


 Cite this: *RSC Adv.*, 2021, **11**, 32236

Recent progress in the use of diaziridine-based sweetener derivatives to elucidate the chemoreception mechanism of the sweet taste receptor

 Makoto Hashimoto, ^{*a} Tomoya Nakagita ^b and Takumi Misaka ^c

All sweeteners are recognized by the sweet taste receptor (T1R2–T1R3). The elucidation of the chemoreception mechanism of receptor–ligand interactions is an attractive topic for researchers. Molecular biology and computational biology techniques can reveal the proposed mechanisms for this topic. Other approaches, including chemical biology (bioorganic chemistry), have helped to identify mechanisms on the basis of molecular structure. In this mini-review, we have summarized the recent progress in the synthesis of sweetener derivatives, which includes the use of photoaffinity labeling of diazirine-based derivatives to elucidate the chemoreception of sweeteners.

 Received 22nd June 2021
 Accepted 21st August 2021

DOI: 10.1039/d1ra024831g

rsc.li/rsc-advances

1. Introduction

Five taste modalities have been identified in humans: sweet, sour, bitter, salty, and umami.¹ Sweetness provides a means to

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

^bDepartment of Agricultural Chemistry, School of Agriculture, Meiji University, Kawasaki, Kanagawa 214-8571, Japan

^cDepartment of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

identify an energy source in the form of carbohydrates. A greater understanding of the chemoreception mechanism of sweet taste and the way in which sweet molecules interact with the sweet receptor will provide food scientists with the knowledge required to develop new sweeteners.

There are three major approaches to this research, as summarized in Fig. 1: (I) molecular biology; (II) computational biology; and (III) chemical biology (bioorganic chemistry).

(I) Molecular biology: the sweet taste receptor is a member of the family of class C G protein-coupled receptors (GPCRs) and forms a heterodimeric structure with T1R2 and T1R3 subunits.



Makoto Hashimoto was born in Hokkaido, Japan in 1966. He received both his B.S. (1989) and PhD (1995) from Hokkaido University and conducted his postdoctoral research at Toyama Medical and Pharmaceutical University (1995–1997) as a Research Fellow of the Japan Society for the Promotion of Science, and at the University of Bath, UK (1999–2000) as a Research Officer. In 1997, he

joined the bioorganic laboratory at Obihiro University of Agriculture and Veterinary Medicine until 2010. He was appointed associate professor from 2010 and professor from 2021 at Graduate School of Agriculture, Hokkaido University. His research is concentrated on defining and understanding the mechanisms of bioactive compounds with bioorganic methods.



Tomoya Nakagita was born in Mie, Japan in 1988. He received his B.S. (2010) from Tokyo University of Science and PhD (2015) from the University of Tokyo. He conducted his postdoctoral research at Kyoto University (2015–2019), and at Ehime University (2019–2021). He learned structural biology and antibodies production at these labs. In 2021, he joined a food science laboratory at

Meiji University as an assistant professor. His research is focused on food science, especially on the molecular mechanism of taste reception.



Each subunit has a large amino-terminal domain (ATD) linked by a cysteine-rich domain (CRD) at the extracellular site to a seven transmembrane helical domain (TMD).⁹ The human heterodimeric sweet taste receptor (hT1R2–hT1R3) responds to a wide variety of chemical substances, including naturally occurring sugars, glycosides, D-amino acids, and artificial chemical compounds, such as sucralose, aspartame, and saccharin.¹⁰ Although these sweeteners have various chemical structures, all the compounds bind to the same sweet taste receptor.^{8,11}

The sweet taste assay in combination with site-directed mutagenesis of the sweet taste receptors revealed the important amino acid residues for the chemoreception of each sweetener.

The structural features of the homodimeric metabotropic glutamate type 1 receptor (mGluR1) have been identified from X-ray crystal structure analysis, and this was the first example of the structure of a class C GPCR.¹²

(II) Computational biology: computational biology, based on homology modeling of the metabolic glutamate receptor (mGluR), can predict the 3D structure for the sweetener-sweet taste receptor complex.¹³ The structural homology between the sweet taste receptor and mGluR1 can predict important amino acid residues for the sweetener binding site from the mutagenesis studies and *in silico* structure modeling. Recent progress in the *in silico* analysis of the ligand–receptor binding modes afforded the useful information for the development of new sweeteners.

(III) Chemical biology (bioorganic chemistry): structure-based elucidation of sweeteners was performed to reveal the interactions of sweeteners with their binding sites. In 1967, Shallenberger and Acree proposed the AH–B theory for the structures of sweeteners. The sweetener must contain a glyco-phore, which consists of a hydrogen bond donor (AH) and a hydrogen bond acceptor (B) at a distance of 2.5 to 4 Å, and the glyco-phore can react with a complementary AH–B site on the

receptor with a pair of hydrogen bonds.² The theory was expanded by a third binding site (x) by Kier³ in 1972 and, subsequently, eight recognition sites were proposed by Nofre and Tinti in 1996.⁴

Based on these findings, the quantitative structure–activity relationship (QSAR) of sweeteners *in silico* is one of the methods for prediction.^{5–7} The models are based on the structure of binding ligands, but do not take into consideration the structure of the sweet receptor.⁸

The development of analytical methods for the study of the mature ligand–receptor binding complex may be one of the complementary methods to understand the mode of sweet receptor chemoreception. Chemical biology/bioorganic chemistry methods are used to elucidate the ligand–receptor binding complex.

The elucidation of protein function based on the structure–activity relationship is a major goal for scientists trying to understand the mechanisms of biological homeostasis. The understanding of the mechanism of the molecular interactions between small bioactive ligands and proteins is an important for rational drug design and discovery. Chemical methods provide an alternative route for the direct identification of target proteins in mixtures of biomolecules, as well as their ligand binding site structure, because these analyses are based on the affinity between the ligand and target protein.

Photoaffinity labeling^{14–17} significantly increased the specific capabilities of tagging. Photochemically generated highly

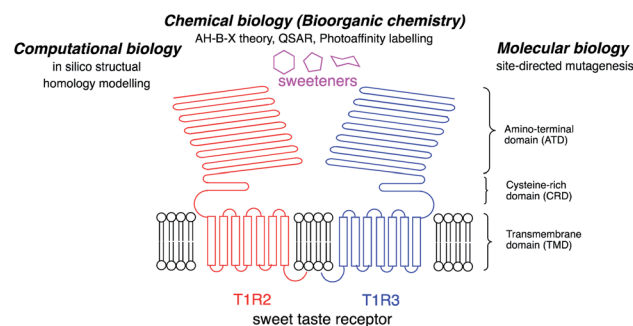


Fig. 1 Schematic structure of the sweet taste receptor and approaches to the structural analysis of the sweetener–sweet taste receptor interactions.



Takumi Misaka was born in Hyogo, Japan in 1971. He received both his B.S. (1993) and PhD (1998) from the University of Tokyo. He worked in a food company (Nissin Food Products Co. Ltd. in Tokyo, 1998–2000), and then became a postdoctoral fellow at the University of Tokyo (2000–2001). He became a research fellow of the Japan Society for the Promotion of Science, and then became a research associate of National Institute for Physiological Sciences in Okazaki. In 2005, he moved to the University of Tokyo, and was appointed an associate professor from 2008 at Graduate School of Agricultural and Life Sciences, the University of Tokyo. His research is focused on food science, especially on the molecular mechanism of taste reception.

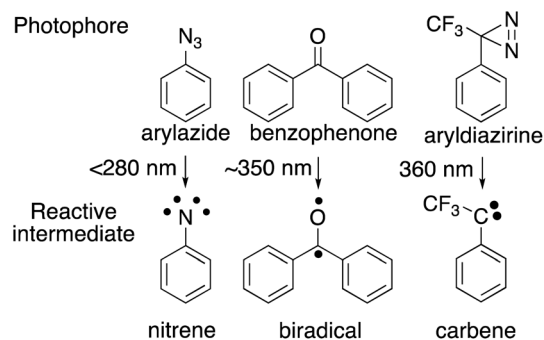


Fig. 2 Photochemical reactions of major photophores used for photoaffinity labeling.



reactive species introduce covalent bonds between the ligand and protein in a nonselective manner and any amino acid in the binding site can be tagged.

From these results, the researchers can select the most suitable approach to determine the sweet taste chemoreception mechanism, as summarized in Fig. 1.

The choice of photophore for effective photoaffinity labelling is important. Typically, aryl azide, benzophenone, and aryl diazirine are used (Fig. 2). Aryl azide is photoactivated by wavelengths below 300 nm, which sometimes causes damage to biomolecules, and generates nitrene¹⁸ as an active species, which is sometimes rearranged to the undesirable side product of ketenimine.¹⁹ Benzophenone²⁰ is photoreactivated at wavelengths over 350 nm and generates the reactive triplet states of carbonyl, which regenerates the ground-state carbonyl compounds and can be re-used for photolabeling; however, the photophore sometimes requires a long photoirradiation period for labeling.

