trehalose are well known to be sweeter than the corresponding β-linked disaccharides cellobiose and lactose [11]. Suitable derivatives of α-linked disaccharides may provide increased hydrophilic bonding ($AH_s$) with those on the receptor ($AH_r$). Using the above concept (1 to 4) based on sweetness inhibitors, 4,6,4′,6′-tetrachloro-4,6,4′,6′-tetradeoxy-α-D-galactopyranosyl-α-D-galactopyranoside (**6**), a derivative of the known sweetness inhibitor was investigated.

The tetrachloro-tetradeoxydisaccharide **6** was found to suppress the perceived intensities of sucrose in our human psychophysical experiments under the procedure reported by Schiffman et al. [7]. Although **6** was less soluble in water than the monosaccharide sweetness inhibitor **1**, 0.008 M solution of **6** showed 20 % w/v reduction in the sweetness of a 15 % w/v sucrose solution. The suppression by the disaccharide derivative **6** appeared at a lower concentration (20–25 % in 0.008 M) when compared with that of the monosaccharide inhibitor **1** (32 % in 0.05 M). Introduction of vicinal diequatorially oriented di-*O*-(L-aminoacyl) groups into **6** led to a new highly potent sweetener, the 2,3,2′,3′-tetra-*O*-(L-alanyl) derivative **7**, up to 600 times sweeter than sucrose. Then we designed another new high-intensity sweetener, based on the dimeric form of the sweet-tasting di-aminoacyl-monosaccharide **5**, by the synthesis from α,α-trehalose of 2,3,2′,3′-tetra-*O*-(L-alanyl)-6,6′-dichloro-α-D-glucopyranosyl-α-D-glucopyranoside (**8**), which was up to 1000 times sweeter than sucrose. The antagonist and agonist relationships noted for **1**, **3**, and **5**, were also observed in the corresponding trehalose-derived disaccharide derivatives, **6**, **7**, and **8** (Fig. 6).

In order to obtain more effective hydrophobic interactions ($X_s$) for sweet taste induction, we examined related compounds by replacing 6-chloro groups by 6-methyl groups (6,7-dideoxyhepto pyranosides), which have a van der Waals radius similar to that of 6-chloro groups (Fig. 7). The observed high potencies of sweetness in the 2,3-di-*O*-(L-aminoacyl)-6,7-dideoxyheptopyranoside **9** and its dimeric trehalose derivative **10** proved that the 6-functionalities in these compounds are effective hydrophobic components (Fig. 7).

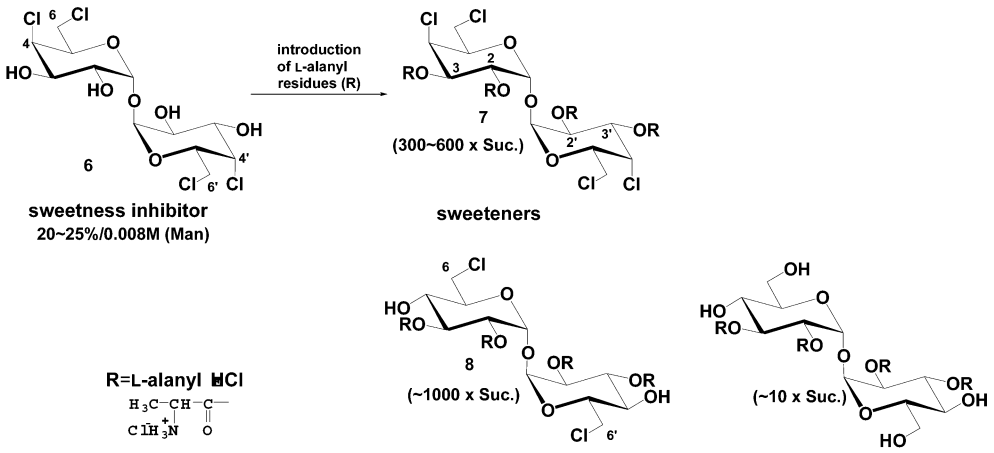


**Fig. 6** Transformation of di- and tetra-chloro-disaccharides into sweet compounds.

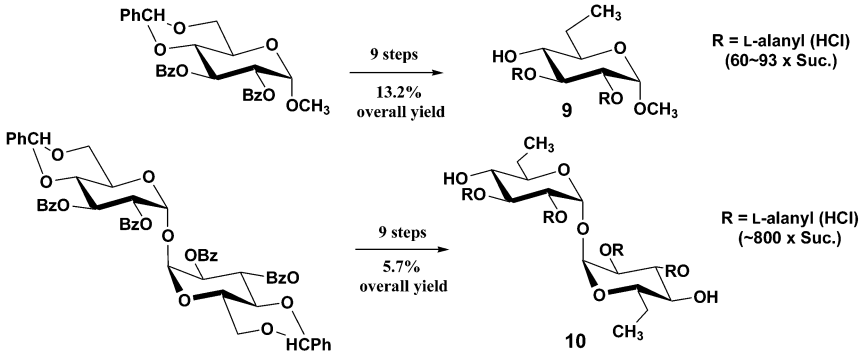


**Fig. 7** Synthesis of sweet 6-methyl derivatives (6,7-dideoxyheptopyranosides). Reagents: (i) $Et_3SiH$, $F_3CCO_2H$; (ii) MOM-Cl/$(iPr)_2NEt$, $CHCl_3$; (iii) $H_2$, Pd/C; (iv) DMSO, DCC, $H_3PO_4$; (v) $Cp_2Ti(Cl)CH_2AlMe_2$ (Tebbe reagent), THF, toluene; (iv) $H_2$, Pd/C; (vii) NaOMe/MeOH; (viii) Boc-L-alanine, EDCI, DMAP, $CH_2Cl_2$; (ix) HCl, dioxane.

## CONCLUSIONS

The transformation of sweetness inhibitors to sweeteners, based on their stereochemical analogy, presented here provides a new basis for the design of new high-intensity sweeteners. The effectiveness of a carbohydrate pyranoside skeleton for providing the stereospecifity for multicenter, multifurcated hydrogen bonds from the sweetener to the receptor has been demonstrated. Hence, the design, through the combination of multiple interaction units for both hydrophilic bonding ($AH_s$) and hydrophobic interaction ($X_s$), employs the pyranoside skeleton for the favorable stereochemical arrangement of these units for the interaction with the receptor and thereby achieves the required intensification of sweetness.

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