Trifluoromethylphenyldiazirine²¹ is also photoreactivated with by wavelengths over 350 nm and generates carbene, which is more reactive than other photophores species, rapidly forming crosslinks to biomolecules with short photoirradiation. Comparative irradiation studies of these three photophores in living cells suggested that the irradiation to generate the active species from azide and benzophenone caused cell death during photolysis because of the long irradiation time needed to incorporate the photophore to the cell membrane surface. In contrast, the carbene precursor (3-trifluoromethyl)phenyldiazirine (TPD) did not promote cell death during the generation of active species. These results indicated that photoaffinity labeling with TPD was the most promising strategy for the investigation of labeled components.

In this mini-review, we have summarized the recent progress in the synthesis of sweetener derivatives, which includes the use of the photoaffinity labeling of diazirine-based derivatives to elucidate the chemoreception of sweeteners.

2. Photoaffinity probes for sweetener derivatives

2.1. Sucrose

Sucrose, which is found in an abundance of natural sources, is recognized as sweet; however, not all sucrose and fructose derivatives are sweet, especially if a substitution is made on one hydroxyl group. The detailed relationship between the structure and biological activity has not been completely clarified. Chemical designs are important when photoaffinity labeling is used to perform a functional analysis of sweetness. It was found that several sucrose-based oligosaccharides have a sweet taste and 1-kestose (GF2), which has an additional fructose, was linked at the 1'-position of the fructose unit of sucrose and acted as a sweetener.²² The results indicated that the 1'-position of the sucrose could accept substitutions. Although selective esterification of 1'-position was achieved by biotransformation,²³ the ester linkage was easily hydrolyzed under physiological conditions and metabolized during analysis. In contrast, ether (phenoxy) linkages appear more stable than esters for functional analysis.²⁴ In general, the reactivity of sucrose primary alcohols toward halogenation was greater for 6- and/or 6'-modification, and lower for the neopentyl-like 1'-position.²⁵

The Mitsunobu reaction is one of the most popular reactions for the formation of ether linkages between different alcohols.²⁶ The *o*- or *p*-nitro substituted phenols, which contain strong electron-withdrawing functional groups, were better substrates for phenoxy modifications at the 1'-position of heptaacetyl sucrose (**1**) over 85% yield. Although the trifluoromethylphenyldiazirine derivatives (**2** and **3**) had moderate electron-withdrawing properties, the Mitsunobu reaction conditions promoted the decomposition of the diazirine three-membered ring and the desired products (**4** and **5**) were afforded at a yield of less than 10%. No improvement was observed for phenoxylation at the 1'-position of heptaacetyl sucrose with various reaction conditions (temperature, amount of reagents, and reaction time) (Fig. 3A).²⁷

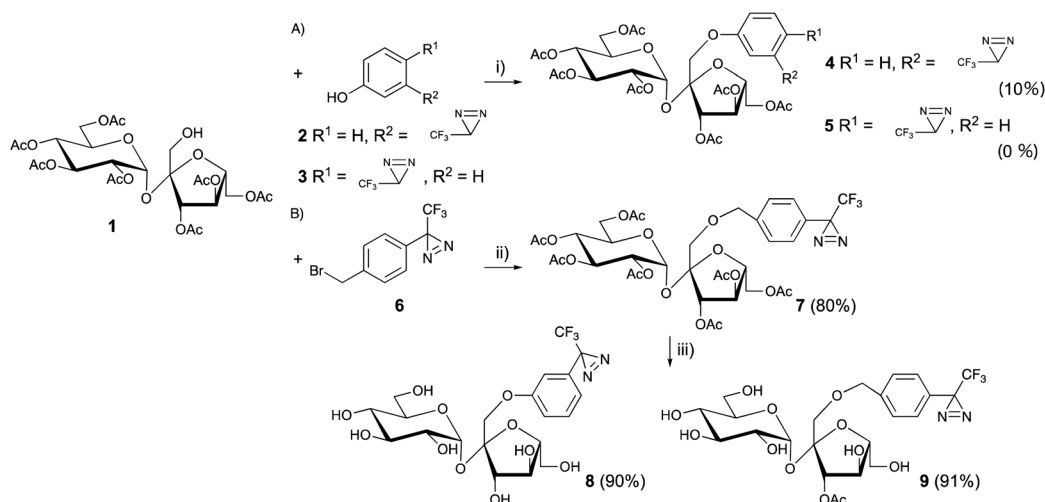


Fig. 3 Synthesis of trifluoromethylphenyldiazirine-modified sucrose derivatives at the 1'-position. (i) **2** or **3**, DIAD, Ph_3P (each 7.5 eq.), toluene, 60 °C, 24 h, (ii) **4** (2 eq.), Ag_2O (3 eq.), MS-4A, CH_2Cl_2 : *n*-hexane = 1 : 4, 60 °C, N_2 , 16 h, (iii) NH_3 - CH_3OH , rt, 12 h.



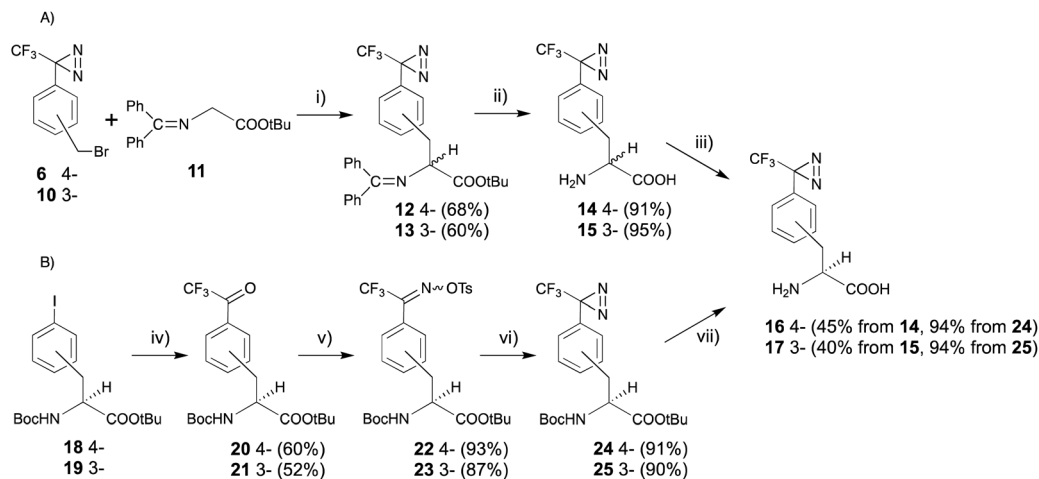


Fig. 4 Synthesis of (3- and 4-trifluoromethyldiazirinyloxy)-D-phenylalanine derivatives. (i) CsOH, Bu₄NBr, CH₂Cl₂, rt, 12 h, (ii) TFA, rt, 2 h, (iii) L-amino acid oxidase, pH 7.0, 37 °C, 12 h, (iv) (1) MeLi, (2) tBuLi, (3) CF₃CO₂Et, toluene, -78 °C, 12 h, (v) (1) NH₂OH·HCl, pyridine, 70 °C, 1.5 h, (2) TsCl, TEA, acetone, rt, 1 h, (vi) NH₃ (l), ether, 80 °C, 9 h, (vii) TFA, rt, 8 h.

However, it has been reported that the more sterically hindered neopentyl-like 1'-hydroxyl modification of sucrose exhibited the lowest reactivity among the primary hydroxyls in many reactions,^{25,28,29} which confers difficulties to the modification of the 1'-position of sucrose.

Benzyl ether modifications at the 1'-position with benzyl halide and silver oxide were performed to overcome the lower reactivity of the 1'-hydroxyl group in sucrose.

1'-Hydroxy heptaacetyl sucrose **1** and the diazirinyl benzyl halide derivative **6** were reacted in the presence of Ag₂O in CH₂Cl₂, which is a common solvent in carbohydrate chemistry. A large excess of diazirinyl benzyl halide derivative (10 eq.) and Ag₂O (15 eq.) at 60 °C for 24 h afforded a moderate yield (~60%) for benzylation.³⁰ However, the conditions with large excess were not suitable for rare benzyl halide derivatives. A detailed analysis of the reaction mixture revealed that the benzyl alcohol derivatives were generated under the reaction conditions. The hydrolysis was inhibited in the hydrocarbon solvents of (cyclo)hexane and *n*-pentane. However, the solubility of the carbohydrate derivatives was quite low in these solvents. The use of a co-solvent in (cyclo)hexane and CH₂Cl₂ (4 : 1) improved the benzylation over 80% in the presence of diazirinyl benzyl halide derivatives (2 eq.) and Ag₂O (3 eq.) at 60 °C under an N₂ atmosphere (Fig. 3B).³¹ Subsequently, deprotection of the acetyl groups in both **5** and **7** afforded the 1'-diazirinyloxy sucrose derivatives **8** and **9**.

2.2. D-Amino acids

As the receptor distinguishes D- and L-amino acids, with a preference for D-amino acids,^{32–34} the structural relationships between D-amino acids and other sweeteners and the structural features of D-amino acid derivatives that favor the activation of the sweet taste receptor have been studied using conformational analysis by crystallography, NMR analysis, and molecular modeling. However, it is still difficult to understand the receptor-bound conformations of the sweeteners, as the

structural information on the ligands complexed with the receptor is limited.

2.2.1. Phenylalanine. Diazirinyloxy phenylalanine derivatives are prepared from the corresponding benzyl bromide derivatives with glycine derivatives (**1**) under racemic conditions, followed by enzymatic resolution; and (**2**) in an asymmetric manner with chiral auxiliary and/or sterically hindered amino acid derivatives. A few cases of the asymmetric synthesis of 4-(3-trifluoromethyl)-diazirinyloxy L-phenylalanine derivatives have been reported.^{35–40} However, D-selective synthesis has not been reported. 3- or 4-Trifluoromethyldiazirinyloxy benzyl bromide (**10** and **6**) were condensed with glycine derivative **11** to afford the phenylalanine skeleton (**12** and **13**) with moderate yields in a racemic manner. After deprotection, the racemic mixtures (**14** and **15**) were subjected to enzymatic resolution with L-amino acid oxidase⁴¹ to afford D-isomers (**16** and **17**) (Fig. 4A).

Table 1 Sweet-taste assay for trifluoromethyldiazirinyloxy D-phenylalanine derivatives. Sweetness potential for known chemicals and synthetic photoreactive compounds. Representative ratiometric images of cells, which consist of the calcium ion indicator (fura-2)-loaded HEK293T cells co-expressing hT1R2–hT1R3 and G16-gust44, were defined as responding positively when the F340/F380 ratio increased after addition of the reagent. The degree of cell response, represented as the number of positively responding cells at 1.25 mM of each chemical against the standard response, was normalized to 10 mM aspartame

	Normalized response
Aspartame	0.83 ± 0.20
D-Tryptophan	0.57 ± 0.06
D-Phenylalanine	0.14 ± 0.12
D-Phenylalanine (4-dia) 16	0.72 ± 0.12
D-Phenylalanine (3-dia) 17	0.97 ± 0.02
16 + lactisole	0
17 + lactisole	0



Recently, our group reported a trifluoromethyldiaziriny moiety constructed on aromatic chiral phenylalanine derivatives in a stereocontrolled manner. The 3- or 4-iodo derivatives with an alkaline-stable protecting group at both the N- and C-termini (**18** and **19**) were converted to the trifluoroacetyl derivatives (**20** and **21**), which were subjected to oximation and *o*-tosylation (**22** and **23**), successively. One-pot conversion, which consisted of the construction and oxidation of the diaziridine in liquid ammonia can be applied to the phenylalanine derivatives without racemization (**24** and **25**). The one-pot reaction with improved reagents, LiNH₂, promoted racemization of the α -amino acid moiety. Acidic deprotection afforded desired products (**16** and **17**) (Fig. 4B).⁴² The synthetic route was applied for the synthesis of deuterated derivatives from the deuterated phenylalanine derivatives.⁴³ The trifluoromethyldiazirinyl *D*-phenylalanine derivatives (**16** and **17**) had stronger sweetness than unmodified *D*-phenylalanine and tryptophan, and the same as that of 1.25 mM aspartame. The responses were drastic decrease in the presence of lactisole, which is an inhibitor of sweetness (Table 1).⁴⁴

A complex model of *D*-phenylalanine in the ATD of the hT1R2 has been constructed using the crystal structure of the ATD of the metabotropic glutamate receptor type-1 (mGluR1).¹² *D*-Phenylalanine was docked into the binding site with a guide of the salt bond between the amino group of the ligand and Glu302 of the receptor, which is conserved and crucial in binding in mGluR1 and was suggested to be crucial for the binding of some sweeteners, such as aspartame, by mutation experiments.¹¹ The phenyl group bound a hydrophobic pocket, which also binds a larger indole moiety of *D*-tryptophan. The docking of the designed diazirinyl *D*-phenylalanines at the same site suggested that the receptor accepts the diazirinyl moiety in the binding pocket.⁴⁴

2.2.2. Tryptophan. Tryptophan is one of the most biologically significant metabolites synthesized from indole. Although the synthesis of tryptophan has been reported from various aromatics,⁴⁵ these methods are too difficult to apply to the diazirinyl derivatives, because of the harsh conditions required for the construction of tryptophan skeletons. For example, (1) the Larock heteroannulation or Mori-Ban-Hegedus indole

synthesis of an *o*-iodoaniline skeleton with Schöllkopf reagent,⁴⁶ (2) the Heck-type synthesis of an *o*-iodoaniline skeleton with pyroglutamate derivatives,⁴⁷ and (3) the Fischer indole synthesis of phenyl hydrazones⁴⁸ were ineffective when starting with diazirinyl derivatives, as the diazirinyl moiety was decomposed during the reactions. The construction of a trifluoromethyldiazirinyl moiety on indole at the 5- or 6-position was started by treating the corresponding bromoindole derivatives (**26** and **27**) with potassium hydride – *t*-BuLi followed by treatment with trifluoroacetylated reagents. The trifluoroacetylation of 5- or 6-bromoindole with ethyl trifluoroacetate or trifluoroacetyl piperidine improved the yield of the products (**28** and **29**), because the leaving groups did not decrease the basicity of the reaction mixture. The trifluoroacetyl groups were converted to oximes with hydroxylamine hydrochloride in pyridine (**30** and **31**), followed by tosylation with tosyl chloride in triethylamine and acetone at 0 °C. The tosylation with tosyl chloride in pyridine under reflux was not acceptable because the product was broken down under these conditions. The isolated yield for **32** and **33** dramatically decreased (yield of purified tosyl oxime **32**: 28%) owing to the instability of the tosyl oxime of the indole. To avoid the decrease in yield, the tosyl oximes **32** and **33** were not isolated and the reaction mixtures were directly subjected to conversion to diaziridine (**34** and **35**) with liquid ammonia. This modification drastically improved the yield (84–87% for the two steps). The oxidation of diaziridine to diazirine (**36** and **37**) with activated MnO₂ can occur without any side reactions in a good yield (80–92%).

Tryptophan has been synthesized from an indole with serine in acetic acid and acetic anhydride under reflux conditions.⁴⁹ In the original report, the active species were generated at high temperatures in the presence of indole derivatives. The diazirinyl moieties of **36** and **37** were also decomposed in acetic acid under reflux conditions. The tryptophan skeletons were constructed following a detailed analysis of the reaction mixture. The active species were generated from L-serine, acetic anhydride, and acetic acid in reflux conditions without indole derivatives. The generated active species could react with diazirinyl indoles at low temperature to prevent the decomposition of the diazirinyl ring. The racemate of diazirinyl *N*-acetyl

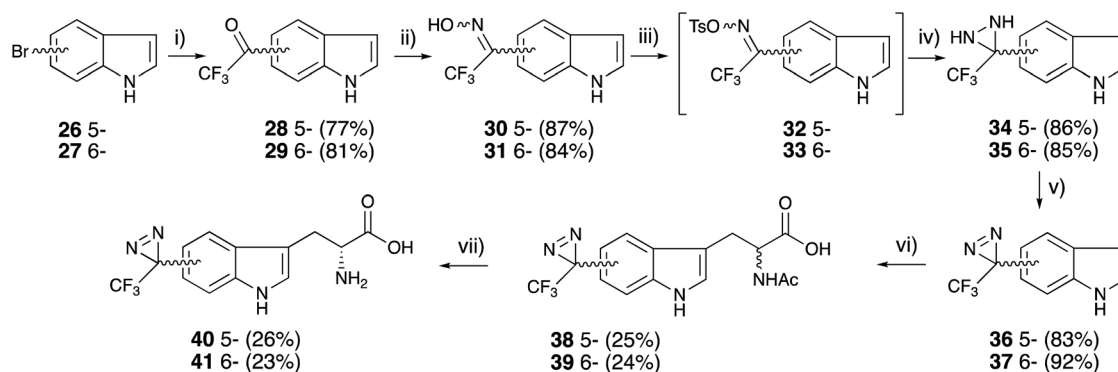


Fig. 5 Synthesis of (5- and 6-trifluoromethyldiazirinyl)-*D*-tryptophane derivatives. (i) (1) KH, *tert*-BuLi, (2) trifluoroacetyl piperidine, THF, –78 °C, 2 h, (ii) NH₂OH·HCl, pyridine, 80 °C, 3 h, (iii) TsCl, TEA, acetone, 0 °C, 1 h, (iv) (1) NH₃ (l), ether, –78 °C, (2) rt, 6 h, (v) MnO₂, ether, rt, 1 h, (vi) (1) L-serine, acetic anhydride, acetic acid, 75 °C, 1 h, (2) rt, 1.5 h, (vii) *D*-aminoacylase, sodium phosphate pH 7.6, CoCl₂, 37 °C, 24 h.





Fig. 6 Synthesis of *o*-(3- and 4-trifluoromethyldiaziriny)benzyl-D-serine derivatives. (i) NaH (3.3 eq.), DMF, 0 °C, 1 h, (ii) diazirinyl benzyl bromide **4** or **10** (1.5 eq.), DMF, rt, 15 min, (iii) 4 M HCl-dioxane, rt, 1.5 h.

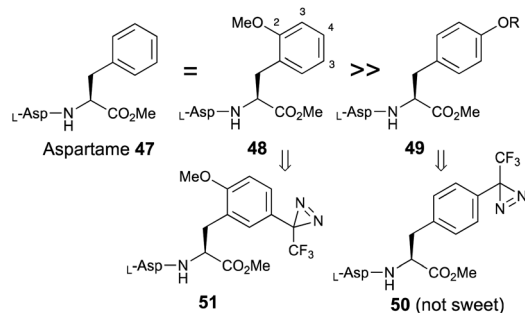


Fig. 7 Effects of substitution of an aromatic moiety on phenylalanine on the sweetness of aspartame.

tryptophan derivatives **38** and **39** was subjected to enzymatic resolution with D-aminoacylase to afford optically pure diazirinyl D-tryptophan (**40** and **41**) without the decomposition of the diazirinyl moiety (Fig. 5).⁵⁰

2.2.3. *o*-Benzyl-D-serine. D-Serine has been reported to be a weaker sweetener than the aromatic amino acids (D-Phe and D-Tyr).⁵¹ It was predicted that the bulky substitutions at β-OH of D-serine gave acceptable sweet potency from the molecular modeling of human heterodimeric sweet taste receptors hT1R2–hT1R3.⁵² Substituents that afforded hydrophilic interactions between the receptors were preferred at the side chain of amino acid. The above results led us to synthesize trifluoromethylphenyldiaziriny derivatives for photoaffinity

labeling. However, bulky substituents must sometimes be introduced to the parent skeleton. The introduction of the photophore to the benzene ring of *o*-benzyl-D-serine appears to be the minimum substitution for the alterations. Several papers on the synthesis of *o*-benzyl-D-ser have been reported by the chemical⁵³ or enzymatic^{54,55} resolution of the racemate, and by the enantioselective synthesis with chiral auxiliary reagents.⁵⁶ The resolution of the racemate is a waste of the opposite isomer and the enantioselective synthesis prepares benzyl halomethyl ether derivatives under acidic conditions to construct the *o*-benzyl moiety. It is difficult to apply these synthetic methods to the trifluoromethylphenyldiazirine derivatives and avoid breaking the trifluoromethyldiaziriny moiety during these conditions. These disadvantages encouraged us to select other routes for the synthesis of *o*-diazirinybenzyl-D-ser derivatives under mild conditions (Fig. 6).

Boc-D-serine (**42**) was chosen for the starting material for the preparation of the *o*-benzyl serine derivative. First, the alkoxide ion generated from the hydroxyl group of a side chain, followed by the reaction with benzyl halide derivatives. A detailed analysis revealed that the chemical yield was influenced by the proportion of reagents and reaction time for each step. Sodium hydride treatment was preferred over an hour with 3.3 equivalents and the benzylation step was optimized within 15 min with 1.5 equivalents of benzyl bromide derivatives at room temperature. There was no difference in the benzylation between stereoisomers. Boc-D-serine (**42**) was treated with NaH (3.3 eq.) in DMF for 60 min, followed by the reaction with diazirinyl benzyl bromides **4** or **10** (1.5 eq.) within 15 min to afford *o*-benzyl-D-serine derivatives **43** and **44** with good yields (>85%) (Fig. 5).⁵⁷

2.2. Aspartame

Aspartame (L-Asp-L-Phe-OMe, **47**) is one of the most well-known artificial sweeteners. Based on structure–activity relationships, the substitution of a methoxy group at the 2-position of phenylalanine (**48**) did not reduce the sweetness and 3-substitution also had less influence on sweet potency compared with aspartame.⁵⁸ In contrast, substitution at the 4-position (**49**) dramatically decreased the sweetness, and the 4-diaziriny

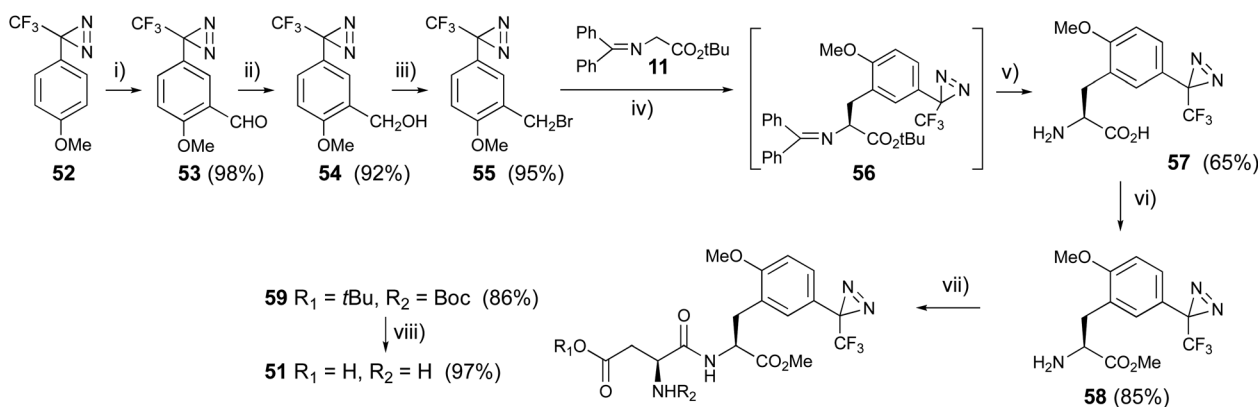


Fig. 8 Synthesis of trifluoromethyldiaziriny aspartame derivative. (i) Cl₂CHOMe, TiCl₄, 0 °C, 1 h, (ii) NaBH₄, EtOH, rt, 4 h, (iii) CBr₄, Ph₃P, CH₂Cl₂, rt, 4 h, (iv) phosphazene base BTPP, *o*-allyl-*N*-(9-anthracenylmethyl)chinchonidium bromide, CH₂Cl₂, –78 °C, 8 h, (v) TFA, 0 °C, 2 h, (vi) SOCl₂, MeOH, rt, 8 h, (vii) *N*-Boc-L-Asp(OtBu)OSu, (*i*-Pr)₂NEt, THF, rt, 8 h, (viii) TFA, 0 °C, 3 h.



aspartame derivative (**50**) had no sweet taste, as shown in a pioneering work on diazirine-based photoaffinity labeling.³⁶ These results indicated that the substitution of 2-methoxy-5-trifluoromethyl diazirinyl on phenylalanine (**51**) maintained sweetness (Fig. 7).

4-Methoxy-trifluoromethylphanyldiazirine (**52**) was prepared from 4'-methoxy-2,2,2-trifluoroacetophenone with general methods for diazirine construction.^{59,60} The formyl group was introduced by a Friedel-Crafts reaction with dichloromethyl methyl ether in the presence of titanium chloride at 0 °C. Benzaldehyde aldehyde derivative (**53**) was reduced with sodium borohydride to afford benzyl alcohol (**54**), which was converted to benzyl bromide derivatives (**55**) with carbon tetrabromide and triphenylphosphine. The diazirinyl benzyl bromide (**55**) was subjected to asymmetric synthesis with glycine derivative (**9** → **11**) in the presence of the phosphazene base BTTP and catalytic amounts of *o*-allyl-*N*-(9-anthracenylmethyl)chinchonidium bromide at -78 °C.⁶¹ The condensed product (**12**) was slightly decomposed during silica gel-column chromatography, so the reaction mixture was directly deprotected with TFA to afford a new photoreactive L-phenylalanine derivative (**57**). The chirality of the synthesized **57** was analyzed using chiral HPLC, to reveal an ee of over 96%. The diazirinyl phenylalanine (**57**) was converted to the methyl ester (**58**) with thionyl chloride in methanol, then reacted with *N*-Boc-L-Asp(*O**t*Bu)-OSu in the presence of diisopropyl ethyl amine at room temperature, followed by the deprotection of protecting groups with TFA to afford the new photoreactive aspartame derivative (**51**) in good yield (Fig. 8). The synthesized photoreactive aspartame derivative **51** was subjected to preliminary sensory evaluations using a filter-paper disk test.⁶² The synthetic compound had lower sweet potency than the parent compound aspartame, but a slightly higher sweet potency than sucrose.

2.3. Saccharin

Saccharin is one of the most common artificial sweeteners in the world and is several hundred times sweeter than sucrose. Suami *et al.* reported on several structure-activity relationships for saccharin, and found that substitutions at the 5- and 6-positions in saccharin were tolerated with regards to its biological activities.⁶³ In addition, some sweet compounds also interact with other taste modalities. 3-(*p*- or *m*-Tolyl)-3-(trifluoromethyl)diazirines (**60** and **61**) were subjected to aromatic chlorosulfonation at adjacent positions of the toluene methyl group with chlorosulfonic acid at -20 °C. The addition of the strong acid at a higher temperature promoted decomposition of the diazirinyl ring.^{43,64} The sulfonyl chloride was converted to *N*-alkylated sulfonamide using *t*BuNH₂ at room temperature. The reaction in the triethylamine and *t*BuNH₂ system both 2 equivalents; experimental conditions reported⁶⁵ afforded a more complex mixture than that in *t*BuNH₂ only (4 equivalents). Oxidative cyclization with H₅IO₆ and CrO₃ between the methyl group and *o*-oriented *N*-*tert*-butyl sulfonamide synthesized the saccharin skeleton (**62** and **63**).

The purification for each step generated lower isolated yields for the cyclized compounds **62** and **63** (less than 10%). We

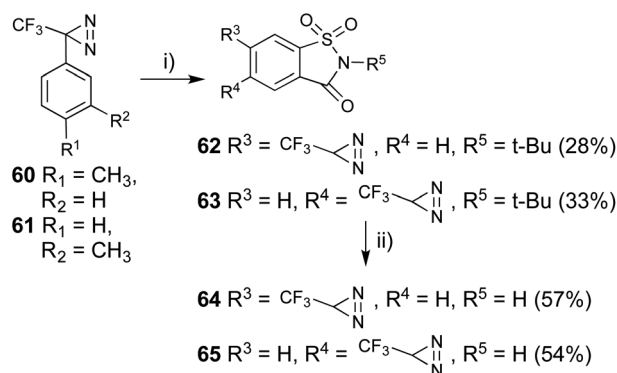


Fig. 9 Synthesis of 5- or 6-trifluoromethyl diazirinyl saccharin derivatives. (i) (1) ClSO₃H, TiCl₄, -20 °C, 1 h, then rt, 4 h, (2) *t*-BuNH₂, CH₂Cl₂, 0 °C, 2 h, then rt, 22 h, (3) H₅IO₆, CrO₃, Ac₂O, CH₃CN, 0 °C, 0.5 h, then rt, 48 h, (ii) CF₃COOH, reflux, 24 h.

Table 2 Sweet taste assay for trifluoromethyl diazirinyl saccharin derivatives. Sweetness for known chemicals and synthetic photoreactive compounds. The degree of cell response, presented as the number of positively responding cells in response to 10 mM of each chemical, is normalized to the response to 10 mM aspartame

	Normalized response
Saccharin	0.86 ± 0.17
Saccharin (6-dia) 64	0.64 ± 0.30
Saccharin (5-dia) 65	0.81 ± 0.05
64 + lactisole	0
65 + lactisole	0

performed these three steps as one-pot reactions (~30% yields). The deprotection of the *t*Bu group with TFA under reflux conditions afforded diazirinyl saccharin derivatives (**64** and **65**, Fig. 9). The synthesized photoreactive saccharin derivatives were insoluble in water. We performed further purification of the synthesized saccharin derivatives by converting them to the corresponding sodium salts with aqueous sodium hydroxide.

The synthesized photoreactive saccharin derivatives were subjected to preliminary gustatory receptor assays at 10 mM. Saccharin, compounds **64** and **65** have 90%, 80%, and 65% relative sweetness activity, respectively, against the same concentration of aspartame.^{52,66} The response to the synthetic compounds was completely inhibited by addition of lactisole, which is one of the specific inhibitors in the sweet taste assay (Table 2).⁶⁷

2.4. 2-Propoxyaniline

5-Nitro-2-propoxyaniline (**66**, Fig. 9) has a sweetness intensity that is approximately 4000-times greater than that of sucrose and is commonly considered as one of the strongest artificial sweeteners.⁶⁸ Despite previous use as an artificial sweetener, it has been banned because of its possible toxicity.⁶⁹ The mechanism of sweetness and toxicity of 5-nitro-2-propoxyaniline has still not been studied. There have been only a few studies using homology modeling to determine the mechanism of action of 5-nitro-2-propoxyaniline at the gustatory receptor.^{70,71} According



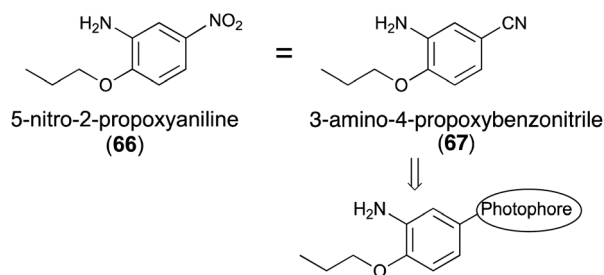


Fig. 10 Substitution effects of the aromatic moiety of the sweetness of 5-nitro-2-propoxyaniline (66) and its derivatives.

to a previous report,⁷² 3-amino-4-propoxybenzonitrile (67), in which the nitro group of 66 was substituted by a cyano group, resulted in almost no decrease in sweetness.

To the best of our knowledge, the synthesis of photoreactive-2-propoxyaniline derivatives for photoaffinity labeling has not yet been reported. The introduction of the diazirinyl photophore at the 5-position of 2-propoxyaniline may be one possible reaction to the introduce photophores into the ligand skeleton (Fig. 10).

4-Propoxybenzaldehyde (68) was treated with $\text{CF}_3\text{-TMS}$ ⁷³ and worked up with 1 M HCl to construct the 2,2,2-trifluoro-1-phenylethan-1-ol skeleton (69), then oxidized with Dess–Martin periodinane⁷⁴ to afford the trifluoroacetophenone derivative (70) in excellent yield. Nitration of compound (70) with fuming HNO_3 in acetic anhydride at 0 °C proceeded selectively (71). The trifluoroacetyl moiety was converted to the corresponding oxime (72) and *o*-tosyloxime (73) to diaziridine (74) using a general method.⁷⁵ The reduction of the nitro group with $\text{Na}_2\text{S}_2\text{O}_4$ (ref. 76) to amine (74) occurred more quickly than oxidation of diaziridine (75) to diazirine (76) (Fig. 11).⁷⁷

3. Photoaffinity probes for inhibition (negative allosteric modulation) of the sweet taste receptor

3.1. Lactisole ((±)-2-(4-methoxyphenoxy)-propionic acid)

Lactisole, which is known as an inhibitor (negative allosteric modulator; NAMs) of the human sweet taste receptor, has a 2-phenoxypropionic acid skeleton and has been shown to interact

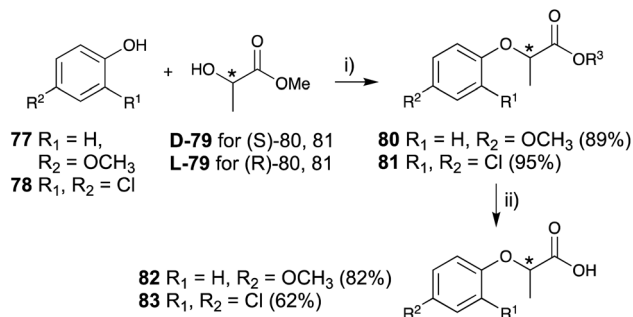


Fig. 12 Asymmetric synthesis of lactisole and (2-(2,4-dichlorophenoxy)propionic acid) derivative. (i) (1) PPh_3 , CH_2Cl_2 , 0 °C, 10 min, (2) DEAD, rt, 12 h, (ii) K_2CO_3 , CH_3OH , H_2O , reflux, 2 h.

with the transmembrane domain of the T1R3 subunit (T1R3-TMD) of the receptor. Lactisole was first isolated from roasted coffee beans,⁷⁸ and has naturally occurring optical isomers owing to the lactic acid structure present in the molecule. In general, *L*-lactic acid [(*S*)-lactic acid] is more abundant in plants than *D*-lactic acid, so the (*S*)-isomer of lactisole is predominant in roasted coffee beans.⁷⁹

The parent skeleton, 2-phenoxypropanoic acid, is found in several phenoxy herbicides, such as 2-(2,4-dichlorophenoxy)propanoic acid (dichlorprop, 2,4-DP) 2-(4-chlorophenoxy)propanoic acid (4-CPP),⁸⁰ and the (*R*)-isomers that have predominantly herbicide activities. Racemic lactisole and 2,4-DP (2-(2,4-dichlorophenoxy)propionic acid) are also used as NAMs for the sweet taste receptors owing to their specific interaction with T1R3-TMD;⁸⁰ thus, the detailed functional analysis of the stereocenter may be important to elucidate the mechanism of sweet taste chemoreception.

The key steps in the construction of a lactisole and 2,4-DP skeleton in an asymmetric manner are achieved by the Mitsunobu reaction of 4-methoxyphenol (77) or 2,4-dichlorophenol (78) and optically pure methyl lactate (79), followed by hydrolysis to afford optically pure lactisole (82) and 2,4-DP (83), respectively (Fig. 12).

The (*S*)-isomers of both lactisole and 2,4-DP showed potent inhibitory activities with the following IC_{50} values: 20 μM for (*S*)-lactisole, 2.6 μM for (*S*)-2,4-DP, and 45 μM for (*R*)-2,4-



Fig. 11 Synthesis of 2-propoxy-5-trifluoromethyl diazirinyl aniline derivative. (i) (1) TMS-CF_3 , CsF , THF, rt, 4 h, (2) 1 M HCl, rt, 6 h, (ii) Dess–Martin periodinane, TFA, CH_2Cl_2 , rt, 12 h, (iii) HNO_3 , Ac_2O , 0 °C, 40 min, (iv) $\text{NH}_2\text{OH}\cdot\text{HCl}$, pyridine, 80 °C, 3 h, (v) TsCl, TEA, 0 °C, 1 h, (vi) NH_3 (l), ether, rt, 6 h, (vii) $\text{Na}_2\text{S}_2\text{O}_4$, THF, EtOH, H_2O , rt, 1 h, (viii) MnO_2 , ether, rt, 1 h.





Fig. 13 Asymmetric synthesis of 3- and 4-trifluoroacetyl lactisole derivatives. (i) LiCl, DMF, reflux, 5 h for **86**, BBr₃, CH₂Cl₂, rt, 2 h for **87**, (ii) (1) PPh₃, CH₂Cl₂, 0 °C, 10 min, (2) DEAD, CH₂Cl₂, rt, 8 h, (iii) K₂CO₃, MeOH, H₂O, reflux, 2 h, (iv) *t*-BuBr, K₂CO₃, tetrabutylammonium bromide (TBAB), DMA, 55 °C, 5 h.

DP was over 10-fold less effective than (*S*)-2,4-DP, and (*R*)-lactisole exerted almost no inhibition at millimolar concentrations. These results were consistent with both point mutations of T1R3 affinity measurements and simulations for molecular dynamics-based energy minimization *in silico*. The detailed *in silico* analysis indicated that the *o*-substitution of the phenol moiety may play an important role in the strong inhibitory activity.⁸¹

Trifluoromethyldiazirine-based lactisole derivatives have not yet been reported. The three membered azi (N N) partial structure of trifluoromethyldiazirine is not stable in the Mitsunobu conditions because the reactant, DEAD, also has azo group in the structure. The reaction with diazirinyl phenol derivatives and 1'-hydroxy peracetylsucrose with DEAD or Tsuda reagents

did not proceed in our previous study.²⁷ The synthetic plan was based on the preparation of trifluoroacetyl modified on the aromatic ring of lactisole, and then the construction of a diazirinyl-three membered ring on the trifluoroacetyl group. The liquid ammonia reagent is essential for the construction of diazirine moiety, but the reagent also reacts with a methyl ester to form amide; *tert*-butyl ester was utilized for this purpose.

p-Trifluoroacetyl anisole **84** was treated with lithium chloride under reflux condition to obtain trifluoroacetyl phenol **86**.⁷⁷ *m*-Trifluoroacetyl phenol **85** was synthesized with boron tribromide at room temperature. The Mitsunobu reactions for these phenols with chiral methyl lactate were performed in an identical manner to construct lactisole methyl ester skeletons **88** and **89**. The hydrolysis of methyl esters with K₂CO₃ under reflux conditions yielded the corresponding trifluoroacetyl-substituted carboxylic acids **90** and **91**, which were difficult to convert diazirine directly. The carboxylic acids were converted to *tert*-butyl esters with *tert*-butyl bromide in the presence of K₂CO₃ and TBAB under reflux conditions. Although the reactions were not completed within 5 h, the decomposition of the diazirinyl moiety of the starting material was observed over 5 h. *tert*-Butyl derivatives **92** and **93** were obtained in moderate yields and the starting materials were recovered from the reaction mixture (Fig. 13). Mitsunobu reactions for trifluoroacetyl phenol **86** and **87** with the chiral *tert*-butyl lactate were also conducted, but no reactions were observed.

2-Trifluoroacetyl phenol, which was prepared by the Fries rearrangement of phenyl 2,2,2-trifluoroacetate with AlCl₃,⁸² was subjected to the Mitsunobu reaction with chiral methyl lactate **79** and did not afford 2-trifluoroacetyl lactisole skeleton. The Mitsunobu reaction of salicylaldehyde and methyl lactate **79**

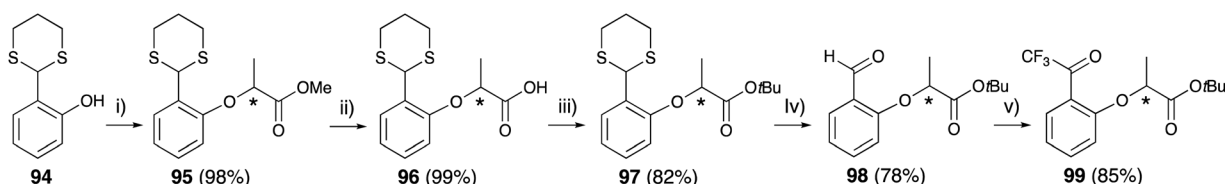


Fig. 14 Asymmetric synthesis of 2-trifluoroacetyl lactisole derivative. (i) (1) **79**, PPh₃, CH₂Cl₂, 0 °C, 10 min, (2) DEAD, rt, 8 h, (ii) K₂CO₃, MeOH, H₂O, reflux, 2 h, (iii) *t*-BuBr, K₂CO₃, TBAB, DMA, 55 °C, 5 h, (iv) CH₃I, NaHCO₃, CH₃CN, H₂O, rt, 24 h, (v) (1) TMS-CF₃, K₂CO₃, DMF, rt, 4 h, (2) 1 M HCl, rt, 4 h, (3) Dess–Martin periodinane, CH₂Cl₂, rt, 3 h.

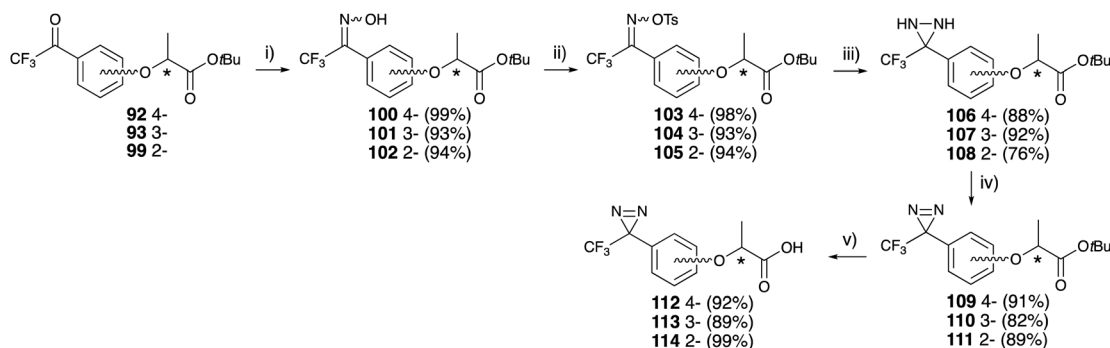


Fig. 15 Asymmetric synthesis of trifluoromethyldiazirinyl lactisole derivatives. (i) H₂NOH·HCl, pyridine, EtOH, 60 °C, 12 h, (ii) tosyl chloride, DMAP, CH₂Cl₂, rt, 45 min, (iii) NH₃ (l), Et₂O, rt, 12 h, (iv) MnO₂, CH₂Cl₂, rt, 2 h, (v) CF₃COOH, CH₂Cl₂, rt, 4 h.



Review

Table 3 IC₅₀ (μM) inhibitory concentrations of synthetic lactisole derivatives

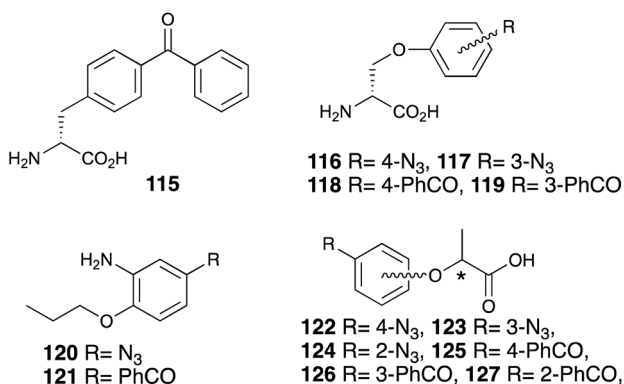
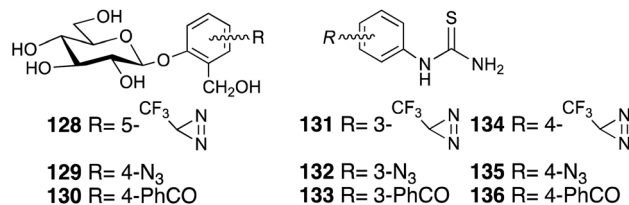
(<i>RS</i>)-lactisole 82	82 ± 0.44	(<i>S</i>)- 112	12 ± 0.067
(<i>S</i>)- 82	20 ± 4.3	(<i>R</i>)- 112	250 ± 1.8
(<i>R</i>)- 82	nd	(<i>S</i>)- 113	19 ± 0.14
(<i>RS</i>)-2,4-DP 83	7.9 ± 0.067	(<i>R</i>)- 113	250 ± 1.7
(<i>S</i>)- 83	2.6 ± 0.51	(<i>S</i>)- 114	37 ± 0.28
(<i>R</i>)- 83	45 ± 6.1	(<i>R</i>)- 114	300 ± 1.8

followed by conversion of the aldehyde to the trifluoroacetyl group also failed owing to the instability of the methyl ester under the conditions for trifluoroacetyl construction.

Thioacetal-protected salicylaldehyde **94** (ref. 83) was selected for trifluoroacetyl substitution at the 2-position of lactisole. Compound **94** was subjected to the Mitsunobu reaction with chiral methyl lactate **79** to construct the lactisole methyl ester **95** in an identical manner to that described above. After hydrolysis of methyl esters with methanolic NaOH under reflux condition, the carboxylic acids **96** were converted to lactisole *tert*-butyl ester **97**, and then deprotected with methyl iodide. The aldehyde **98** was treated TMS-CF₃ and then oxidized with Dess–Martin periodinane to afford 2-trifluoroacetyl-substituted lactisole *tert*-butyl ester **99** (Fig. 14).

Each trifluoroacetyl-substituted lactisole *tert*-butyl ester (**92**, **93**, **99**) was subjected to diazirine construction, oximation with hydroxylamine hydrochloride **100–102**, tosylation of the oxime **103–105**, diaziridine formation in liquid ammonia **106–108**, and oxidation with activated MnO₂ to obtain **109–111**, which were examined for their chiral center configuration. The *tert*-butyl esters were hydrolyzed with TFA to afford trifluoromethyldiazirine-based lactisole derivatives **112–114** in good yields (Fig. 15).

Human sweet receptor assays for diazirine-containing lactisole derivatives revealed that (*S*)-isomers at the 4- and 3-position of lactisole derivatives **112** and **113** had identical affinity to optically pure (*S*)-lactisole **82**. These results indicated that photoaffinity labeling with synthetic lactisole derivatives would be useful for the functional analysis of the sweet taste receptor (Table 3).⁸⁴

**Fig. 16** Photoreactive sweetener derivatives containing azide or benzophenone.**Fig. 17** Photoreactive bitter substance derivatives.

4. Conclusions

There are many challenges to elucidating the sweet taste chemoreception mechanism using photoaffinity labeling, which is one of the chemical biology/bioorganic chemistry methods. Other photophores (phenylazide and benzophenone) have also been utilized for phenylalanine **115** (ref. 85) *o*-benzyl-*D*-serine **116–119** (ref. 57) 2-propoxyaniline **120**, **121** (ref. 77) and lactisole **122–127** (ref. 84) (Fig. 16).

It is often observed that photophore substitution reduces the sweet taste properties of the photoreactive benzophenone and phenylazide derivatives. The introduction of the trifluoromethyl diazirinyl group on the aromatic moiety of sweeteners has become the first choice for this functionality.

Photoaffinity labeling strategies have also been developed to elucidate the bitter taste receptors, which are mediated by the T2R family of GPCRs. Photoreactive salicin **128–129** (ref. 86, 87) and phenylthiourea **131–136** (ref. 88) and highly concentrated saccharine derivatives will be used in bitter taste assays to elucidate the chemoreception mechanism (Fig. 17).

The combination of analysis techniques from molecular biology, computational biology, and chemical biology will play a key part in our understanding of gustatory chemoreception.

Author contributions

M. H. conceived the concept and idea of the present review and worked on the study design strategy. T. N. performed the literature searches and analyzed the data. T. M. selected the topics to be discussed. All authors have read and approved the final draft.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Part of this work was performed under the Cooperative Research Program of “Network Joint Research Center for Materials and Devices”.

Notes and references

- 1 P. Temussi, *J. Mol. Recognit.*, 2006, **19**, 188.
- 2 R. S. Shallenberger and T. E. Acree, *Nature*, 1967, **216**, 480.
- 3 L. B. Kier, *J. Pharm. Sci.*, 1972, **61**, 1394.



- 4 C. Nofre and J. M. Tinti, *Food Chem.*, 1996, **56**, 263.
- 5 X. Yang, Y. Chong, A. Yan and J. Chen, *Food Chem.*, 2011, **128**, 653.
- 6 M. Zhong, Y. Chong, X. Nie, A. Yan and Q. Yuan, *J. Food Sci.*, 2013, **78**, S1445.
- 7 C. Rojas, D. Ballabio, V. Consonni, P. Tripaldi, A. Mauri and R. Todeschini, *Theor. Chem. Acc.*, 2016, **135**, ARTN 66.
- 8 M. Cui, P. H. Jiang, E. Maillat, M. Max, R. F. Margolskee and R. Osman, *Curr. Pharm. Des.*, 2006, **12**, 4591.
- 9 J. Chandrashekar, M. A. Hoon, N. J. Ryba and C. S. Zuker, *Nature*, 2006, **444**, 288–294.
- 10 X. Li, L. Staszewski, H. Xu, K. Durick, M. Zoller and E. Adler, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 4692–4696.
- 11 H. Xu, L. Staszewski, H. Tang, E. Adler, M. Zoller and X. Li, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 14258–14263.
- 12 N. Kunishima, Y. Shimada, Y. Tsuji, T. Sato, M. Yamamoto, T. Kumasaka, S. Nakanishi, H. Jingami and K. Morikawa, *Nature*, 2000, **407**, 971–977.
- 13 K. Masuda, A. Koizumi, K. Nakajima, T. Tanaka, K. Abe, T. Misaka and M. Ishiguro, *Plos One*, 2012, **7**, e35380.
- 14 J. Brunner, *Annu. Rev. Biochem.*, 1993, **62**, 483–514.
- 15 T. Tomohiro, M. Hashimoto and Y. Hatanaka, *Chem. Rec.*, 2005, **5**, 385–395.
- 16 M. Hashimoto and Y. Hatanaka, *Eur. J. Org. Chem.*, 2008, 2513–2523.
- 17 Y. Hatanaka, *Chem. Pharm. Bull.*, 2015, **63**, 1–12.
- 18 M. S. Platz, *Acc. Chem. Res.*, 2002, **28**, 487–492.
- 19 W. L. Karney and W. T. Borden, *J. Am. Chem. Soc.*, 1997, **119**, 3347–3350.
- 20 R. E. Galardy, L. C. Craig and M. P. Printz, *Nat. New Biol.*, 1973, **242**, 127–128.
- 21 R. A. Smith and J. R. Knowles, *J. Am. Chem. Soc.*, 1973, **95**, 5072–5073.
- 22 T. Oku, T. Tokunaga and N. Hosoya, *J. Nutr.*, 1984, **114**, 1574–1581.
- 23 G. Carrea, S. Riva, F. Secundo and B. Danieli, *J. Chem. Soc., Perkin Trans.*, 1989, **1**, 1057–1061.
- 24 R. Matsumi, H. Atomi, A. J. Driessen and J. van der Oost, *Res. Microbiol.*, 2011, **162**, 39–52.
- 25 S. Jarosz and M. Mach, *Eur. J. Org. Chem.*, 2002, **2002**, 769–780.
- 26 O. Mitsunobu, *Synthesis*, 1981, **1981**, 1–28.
- 27 Y. Tsunekawa, K. Masuda, M. Muto, Y. Muto, Y. Murai, Y. Hashidoko, Y. Orikasa, Y. Oda, Y. Hatanaka and M. Hashimoto, *Heterocycles*, 2012, **84**, 283–290.
- 28 C. Chauvin and D. Plusquellec, *Tetrahedron Lett.*, 1991, **32**, 3495–3498.
- 29 D. M. Clode, D. McHale, J. B. Sheridan, G. G. Birch and E. B. Rathbone, *Carbohydr. Res.*, 1985, **139**, 141–146.
- 30 L. Wang, Z. P. Tachrim, N. Kurokawa, F. Ohashi, H. Wakasa, Y. Sakihama, Y. Hashidoko, T. Suzuki and M. Hashimoto, *Arkivoc*, 2018, 58–65.
- 31 L. Wang, Y. Hashidoko and M. Hashimoto, *J. Org. Chem.*, 2016, **81**, 4464–4474.
- 32 T. Kaneko, *Nippon Kagaku Kaishi*, 1938, **59**, 433–439.
- 33 T. Kaneko, *Nippon Kagaku Kaishi*, 1939, **60**, 531–538.
- 34 J. W. Finley and M. Friedman, *J. Agric. Food Chem.*, 1973, **21**, 33–34.
- 35 M. Nassal, *J. Am. Chem. Soc.*, 1984, **106**, 7540–7545.
- 36 L. B. Shih and H. Bayley, *Anal. Biochem.*, 1985, **144**, 132–141.
- 37 G. Baldini, B. Martoglio, A. Schachenmann, C. Zugliani and J. Brunner, *Biochemistry*, 1988, **27**, 7951–7959.
- 38 C. W. G. Fishwick, J. M. Sanderson and J. B. C. Findlay, *Tetrahedron Lett.*, 1994, **35**, 4611–4614.
- 39 M. Hashimoto, Y. Hatanaka, Y. Sadakane and K. Nabeta, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2507–2510.
- 40 H. Nakashima, M. Hashimoto, Y. Sadakane, T. Tomohiro and Y. Hatanaka, *J. Am. Chem. Soc.*, 2006, **128**, 15092–15093.
- 41 M. T. Stankovich and B. G. Fox, *Biochim. Biophys. Acta*, 1984, **786**, 49–56.
- 42 L. Wang, Y. Murai, T. Yoshida, A. Ishida, K. Masuda, Y. Sakihama, Y. Hashidoko, Y. Hatanaka and M. Hashimoto, *Org. Lett.*, 2015, **17**, 616–619.
- 43 L. Wang, Y. Murai, T. Yoshida, M. Okamoto, K. Masuda, Y. Sakihama, Y. Hashidoko, Y. Hatanaka and M. Hashimoto, *Biosci., Biotechnol., Biochem.*, 2014, **78**, 1129–1134.
- 44 K. Masuda, A. Koizumi, T. Misaka, Y. Hatanaka, K. Abe, T. Tanaka, M. Ishiguro and M. Hashimoto, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1081–1083.
- 45 S. Bittner, R. Scherzer and E. Harlev, *Amino Acids*, 2007, **33**, 19–42.
- 46 J. Ma, W. Yin, H. Zhou, X. Liao and J. M. Cook, *J. Org. Chem.*, 2009, **74**, 264–273.
- 47 P. S. Baran, B. D. Hafensteiner, N. B. Ambhaikar, C. A. Guerrero and J. D. Gallagher, *J. Am. Chem. Soc.*, 2006, **128**, 8678–8693.
- 48 H. Eto, C. Eguchi and T. Kagawa, *Bull. Chem. Soc. Jpn.*, 1989, **62**, 961–963.
- 49 H. R. Snyder and J. A. MacDonald, *J. Am. Chem. Soc.*, 2002, **77**, 1257–1259.
- 50 Y. Murai, K. Masuda, Y. Sakihama, Y. Hashidoko, Y. Hatanaka and M. Hashimoto, *J. Org. Chem.*, 2012, **77**, 8581–8587.
- 51 S. S. Schiffman, K. Sennewald and J. Gagnon, *Physiol. Behav.*, 1981, **27**, 51–59.
- 52 K. Nakajima, T. Asakura, J. Maruyama, Y. Morita, H. Oike, A. Shimizu-Ibuka, T. Misaka, H. Sorimachi, S. Arai, K. Kitamoto and K. Abe, *Appl. Environ. Microbiol.*, 2006, **72**, 3716–3723.
- 53 G. Barcelo, J.-P. Senet, G. Sennyey, J. Bensoam and A. Loffet, *Synthesis*, 1986, **1986**, 627–632.
- 54 J. T. Spletstoser, P. T. Flaherty, R. H. Himes and G. I. Georg, *J. Med. Chem.*, 2004, **47**, 6459–6465.
- 55 N. Umezawa, N. Matsumoto, S. Iwama, N. Kato and T. Higuchi, *Bioorg. Med. Chem.*, 2010, **18**, 6340–6350.
- 56 M. Sechi, F. Carta, L. Sannia, R. Dallochio, A. Dessi, R. I. Al-Safi and N. Neamati, *Antiviral Res.*, 2009, **81**, 267–276.
- 57 M. Hashimoto, T. Yoshida, Z. P. Tachrim, Y. Sakihama, Y. Hashidoko, Y. Hatanaka and Y. Kanaoka, *Heterocycles*, 2017, **95**, 462–473.
- 58 M. Kawai, M. Chorev, J. Marin-Rose and M. Goodman, *J. Med. Chem.*, 1980, **23**, 420–424.



- 59 Y. Hatanaka, M. Hashimoto, H. Kurihara, H. Nakayama and Y. Kanaoka, *J. Org. Chem.*, 1994, **59**, 383–387.
- 60 M. Hashimoto, Y. Kato and Y. Hatanaka, *Tetrahedron Lett.*, 2006, **47**, 3391–3394.
- 61 E. J. Corey, F. Xu and M. C. Noe, *J. Am. Chem. Soc.*, 1997, **119**, 12414–12415.
- 62 K. Berling, J. Knutsson, A. Rosenblad and M. von Unge, *Acta Otolaryngol.*, 2011, **131**, 488–493.
- 63 T. Suami, L. Hough, T. Machinami, T. Saito and K. Nakamura, *Food Chem.*, 1998, **63**, 391–396.
- 64 R. A. Moss, J. M. Fede and S. Yan, *Org. Lett.*, 2001, **3**, 2305–2308.
- 65 L. Xu, H. Shu, Y. Liu, S. Zhang and M. L. Trudell, *Tetrahedron*, 2006, **62**, 7902–7910.
- 66 T. Ueda, S. Ugawa, H. Yamamura, Y. Imaizumi and S. Shimada, *J. Neurosci.*, 2003, **23**, 7376–7380.
- 67 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, **2015**, 3129–3134.
- 68 W. S. Cox, *J. AOAC Int.*, 1953, **36**, 749–750.
- 69 O. G. Fitzhugh, A. A. Nelson and J. P. Frawley, *J. Am. Pharm. Assoc.*, 1951, **40**, 583–586.
- 70 A. Bassoli, M. G. Drew, L. Merlini and G. Morini, *J. Med. Chem.*, 2002, **45**, 4402–4409.
- 71 G. Morini, A. Bassoli and P. A. Temussi, *J. Med. Chem.*, 2005, **48**, 5520–5529.
- 72 H. Iwamura, *J. Med. Chem.*, 1980, **23**, 308–312.
- 73 A. Chatterjee, D. J. Oh, K. M. Kim, K. S. Youk and K. H. Ahn, *Chem.-Asian J.*, 2008, **3**, 1962–1967.
- 74 R. J. Linderman and D. M. Graves, *J. Org. Chem.*, 2002, **54**, 661–668.
- 75 Y. Hatanaka, M. Hashimoto, H. Nakayama and Y. Kanaoka, *Chem. Pharm. Bull.*, 1994, **42**, 826–831.
- 76 S. Kosemura, H. Emori, S. Yamamura, T. Anai, K. Tomita and K. Hasegawa, *Tetrahedron Lett.*, 1997, **38**, 2125–2128.
- 77 Y. Murai, T. Yoshida, L. Wang, K. Masuda, Y. Hashidoko, K. Monde, Y. Hatanaka and M. Hashimoto, *Synlett*, 2016, **27**, 946–950.
- 78 E. B. Rathbone, G. D. Patel, R. W. Butters, D. Cookson and J. L. Robinson, *J. Agric. Food Chem.*, 1989, **37**, 54–58.
- 79 E. B. Rathbone, R. W. Butters, D. Cookson and J. L. Robinson, *J. Agric. Food Chem.*, 1989, **37**, 58–60.
- 80 E. L. Maillet, R. F. Margolskee and B. Mosinger, *J. Med. Chem.*, 2009, **52**, 6931–6935.
- 81 T. Nakagita, A. Ishida, T. Matsuya, T. Kobayashi, M. Narukawa, T. Hirokawa, M. Hashimoto and T. Misaka, *PLoS One*, 2019, **14**, e0213552.
- 82 D. V. Sevenard, M. Vorobyev, V. Y. Sosnovskikh, H. Wessel, O. Kazakova, V. Vogel, N. E. Shevchenko, V. G. Nenajdenko, E. Lork and G.-V. Rösenthaler, *Tetrahedron*, 2009, **65**, 7538–7552.
- 83 A. Neogi, T. P. Majhi, B. Achari and P. Chattopadhyay, *Eur. J. Org. Chem.*, 2008, **2008**, 330–336.
- 84 T. Nakagita, A. Ishida, Z. P. Tachrim, L. Wang, T. Misaka and M. Hashimoto, *Molecules*, 2020, **25**, 2790.
- 85 Y. Murai, L. Wang, Y. Muto, Y. Sakihama, Y. Hashidoko, Y. Hatanaka and M. Hashimoto, *Heterocycles*, 2013, **87**, 2119–2126.
- 86 M. Sakurai, T. Yoshida, L. Wang, Y. Murai, K. Masuda, Y. Sakihama, Y. Hashidoko, Y. Hatanaka and M. Hashimoto, *Heterocycles*, 2015, **90**, 698–705.
- 87 T. Yoshida, Y. Hashidoko and M. Hashimoto, *Heterocycles*, 2016, **93**, 355–361.
- 88 A. Ishida, L. Wang, Z. P. Tachrim, T. Suzuki, Y. Sakihama, Y. Hashidoko and M. Hashimoto, *Chemistryselect*, 2017, **2**, 160–164.

